

A New Blood Collection Tube and Automated Extraction Method for Stabilization and Analysis of ccfDNA

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Background

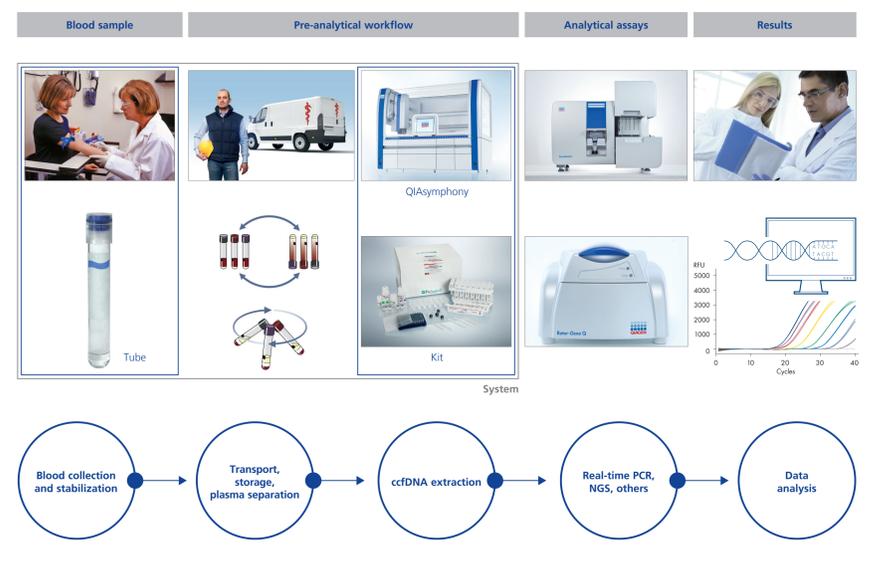
Introduction: Circulating cell-free DNA (ccfDNA) has become an emerging tool in non-invasive prenatal testing (NIPT) and in cancer diagnostics. PreAnalytiX has developed the PAXgene® Blood ccfDNA System, consisting of a blood collection tube, which stabilizes whole blood during transport and storage, and the QIASymphony® PAXgene Blood ccfDNA workflow, for automated extraction of ccfDNA from plasma. Here, we demonstrate the performance of this system.

Methods: Whole blood was drawn into PAXgene Blood ccfDNA tubes, Streck Cell-Free DNA BCT® or spray-dried EDTA tubes and stored for up to 10 days. Genomic DNA (gDNA) was purified from stored blood using the QIAamp® DNA Mini Kit and analyzed by gel electrophoresis. ccfDNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit or the QIASymphony PAXgene Blood ccfDNA protocol and the obtained ccfDNA was analyzed using an Agilent Bioanalyzer and quantitative real-time PCR. Fetal ccfDNA from pregnant donors was analyzed by PrenaTest® (LifeCodexx AG).

Results: The PAXgene Blood ccfDNA stabilization reagent prevented cell lysis and release of gDNA and hemoglobin into the plasma. The ccfDNA fraction in blood stored for up to 10 days in EDTA tubes showed a strong increase of 18S rDNA fragments. By contrast, copy numbers of these fragments remained constant in PAXgene Blood ccfDNA stabilized blood. An initial feasibility study of a small pregnant cohort revealed that the prototype PAXgene Blood ccfDNA tube performs comparably with the Cell-Free DNA BCT in the NGS-based PrenaTest.

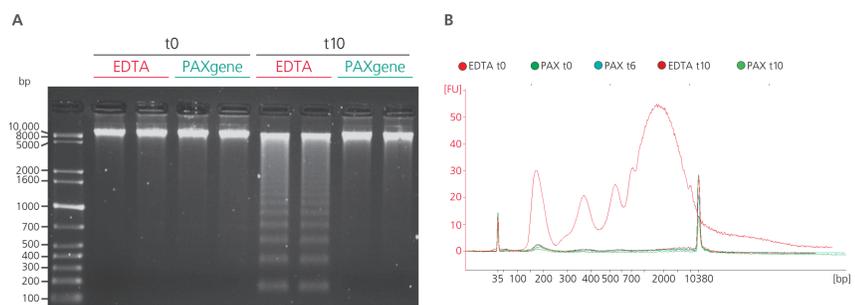
Conclusion: The PAXgene Blood ccfDNA tube enables whole blood stabilization needed for NIPT. The fully automated QIASymphony PAXgene Blood ccfDNA workflow is an efficient method for extraction of ccfDNA.

PAXgene Blood ccfDNA System Workflow



Prevention of Blood Cell Lysis and Release of gDNA

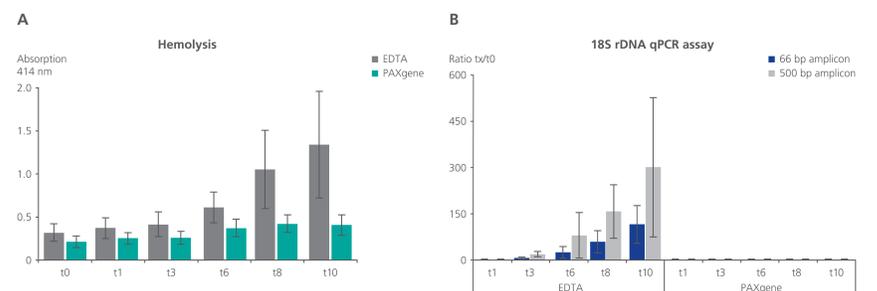
- PAXgene Blood ccfDNA stabilization reagent inhibits the apoptosis of blood cells.
- Release of genomic DNA into the plasma is prevented by stabilizing blood with PAXgene Blood ccfDNA stabilization reagent.



Blood cell lysis and genomic DNA release. Whole blood from 2 healthy donors was drawn in EDTA BCT. After draw, blood in EDTA tubes was either stored or directly transferred into tubes containing PAXgene Blood ccfDNA stabilization reagent (PAXgene). Stabilized blood was immediately processed (t0) or stored for up to 10 days at room temperature (t10). **A** Genomic DNA was extracted from stored blood using the QIAamp Blood Mini Kit and 400 ng DNA was separated by agarose gel electrophoresis. **B** Plasma was generated by double centrifugation after 6 and 10 days of storage and ccfDNA was extracted from 2.4 ml plasma using the PAXgene Blood ccfDNA QIASymphony protocol. 1 µl eluate was analyzed using the Agilent High Sensitivity DNA Kit.

Stabilization of Whole Blood and ccfDNA During Transport and Storage

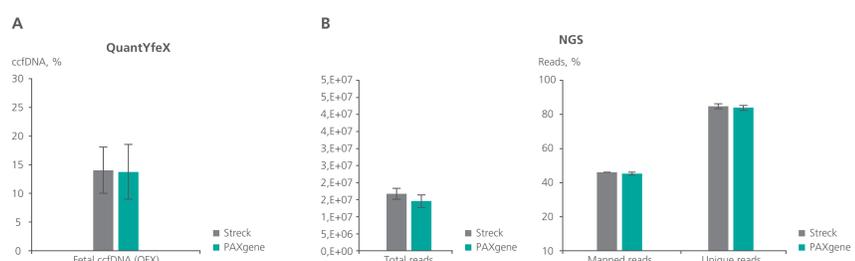
- PAXgene Blood ccfDNA stabilization reagent prevents the hemolysis of erythrocytes.
- Blood stored in EDTA BCT shows a strong increment of DNA in plasma.
- Blood stabilized with PAXgene Blood ccfDNA stabilization reagent can be stored for 10 days at room temperature without increase in ccfDNA levels.



Stabilization during transport and storage. Whole blood from 6 healthy donors was drawn in EDTA BCT. After draw, blood in EDTA tubes was either stored or directly transferred into tubes containing PAXgene Blood ccfDNA stabilization reagent (PAXgene). Stabilized blood was immediately processed (t0) or stored for up to 10 days at room temperature (t10) until plasma was generated. **A** Hemolysis was determined by measuring the absorption of free hemoglobin in plasma at 414 nm. Mean absorption and standard deviation of plasma from 8 donors is shown. **B** ccfDNA from 2.4 ml plasma was extracted using the PAXgene Blood ccfDNA QIASymphony protocol and ccfDNA yield was quantified by real-time PCR (18S rDNA gene, 66 bp/500 bp amplicon). The ratio of copy numbers at day of storage (tx) to immediately processed plasma (t0) is shown.

NIPT From Stabilized Maternal Blood

- All specimens collected in prototype PAXgene Blood ccfDNA tubes fulfilled the quality criterion 'fetal fraction >4%'.
- Absolute fetal ccfDNA yield and fetal-to-maternal DNA ratio were comparable with the current method.
- Fetal gender was concordant between collection tubes for all specimens.
- All 18 specimens stored in PAXgene Blood ccfDNA tubes then analyzed with NGS and the PrenaTest DAP,plus software were concordant with the current method.
- All samples were negative for trisomies 13, 18 and 21.



Comparable performance between prototype PAXgene Blood ccfDNA tube and Streck Cell-Free DNA BCT. Whole blood specimens from pregnant women were collected into prototype PAXgene Blood ccfDNA tubes and Streck Cell-Free DNA BCT. Blood was transported to LifeCodexx and ccfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit. **A** Circulating fetal DNA and fetal gender of 18 samples were determined using the QuantYfeX® assay. **B** Eluates were subjected to genomic library preparation and NGS (Illumina). With PrenaTest DAP,plus software analysis total reads of NGS were analyzed into percentage of mapped and unique mapped reads to determine chromosomal aberrations (n=18).

Handling Benefits of PAXgene Blood ccfDNA Tube

- PAXgene Blood ccfDNA stabilization reagent integrated into a BD plastic Vacutainer® Tube.
- Plastic material mitigates risk of tube breakage during transport and centrifugation.
- Safety BD Hemogard® closure helps protecting laboratory personnel from contact with blood.
- PAXgene Blood ccfDNA tube allows distinct separation of plasma from buffy coat.
- PAXgene Blood ccfDNA tube and automated QIASymphony kit: Integrated collection-stabilization-preparation (CSP) system.

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