Quality Assessment of Blood Collection Tubes for ccfDNA at the End of Their Shelf Life

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Introduction

The analysis of circulating cell-free DNA (ccfDNA) from blood has become routine in prenatal testing and is expanding in cancer diagnosis and therapy monitoring. Laboratories performing these tests require the implementation of standardized preanalytical procedures for sample collection, stabilization, transport, plasma processing, and ccfDNA extraction to obtain reliable results.

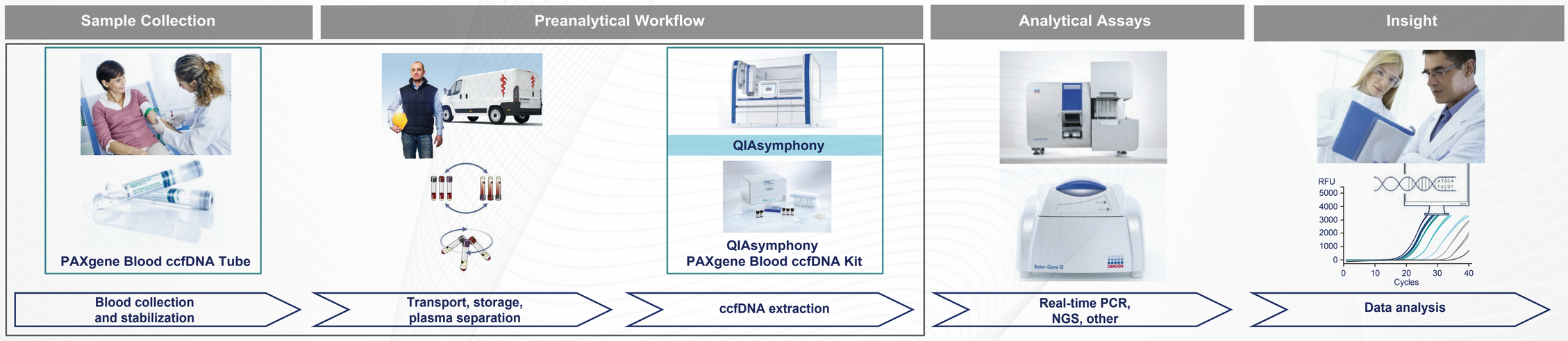
Blood collection and stabilization tubes for ccfDNA testing help prevent the release of genomic DNA from white blood cells into the plasma during blood storage and transport. The decision to use a specific blood collection tube is often based on the performance in downstream assays and consistent performance over the product shelf life.

This research study compared the performance of blood collection tubes based on non-crosslinking (PAXgene® Blood ccfDNA Tube*) and formaldehyde-releasing (Streck Cell-Free DNA BCT) stabilization technologies at the end of each product's shelf life.

Methods

Whole venous blood from 30 consented, healthy donors was collected in random order into PAXgene Blood ccfDNA Tubes† (PreAnalytiX) and Streck Cell-Free DNA BCT® (Streck) which were close to their respective expiry date (old) or used directly after arrival of ordered shipment (new). Samples from 12 donors had to be excluded due to blood clotting in Streck tubes after phlebotomy (Figure 1). Two different targets were spiked into samples after blood draw: A) male plasma was spiked into 8 female blood samples, B) sheared DNA containing EGFR mutations was spiked into the 10 remaining samples. Plasma from paired tubes was either generated directly after blood draw (T0) or after blood storage for 7 days (T7d) at 30°C. Automated ccfDNA extraction was performed on the QIAsymphony® instrument† (QIAGEN) using tube-specific kits and protocols. Native ccfDNA (18S rDNA assay), spiked male ccfDNA (DYS14 assay, Y chromosome-specific) and spiked EGFR mutations were analyzed by qPCR and ccfDNA fragment distribution was assessed using the Bioanalyzer instrument (Agilent).

PAXgene Blood ccfDNA workflow



Results

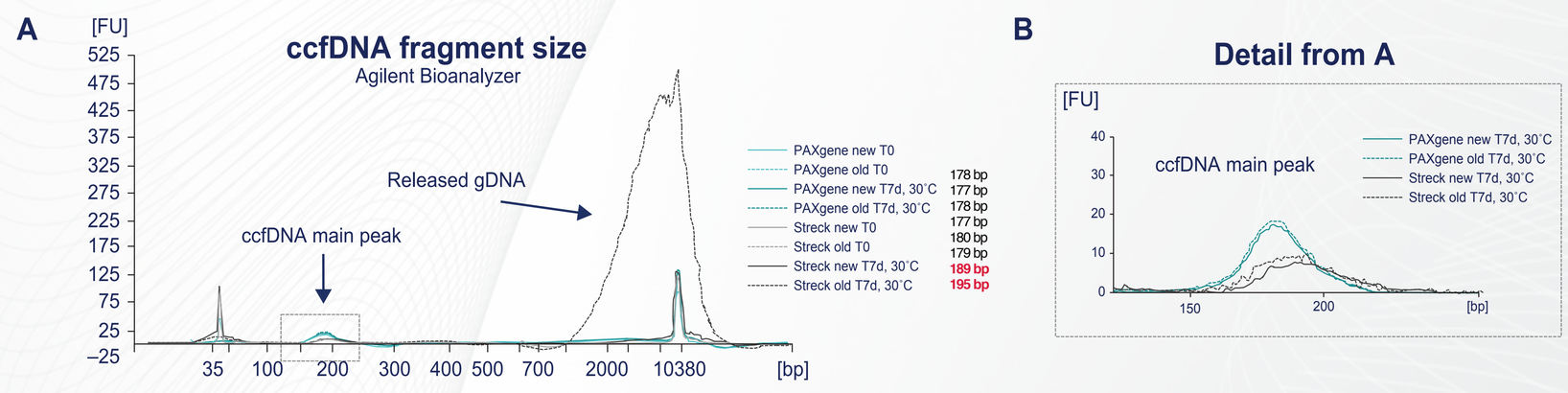
Study issue: clotted tubes

- Using both old and new Streck tubes in phlebotomy resulted in clotted blood after collection for 38 out of 150 Streck samples. 31% of old Streck tubes and 17% of all new Streck tubes were affected. No PAXgene tubes showed clotting.
- The planned study was adjusted due to sample clotting. Samples from only 18 of the 30 donors could be analyzed.

Blood in Streck tubes clotted directly after blood draw although tubes were inverted according to manufacturer's instructions.

Fragment size analysis

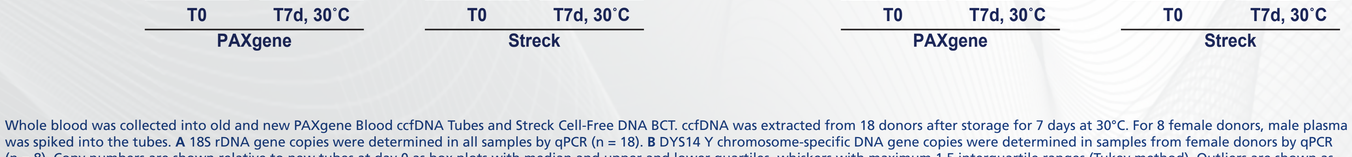
- Both old and new PAXgene Blood ccfDNA Tubes helped prevent gDNA release from white blood cells into plasma after blood storage for 7 days at 30°C
- Blood stored in old Streck tubes showed a strong gDNA release from white blood cells as seen in the high molecular weight area of Agilent fragment size analysis
- Old and new Streck tubes led to a shift of the main nucleosomal peak towards a higher fragment size, possibly due to DNA modifying agents in the Streck stabilizing solution



Whole blood was collected into old and new PAXgene Blood ccfDNA Tubes and Streck Cell-Free DNA BCT. ccfDNA was extracted from 18 donors after storage for 7 days at 30°C. **A** ccfDNA profiles of one exemplary donor analyzed on the Agilent Bioanalyzer using the High Analytical Sensitivity DNA Kit with main ccfDNA peak size in base pairs (bp). **B** Detail of indicated region from panel A showing the ccfDNA main peak.

ccfDNA yield and stability

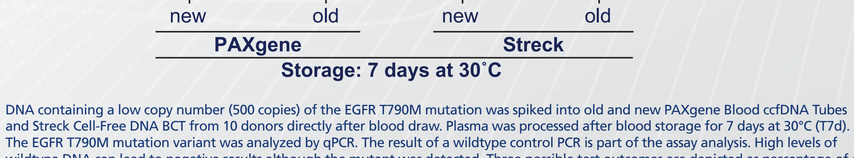
- PAXgene Blood ccfDNA Tubes efficiently stabilized native ccfDNA yield and enabled improved retrieval of spiked in DNA (90% ccfDNA stability) after 7-day blood storage at 30°C at the end of the product's shelf life
- Storage of blood in aged Streck tubes for 7 days at 30°C resulted in 3-fold increase of total DNA in plasma, and a reduced recovery rate (70%) of spiked male plasma DNA



Whole blood was collected into old and new PAXgene Blood ccfDNA Tubes and Streck Cell-Free DNA BCT. ccfDNA was extracted from 18 donors after storage for 7 days at 30°C. For 8 female donors, male plasma was spiked into the tubes. **A** 18S rDNA gene copies were determined in all samples by qPCR (n = 18). **B** DYS14 Y chromosome-specific DNA gene copies were determined in samples from female donors by qPCR (n = 8). Copy numbers are shown relative to new tubes at day 0 as box plots with median and upper and lower quartiles, whiskers with maximum 1.5 interquartile ranges (Tukey method). Outliers are shown as dots, extremes were excluded from analysis.

High analytical sensitivity EGFR mutation detection

- Higher analytical sensitivity in EGFR mutation detection was observed using old and new PAXgene Blood ccfDNA Tubes compared to old and new Streck tubes; spiked EGFR mutants were detected in all cases
- EGFR mutation detection failed in 30% of all samples using Streck tubes
- False negative results increased when using Streck tubes at the end of their shelf life



DNA containing a low copy number (500 copies) of the EGFR T790M mutation was spiked into old and new PAXgene Blood ccfDNA Tubes and Streck Cell-Free DNA BCT from 10 donors directly after blood draw. Plasma was processed after blood storage for 7 days at 30°C (T7d). The EGFR T790M mutation variant was analyzed by qPCR. The result of a wildtype control PCR is part of the assay analysis. High levels of wildtype DNA can lead to negative results although the mutant was detected. Three possible test outcomes are depicted as percentage of all analyzed samples: 1) detected mutants, 2) negative assay outcome, mutant detected and 3) undetected mutants (n = 10).

Conclusions

- The PAXgene Blood ccfDNA Tube stabilized blood for ccfDNA detection with consistent performance over the product's shelf life.
- Stabilization reagents, which have the potential to chemically modify ccfDNA, like in the Streck Cell-Free DNA BCT, exhibited decreased performance in blood stabilization and decreased analytical sensitivity in downstream reactions. This effect is more severe at the end of shelf life.

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