Integrated Workflow for Blood Collection, ccfDNA Stabilization and Extraction for Non-Invasive Prenatal Testing

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Background

Non-invasive prenatal testing (NIPT) uses fetal circulating cell-free DNA (ccfDNA) in the maternal blood circulation. The release of maternal genomic DNA from blood cells during blood collection, transport and storage significantly reduces the sensitivity of the next-generation sequencing (NGS) based NIPT assays. Moreover, the reliable and reproducible extraction of the low abundance and short fetal ccfDNA fragments is technically challenging. Both difficulties are addressed by the PAXgene® Blood ccfDNA System*, which consists of a blood collection tube (PAXgene Blood ccfDNA Tube*) and a ccfDNA purification kit (QIAsymphony® PAXgene Blood ccfDNA Kit*). The PAXgene Blood ccfDNA Tube uses a plastic BD Vacutainer® tube with a unique, non-crosslinking chemistry for stabilization of blood cells. The QIAsymphony PAXgene Blood ccfDNA Kit can be used for automated isolation of either small ccfDNA fragments or co-isolation of small and large ccfDNA fragments. The performance of the new system was evaluated in two research studies in comparison to K_2EDTA tubes and the Streck Cell-Free DNA BCT®.

PAXgene Blood ccfDNA Tube (RUO)

QIAsymphony PAXgene Blood ccfDNA Kit (RUO)

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

Kit features:

- Dedicated ccfDNA isolation technology for use with the PAXgene Blood ccfDNA Tube
  - Binding chemistry optimized for use with PAXgene Blood ccfDNA Tube reagent
  - Optimized input volumes to accommodate higher volume plasma
- Two protocol lines
  - 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
- Prefilled cartridges are ready to use

QIAsymphony PAXgene Blood ccfDNA Protocols

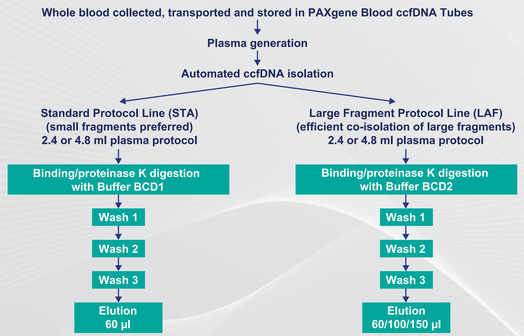

Figure 1. Standard and large fragment protocol lines. With the standard protocols (STA) predominately small ccfDNA fragments are isolated while large fragment protocols (LAF) efficiently co-purify large and small ccfDNA fragments. Both protocol lines consist of protocols for 2.4 ml or 4.8 ml plasma input.

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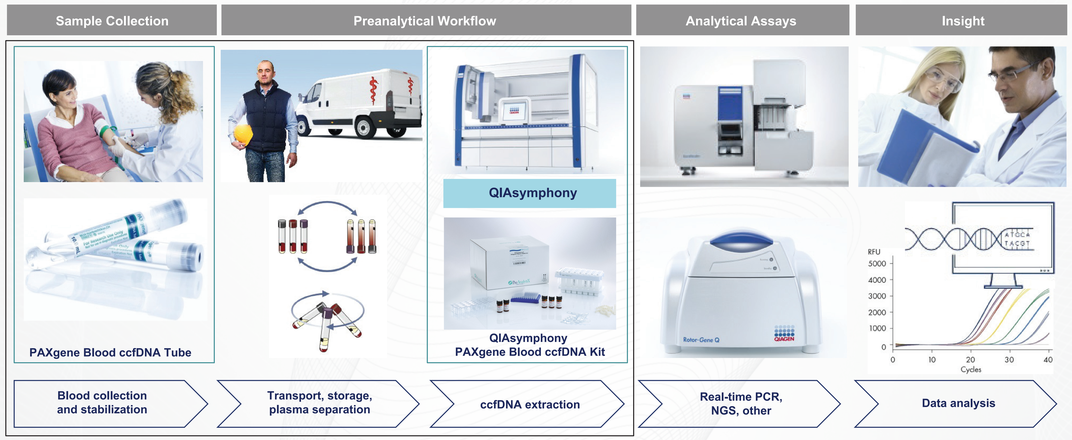
PAXgene Blood ccfDNA System Workflow: Integrated Collection-Stabilization-Preparation (CSP) System



Study 1: Verification of the PAXgene Blood ccfDNA System

Table 1. System performance evaluation of the PAXgene Blood ccfDNA System

Study 1A	
Comparison of PAXgene automated kit vs. manual procedure[1]	
Study Parameters	
Samples tested	825
Subjects	189
Runs	42
Instruments	6
Study Results	
ccfDNA yield	104%
Portion of dsDNA[2]	> 96%
Total failures	0
Study 1B	
Evaluation of PAXgene system performance[3]	
Study Parameters	
Samples tested	1636
Subjects	204
Runs	71
Instruments	6
Study Results	
Yield compared to EDTA plasma[4]	No significant difference
ccfDNA stability at room temperature[5]	7 days
Cross-contamination	No evidence
Eluate freeze/thaw cycles	3
Sample throughput per day[6]	192
Open reagent cartridge stability	6 weeks
Runtime for 96 samples	5 h 40 min

[1]QIAamp Circulating Nucleic Acid Kit; [2]Tested with restriction enzyme based assay for subset of eluates; QIAamp circulating Nucleic Acid Kit eluates with comparable portion of double-stranded DNA (dsDNA); [3]Performance evaluated against RUO product specifications; [4]Tested with 60 samples from 20 subjects, directly after blood collection; [5]Tested with 60 samples from 20 subjects; [6]Includes plasma processing and overnight runs

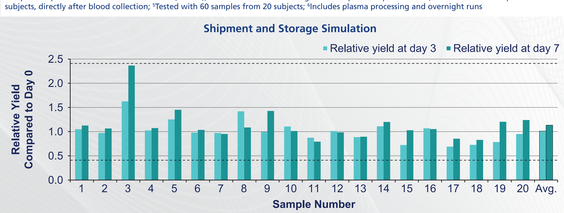


Figure 2. Relative ccfDNA yield compared to eluates generated from plasma separated directly after blood collection.

Plasma was generated from blood collected in PAXgene Blood ccfDNA Tubes after 5 hours of agitation (5 rpm) and 3 or 7 days of horizontal storage at 25°C to simulate typical transport and storage conditions. ccfDNA was purified manually using the QIAamp Circulating Nucleic Acid Kit.

Acceptance criteria: Relative yield must be ≥ 0.5 and ≤ 2.0 compared to Day 0, which equals, including the assay's imprecision [dotted line], a relative yield of ≥ 0.41 and ≤ 2.41.

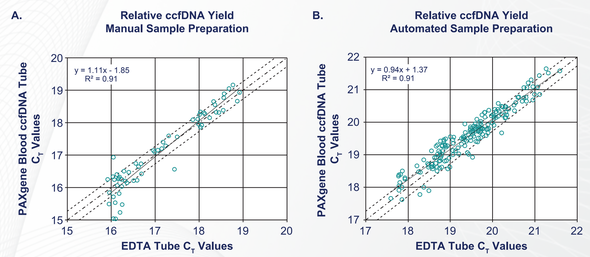


Figure 3. Relative ccfDNA yield of paired plasma samples collected in PAXgene Blood ccfDNA Tubes as compared to EDTA tubes.

C_T values of a validated 18S rDNA PCR assay (precision range ±3σ = 0.27 ΔC_T indicated as dotted lines) are shown in a scatter plot. ccfDNA was either isolated from plasma manually with the QIAGEN QIAamp Circulating Nucleic Acid Kit (n = 60) (A) or with the QIAsymphony (QS) SP instrument using the QIAsymphony PAXgene Blood ccfDNA Kit and 4.8 ml plasma protocol for PAXgene Blood ccfDNA Tubes or the QIAGEN QIAsymphony Circulating DNA Kit for EDTA tubes (n = 180) (B).

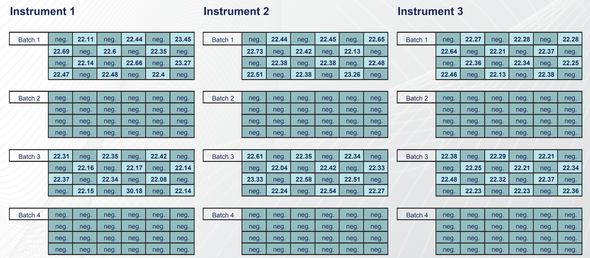


Figure 4: Cross Contamination Assessment.

C_T values of a validated Y-chromosomal DYS14 PCR assay are shown. Three cross contamination test runs of 96 blood samples collected into PAXgene Blood ccfDNA Tubes, were carried out on 3 different QIAsymphony SP instruments using the QIAsymphony PAXgene kit and LAF protocol with 4.8 ml plasma. Per run 24 DYS14-positive (male) and 72 DYS14-negative (female) plasma samples were processed in a checkerboard setup interrupted by negative batches to test for batch to batch contamination.

Study 2: Validation of the PAXgene Blood ccfDNA System for non-invasive prenatal testing (NIPT) application

Study Setup:

- Blood samples were collected from 62 consented pregnant women
- Two blood collection tubes were collected per patient:
  - Tube 1: Streck Cell-Free DNA BCT
  - Tube 2: PAXgene Blood ccfDNA Tube
- Plasma processing from half of the blood samples collected into PAXgene tubes was performed with one centrifugation step, the remainder with two centrifugation steps
- All samples underwent the regular PrenaTest procedure per the standard assay protocol
- 25 plasma samples collected into PAXgene Blood ccfDNA Tubes were selected for downstream analysis (12 with one, 13 with two centrifugation steps)

QuantYfeX: Relative Fetal ccfDNA Content

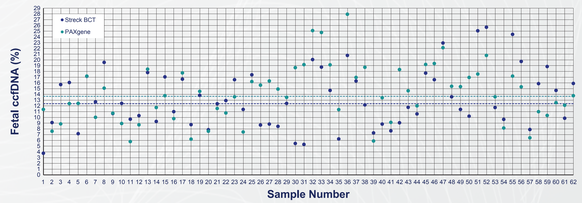


Figure 5: Relative fetal fraction of paired plasma samples collected in PAXgene Blood ccfDNA Tubes or Streck Cell-Free DNA BCT tubes.

Relative amount of fetal ccfDNA in maternal background was quantified with methylation sensitive QuantYfeX QC Assay. Relative fetal ccfDNA fractions (PAXgene median: 13.7%; Streck BCT median: 12.4%) in maternal blood were not significantly different between the two different tubes (p-value: 0.20 paired T-Test, two-tailed distribution).

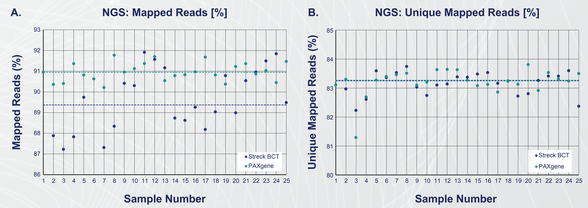


Figure 6: NGS read out quality for paired plasma samples collected in PAXgene Blood ccfDNA Tubes or Streck Cell-Free DNA BCT tubes.

ccfDNA from Streck BCTs and PAXgene tubes sequenced on Illumina HiSeq™ with NEB library preparation. There were significantly more mapped reads with ccfDNA from PAXgene tubes (p-value: 1.78E-04, two-tailed paired T-test; A).

There was no significant difference in unique mapped reads (B).

Study 2 Results

1. Relative fetal ccfDNA fractions in maternal blood in both tubes were not significantly different
2. Significantly more mapped reads with ccfDNA from PAXgene tubes
3. No significant difference in uniquely mapped reads
4. No significant difference with PAXgene tube samples processed with one or two centrifugation steps
5. All samples which passed the internal quality standards produced equivalent results with the PrenaTest assay

Conclusions

Study 1:

- PAXgene Blood ccfDNA Tubes stabilize whole blood samples for up to 7 days of storage at room temperature (15–25°C) for efficient extraction of ccfDNA from plasma.
- Extraction of ccfDNA can be performed manually with the QIAamp Circulating Nucleic Acid Kit or automatically using the QIAsymphony PAXgene Blood ccfDNA Kit.
- Automated ccfDNA extraction with the QIAsymphony protocol offers minimal variation between replicates, highly reliable results and no cross-contamination.
- ccfDNA yield from PAXgene Blood ccfDNA Tubes stored for up to 7 days at room temperature is comparable to unstabilized EDTA blood on day 0.

Study 2:

- PAXgene Blood ccfDNA System fulfills requirements for whole blood stabilization and ccfDNA purification for NIPT.
- PAXgene Blood ccfDNA System is fully compatible with the NGS-based LifeCodexx PrenaTest.

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