# A Shortened, Automated Workflow for Processing Plasma Directly From Primary Blood Collection Tubes Reduces Turnaround Time and Risk of Sample Mix-up in ccfDNA Based Applications

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## Introduction

Plasma separation and harvest is the most time-consuming upfront handling step in the ccfDNA isolation workflow. For many downstream applications, such as non-invasive prenatal testing and circulating tumor DNA testing, a two step centrifugation protocol with two manual transfer steps into fresh tubes is used. This procedure increases the risk of blood exposure and sample mix-up or mislabelling errors, and generates additional biohazardous waste. In the new workflow, one centrifugation step and all manual sample transfer steps are eliminated, as the blood collection tubes are used directly for automated ccfDNA isolation.

Here we show the performance of this new workflow with the recently developed PAXgene® Blood ccfDNA System* in two research studies. As downstream methods, quantitative PCR as well as NGS were used in routine, high throughput setups to analyze isolated ccfDNA.

*For research use only. Not for use in diagnostic procedures.

**Tube features:**

- Effective stabilization of:
  - White blood cells – Helps prevent release of gDNA
  - Red blood cells – Helps minimize hemolysis
- Helps maximize ccfDNA yield from plasma, minimize background gDNA
- Non-crosslinking, no DNA modification
- BD Vacutainer® plastic tube with BD Hemogard™ safety closure
  - Helps minimize risk of tube breakage
  - Enhances safety for healthcare and lab personnel
  - Helps minimize contamination between samples
  - Consistent blood draw volume

**PAXgene Blood ccfDNA System**

**PAXgene Blood ccfDNA Tube (RUO)**


**QIAsymphony® PAXgene Blood ccfDNA Kit (RUO)**


**Kit features:**

- Dedicated ccfDNA isolation technology for use with the PAXgene Blood ccfDNA Tube
  - Binding chemistry optimized for use with PAXgene ccfDNA Tube reagent
  - Optimized input volumes to accommodate higher volume plasma
- Automated ccfDNA extraction
  - Standard protocol for preferred extraction of small fragments (≤ 500 bp) with 60 µl elution volume
  - Large fragment protocol for co-isolation of large fragments (> 500 bp) with flexible elution volume (60, 100, 150 µl)
- 2.4 ml or 4.8 ml plasma processing options
- Prefilled cartridges are ready to use

## Methods

**Study Design and Methods**

**Study 1:** Whole blood was collected into multiple PAXgene Blood ccfDNA Tubes (PreAnalytiX)* from 22 consented apparently healthy subjects. Plasma was harvested either directly (within two hours) after blood draw or after 7 days of storage at 25°C (room temperature, RT). ccfDNA was extracted from plasma using the QIAsymphony PAXgene Blood ccfDNA Kit (PreAnalytiX)* with either the new primary tube handling workflow (figure 2) or the commonly used workflow with two centrifugation steps based on the study of Chiu et al. (2001)[†]. Relative ccfDNA yield was quantified by a probe-based qPCR assay amplifying a 66 bp fragment of the 18S rDNA gene.

**Study 2:** Compatibility of the new workflow with the PrenaTest® (LifeCodexx) was evaluated in a follow up research study. Paired blood samples from 25 consented pregnant women were collected into PAXgene Blood ccfDNA Tubes and Cell-Free DNA BCT® (Streck) and processed in parallel. All samples were processed using the regular PrenaTest Routine (figure 1) with exception of the plasma preparation and ccfDNA isolation steps. PAXgene Blood ccfDNA Tubes were processed using the new workflow while Streck tubes were processed using the manufacturer's protocol including manual plasma sample transfer. ccfDNA yield and fetus specific ccfDNA was quantified by QuantYfeX® qPCR quality control assay. Finally, ccfDNA eluates were analyzed using the NGS-based PrenaTest for chromosomal disorders.

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[†]Chiu, et al. *Clin Chem*. 2001 Sep;47(9):1607-13.

**PrenaTest Routine**

1. Blood collection
2. Plasma preparation
3. Extraction of cell free DNA
4. Quantification of fetal DNA (QuantYfeX)
5. Preparation of a genomic library
6. Next Generation Sequencing
7. BioIT analysis

**Figure 1.** LifeCodexx PrenaTest Routine

**Workflow Options for the PAXgene Blood ccfDNA System**

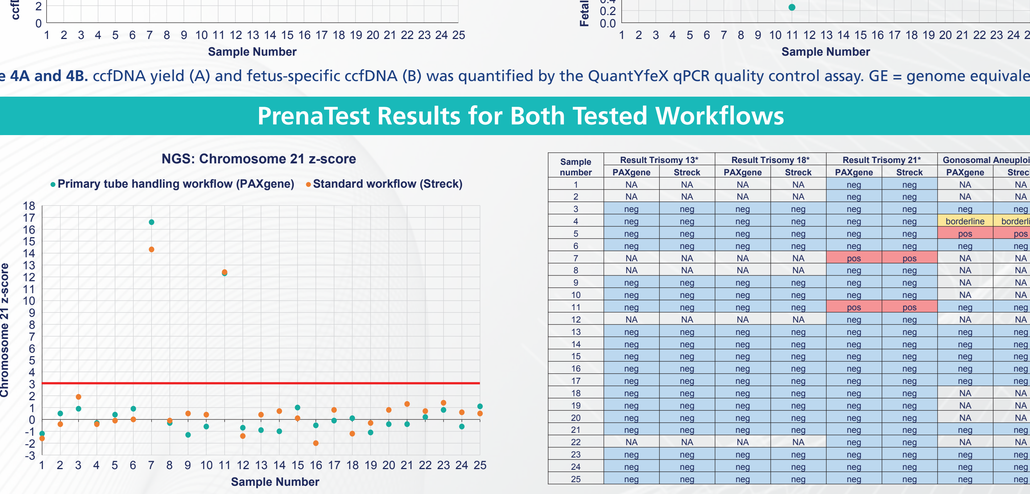


**Figure 2.** Option A is the workflow described in the *QIAsymphony PAXgene Blood ccfDNA Kit Handbook* using the optional second centrifugation step. Option B is the new primary tube handling workflow using the PAXgene Blood ccfDNA Tube to be processed directly on the QIAsymphony SP instrument. Time savings based on processing a 24 sample batch.

## Results

**Study 1:** DNA yields were measured for each blood storage time and protocol used (2.4 and 4.0 ml plasma from direct sampling). Overall, high concordance was observed between ccfDNA yields of the two step centrifugation workflow and the new primary tube handling workflow. In addition, similar ccfDNA yields were seen between plasma generated directly after blood collection or after 7 day storage at room temperature. As expected, smaller plasma volumes resulted in reduced yields, shown by increased $C_T$ values for the 2.4 ml plasma samples in comparison to the 4.0 ml plasma samples. The smaller plasma volumes had correspondingly higher mean $C_T$ values of 0.80 and 0.95 on average when plasma was separated directly after blood collection, and 0.73 and 0.72 on average when tubes were processed after 7 days of sample storage for the secondary and primary workflows, respectively. This is close to the theoretical difference of 0.71 $C_T$.

**Study 2:** Levels of ccfDNA and fetus-specific ccfDNA isolated from plasma generated from maternal blood which was collected in PAXgene Blood ccfDNA Tubes and Streck Cell-Free DNA BCT were not significantly different (Standard workflow: median 7.01 GE/µL; Primary tube handling workflow: median 6.68 GE/µL; *p*-value: 0.619 [paired T-Test, two-tailed distribution]).

All samples produced equivalent results with PrenaTest, independent of the workflow used. All samples pass the NGS QC criteria for mapped reads, unique mapped reads, and GC content. Results for investigated aneuploidies matched 100% between the different workflows.

### Study 1

- High concordance between ccfDNA yields of two step centrifugation workflow and new primary tube handling workflow.
- High concordance in ccfDNA yields over sample storage for 7 days.
- Expected differences in ccfDNA yield depending on used amount of plasma.

**Comparison of ccfDNA Yields From 2.4 and 4.0 ml Input Plasma Using the Protocols With Secondary and Primary Tube Handling**

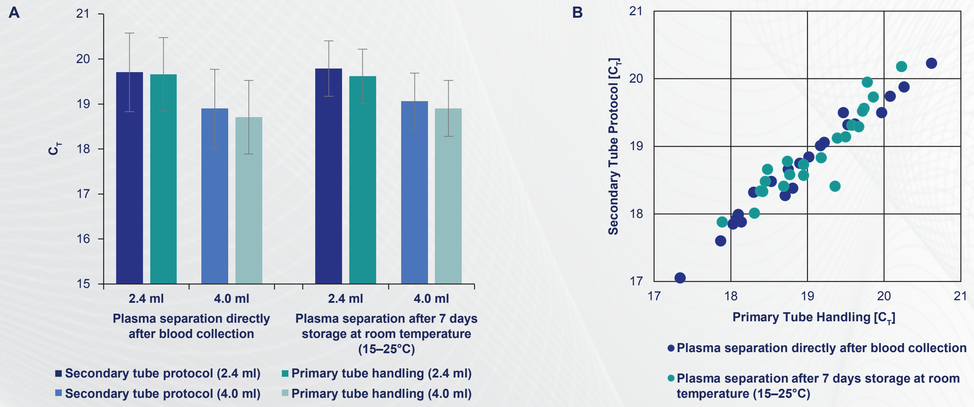


**Figure 3A and 3B. A:** Yield from 22 whole blood samples was qualified with a validated qPCR (18S rDNA, 66 bp amplicon). **B:** $C_T$ values obtained with the two protocols show high concordance.

### Study 2

- All samples yielded enough plasma for direct processing using the PAXgene ccfDNA tube as primary tube.
- No significant difference in obtained absolute ccfDNA levels.
- No significant difference in isolated absolute fetus-specific ccfDNA levels.
- All samples pass our NGS QC criteria for mapped reads, unique mapped reads, and GC content.
- Results for investigated aneuploidies match 100% between the different workflows.

**QuantYfeX Quality Control Assay Results for Both Tested Workflows**

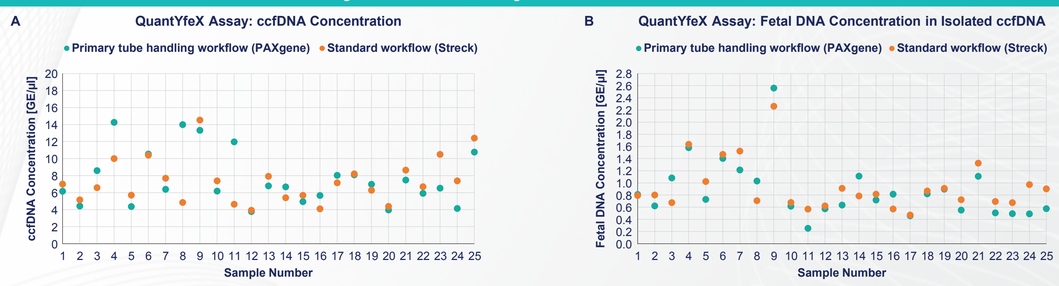


**Figure 4A and 4B.** ccfDNA yield (A) and fetus-specific ccfDNA (B) was quantified by the QuantYfeX qPCR quality control assay. GE = genome equivalents.

**PrenaTest Results for Both Tested Workflows**




**Figure 5.** NGS – Chromosome 21 z-score. Consolidated output of the PrenaTest specific algorithm based on NGS results for chromosome 21. Red line indicates the threshold for trisomy 21. PrenaTest results for both tested workflows.

| Sample number | Result Trisomy 13* | | Result Trisomy 18* | | Result Trisomy 21* | | Gonosomal Aneuploidy* | |
|---|---|---|---|---|---|---|---|---|
| | PAXgene | Streck | PAXgene | Streck | PAXgene | Streck | PAXgene | Streck |
| 1 | NA | NA | NA | NA | neg | neg | NA | NA |
| 2 | NA | NA | NA | NA | neg | neg | NA | NA |
| 3 | neg | neg | neg | neg | neg | neg | neg | neg |
| 4 | neg | neg | neg | neg | neg | neg | borderline | borderline |
| 5 | neg | neg | neg | neg | neg | neg | pos | pos |
| 6 | neg | neg | neg | neg | neg | neg | neg | neg |
| 7 | NA | NA | NA | NA | pos | pos | NA | NA |
| 8 | NA | NA | NA | NA | neg | neg | NA | NA |
| 9 | neg | neg | neg | neg | neg | neg | NA | NA |
| 10 | neg | neg | neg | neg | neg | neg | NA | NA |
| 11 | neg | neg | neg | neg | pos | pos | neg | neg |
| 12 | NA | NA | NA | NA | neg | neg | NA | NA |
| 13 | neg | neg | neg | neg | neg | neg | neg | neg |
| 14 | neg | neg | neg | neg | neg | neg | neg | neg |
| 15 | neg | neg | neg | neg | neg | neg | neg | neg |
| 16 | neg | neg | neg | neg | neg | neg | neg | neg |
| 17 | neg | neg | neg | neg | neg | neg | neg | neg |
| 18 | neg | neg | neg | neg | neg | neg | NA | NA |
| 19 | neg | neg | neg | neg | neg | neg | NA | NA |
| 20 | neg | neg | neg | neg | neg | neg | NA | NA |
| 21 | neg | neg | neg | neg | neg | neg | neg | neg |
| 22 | NA | NA | NA | NA | neg | neg | NA | NA |
| 23 | neg | neg | neg | neg | neg | neg | neg | neg |
| 24 | neg | neg | neg | neg | neg | neg | neg | neg |
| 25 | neg | neg | neg | neg | neg | neg | neg | neg |

**Table 1.** Overview of all PrenaTest results for both tested workflows. *Analyzed only if requested by patient.

## Conclusions

In these research studies, we observed comparable results with both tested workflows. Importantly, results obtained in the aneuploidy testing were 100% concordant.

The testing indicates that the primary tube handling workflow can be a viable option for laboratories with high sample throughput. Upfront handling time can be reduced by 66% for a 24 sample batch, with potentially greater time savings for multiple batches. In addition, this workflow helps prevent sample mix-up errors and reduces the amount of waste generated without impacting ccfDNA yield and test results.

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**The PAXgene Blood ccfDNA System is For Research Use Only. Not for use in diagnostic procedures.**

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit and PreAnalytiX handbook or user manual. Handbooks and user manuals are available at **www.qiagen.com** and **www.preanalytix.com** or can be requested from QIAGEN Technical Services or your local distributor.



**No Sample Transfer**

**Reduces risk of exposure and sample mixup**

**Direct Processing**

**Saves time**

**No Secondary Tubes**

**Reduced costs and biohazardous waste**