PreAnalytiX

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Technical Note
PAXgene® Blood ccfDNA System*
Primary Tube Handling

Shortened workflow processing PAXgene Blood ccfDNA Tubes* directly with the QIAsymphony® PAXgene Blood ccfDNA Kit* and protocols to increase usability and generate ccfDNA yields comparable to a workflow using secondary tubes

Introduction

Plasma separation and harvest is the most time-consuming upfront handling step in the ccfDNA isolation workflow and requires the most hands-on time. For many downstream applications, a two-step centrifugation protocol with two manual transfer steps into fresh tubes is recommended.† In comparison to workflows without two manual transfer steps, this procedure increases the risk of sample mix-up or mislabelling errors, and raises overall workflow costs with longer operator hands-on times, increased use of plastic consumables and greater biohazardous waste disposal.

The processing workflow for PAXgene Blood ccfDNA Tubes described in the *QIAsymphony PAXgene Blood ccfDNA Kit Handbook* allows the use of a single centrifugation step with only one transfer of plasma into a secondary tube. To further improve this procedure, custom protocols were developed to use the centrifuged PAXgene Blood ccfDNA Tube directly on the QIAsymphony SP instrument, without the need for a secondary tube. This increases sample throughput, reduces hands-on time and helps prevent subject sample misidentification errors. This technical note describes this procedure and compares it to a double-centrifugation workflow with manual plasma transfers.

Study Design

Human whole blood was collected into 8 PAXgene Blood ccfDNA Tubes per subject from 22 consented apparently healthy adult subjects. Plasma was harvested either directly after blood draw (within 2 hours after blood collection) or after storage for 7 days at 25°C. ccfDNA was extracted from plasma using either the new primary tube handling workflow with custom protocols‡ (Figure 1), or the workflow with an optional second centrifugation step and the protocol **PAXcircDNA LAF2400** described in the

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* For research use only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

† Chiu et al. (2001) *Clin Chem* 47; 9: 1607-1613.

‡ The custom protocols listed are examples only. Protocols with other plasma volumes are available as well.

QIAsymphony PAXgene Blood ccfDNA Kit Handbook. For each subject, one tube was processed for each combination of sample storage time (0 or 7 days), workflow (single centrifugation with primary tube processing or double centrifugation with secondary tube processing) and plasma input volume (2.4 or 4.0 ml). 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 cycler.

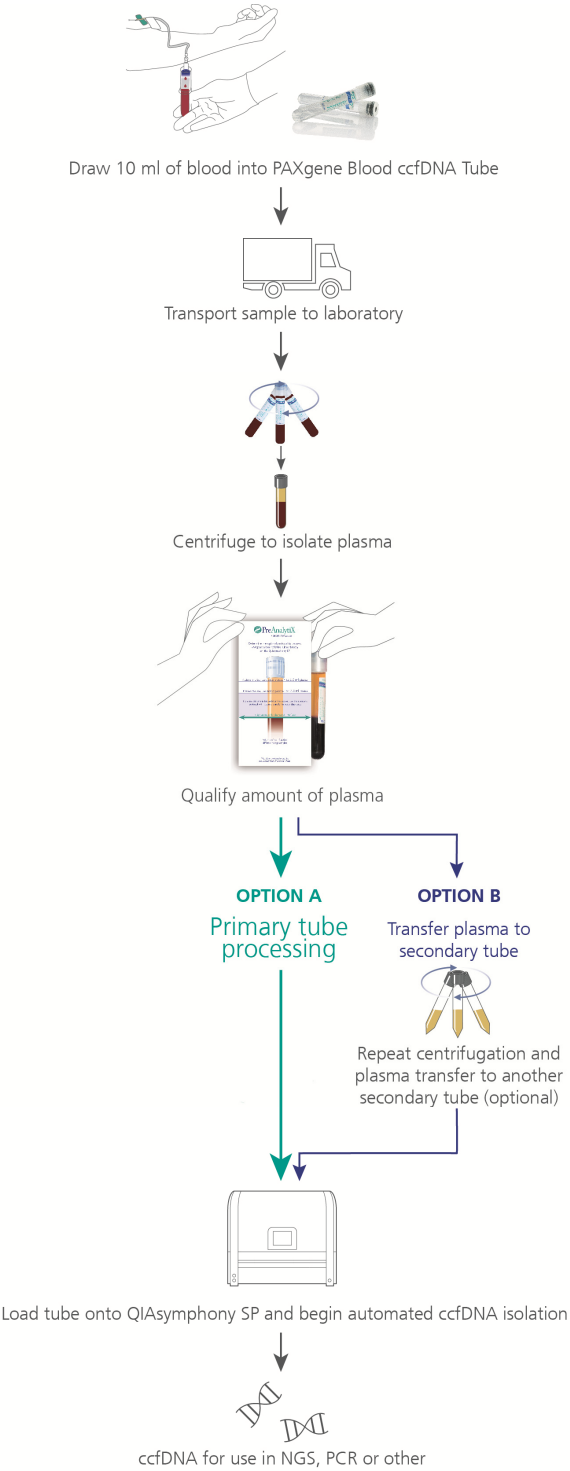

Figure 1. Workflow options for the PAXgene Blood ccfDNA System.
Option A is a streamlined custom workflow using the PAXgene Blood ccfDNA Tube as primary tube to be processed directly on the QIAsymphony SP instrument. Option B is the workflow described in the *QIAsymphony PAXgene Blood ccfDNA Kit Handbook*.

Tube processing via the primary tube handling workflow

Prior to initiating the processing run on the QIAsymphony SP, the instrument was loaded with the reagent cartridges and proteinase K from the QIAsymphony PAXgene Blood ccfDNA Kit and the required plastic consumables. PAXgene Blood ccfDNA Tubes were centrifuged at room temperature (15–25°C) for 15 minutes at 1900 x *g* using a balanced centrifuge.

The plasma volume in each tube was qualified during removal from the centrifuge bucket, either with the PAXgene Blood ccfDNA Purification Protocol Selection Tool (Figure 2) or a suitable standard ruler (Table 1). PAXgene Blood ccfDNA Tubes that contained sufficient plasma for the custom primary tube handling workflow were placed directly into the QIAsymphony Sample Tube Carrier, taking care to avoid agitation while loading the carrier into the sample input area of the QIAsymphony SP. These tubes were processed using the custom protocol corresponding to the measured plasma volume (Table 1). Tubes that did not contain sufficient plasma for the custom primary tube handling workflow were processed following instructions described in the *QIAsymphony PAXgene Blood ccfDNA Kit Handbook,* such that for each subject, one tube was processed per treatment (combination of storage time, workflow and plasma volume).

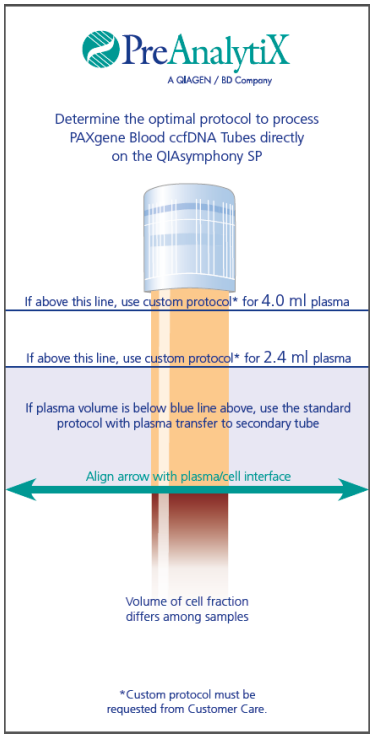

Figure 2. Plasma volume determination using the PAXgene Blood ccfDNA Purification Protocol Selection Tool.
Plasma volume in each PAXgene Blood ccfDNA Tube was qualified after centrifugation. Upon removing the tube from the centrifuge, the teal arrow on the tool was aligned with the plasma/cell interface. The blue lines indicated if the plasma level was sufficient for the 2.4 or 4.0 ml custom primary tube handling protocols.

Custom protocol plasma volume (ml)	Minimum plasma height required (cm)
2.4	2.3
4.0	3.4

Table 1. Minimum plasma height requirement.
A minimum plasma column height is needed for the corresponding custom primary tube handling protocol.

Results

DNA Yield Comparison of 2.4 and 4.0 ml plasma input volumes

DNA yields were measured for each blood storage time and workflow used (Figure 3A). The theoretical difference in C_Ts between a 4.0 and 2.4 ml input plasma volume is 0.71, with a reduced plasma volume resulting in an increased C_T value. As expected, the smaller plasma volumes had correspondingly lower mean C_T values. In paired samples, C_T values were lower by 0.80 and 0.95 C_T on average when plasma was separated directly after blood collection for the secondary and primary tube workflows, respectively. Similarly, C_T values were lower by 0.73 and 0.72 C_T on average when tubes were processed after 7 days of sample storage for the secondary and primary tube workflows.

DNA Yield Comparison of secondary and primary tube workflows

The secondary and primary tube workflows, using both 4.0 and 2.4 ml plasma input volume, had similar mean C_T values for paired samples. In addition, results were concordant between both workflows for samples processed directly and those stored for 7 days at 15–25°C.

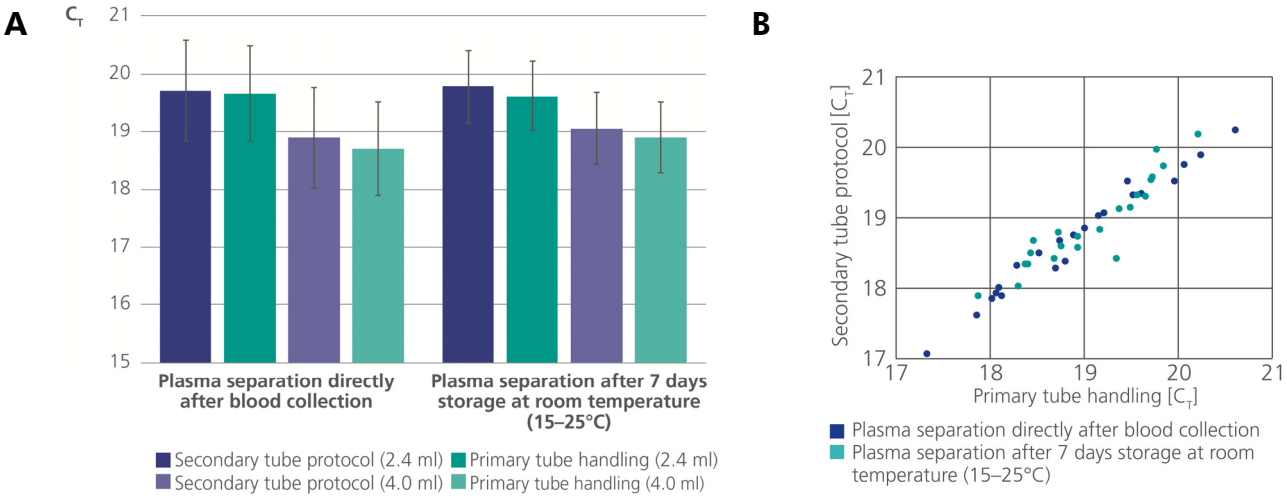

Figure 3. Comparison of ccfDNA yield from the custom primary tube handling workflow and the workflow with two centrifugation steps and secondary tubes. Plasma was generated either directly after blood draw or after seven days of storage at room temperature (15–25°C). The workflows were compared using a validated qPCR assay (target 18S rDNA gene, 66 bp amplicon; n = 22 per treatment). **A** Mean C_T values with standard deviation. **B** C_T values in a scatter plot.

Conclusion

The custom primary tube handling workflow for the PAXgene Blood ccfDNA System is a viable option for laboratories that need increased sample throughput and reduced hands-on time, or would like to reduce the risk of subject sample misidentification errors that may occur during sample transfer into secondary tubes. This workflow uses a single centrifugation step followed by visual qualification of the plasma volume and direct loading of PAXgene Blood ccfDNA Tubes into the QIAsymphony SP. Upfront handling time is reduced by 40 minutes (20 minutes instead of 60 minutes for 24 tubes). In addition, the custom primary tube handling workflow helps prevent subject sample mix-up and minimizes the amount of plastic consumables used and the waste generated without impacting ccfDNA yield.

Products used

Product	Catalog No.
PAXgene Blood ccfDNA Tube (100)	768115
QIAsymphony PAXgene Blood ccfDNA Kit (192)	768536
QIAsymphony SP (QIAGEN)	9001297
Rotor-Gene Q (QIAGEN)	9001550

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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