Multimodal Analysis of Circulating Cell-free RNA (ccfRNA), Circulating Cell-free DNA (ccfDNA) and Genomic DNA (gDNA) From Blood Samples Collected in PAXgene Blood ccfDNA Tubes

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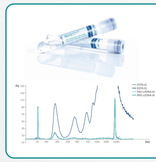
Introduction

While circulating cell-free DNA (ccfDNA) from blood is widely used as an analyte in liquid biopsy research applications, circulating cell-free RNA (ccfRNA) has recently gained relevance for biomarker studies. Combined insights from both analytes promise to increase the understanding of underlying molecular processes. However, challenges in the preanalytical workflow, such as choosing blood collection tubes and extraction methods suitable for multimodal analysis, must still be overcome.

We investigated multimodal extraction and analyses of ccfRNA, ccfDNA and gDNA from one blood sample collected using the PAXgene® Blood ccfDNA Tube (RUO)*[†].

PAXgene Blood ccfDNA Tube



The PAXgene Blood ccfDNA Tubes (RUO)* enable:

- Stabilization of ccfDNA for **10 days up to 25°C, 7 days up to 30°C and 3 days up to 37°C**
- Single tube collection, stabilization, transport and storage
- Standardized preanalytical sample processing

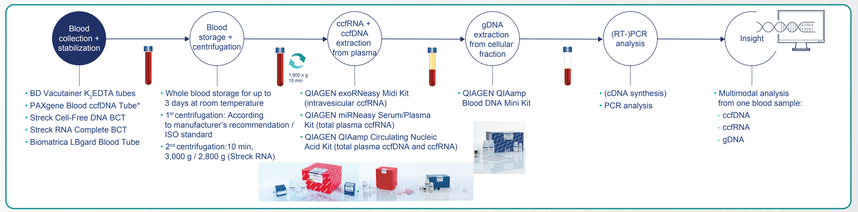
PAXgene Blood ccfDNA stabilization reagent helps prevent release of gDNA into plasma

Data from www.preanalytix.com

Methods

Whole blood samples were collected from healthy consented donors into PAXgene Blood ccfDNA Tubes* (PreAnalytiX®), BD Vacutainer® K_2EDTA tubes (BD), Cell-Free DNA BCT® (Streck®), RNA Complete BCT™ (Streck) and LBgard® Blood Tubes (Biomatrica®). Plasma was generated by double centrifugation immediately after blood collection or after storage for up to three days. Cell-free nucleic acids were extracted as shown in the workflow.

Multimodal workflow for the analysis of ccfRNA, ccfDNA and gDNA

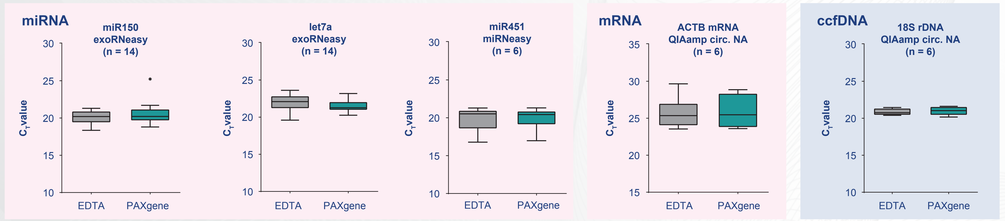


Results

ccfRNA yield in plasma after blood storage in EDTA and PAXgene Blood ccfDNA tubes

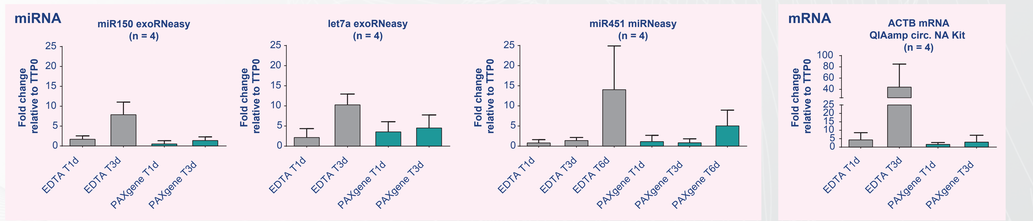
- Quantitative PCR analysis revealed comparable yields of miRNA, mRNA and ccfDNA targets from plasma of blood collected in PAXgene Blood ccfDNA Tubes and EDTA tubes
- After blood storage in PAXgene Blood ccfDNA Tubes* for up to 3 days, RNA targets (both intra- and extravesicular extracted with exoRNeasy and miRNeasy, respectively) could still be detected with improved stabilization over EDTA.

A Comparison at TTP0



C_t values of qPCR analysis of miR150, let7a and miR451 micro RNAs, ACTB mRNA and 18S rDNA (ccfDNA) in PAXgene and EDTA plasma, generated directly after blood collection (test time point = TTP0) and extracted using the indicated kit.

B Relative fold change upon whole blood storage

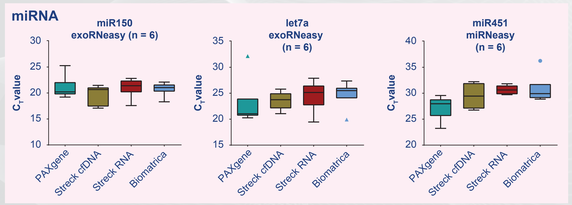


Calculated fold change (relative to TTP0) of qPCR analysis of miR150, let7a, miR451 micro RNAs and ACTB mRNA of PAXgene and EDTA plasma, generated after 1, 3 or 6 days of whole blood storage. RNA was extracted using the indicated kit.

miRNA yield in plasma after blood storage in stabilization tubes

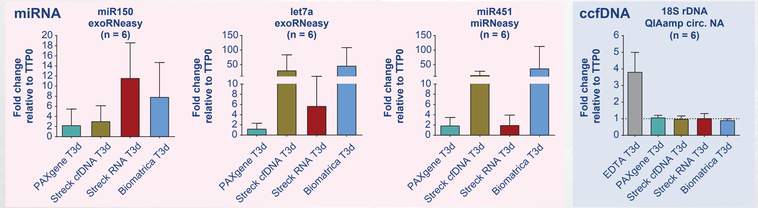
- RNA extraction and detection sensitivity was impacted by blood collection tubes containing formaldehyde-releasing formulations (Streck and Biomatrica) as indicated by slightly higher C_t values at TTP0 and lower RNA stabilization efficiency after 3 days of storage.

A Comparison at TTP0



C_t values of qPCR analysis of miR150, let7a and miR451 micro RNAs in PAXgene, Streck cfDNA, Streck RNA and Biomatrica plasma, generated directly after blood collection (TTP0) and extracted using the indicated kit.

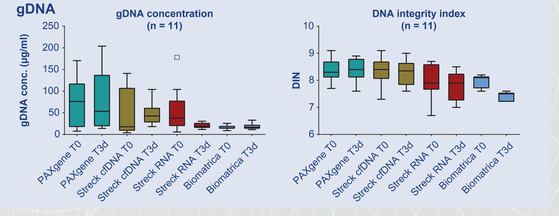
B Relative fold change upon whole blood storage



Calculated fold change (relative to TTP0) of qPCR analysis of miR150, let7a, miR451 micro RNAs and 18S rDNA in PAXgene, Streck cfDNA, Streck RNA and Biomatrica plasma, generated after 3 days of storage (T3d). RNA was extracted using the indicated kit.

Genomic DNA yield and integrity

- PAXgene Blood ccfDNA Tubes* enabled efficient gDNA extraction from residual blood cells after plasma separation following 3 days of whole blood storage with intact DNA as indicated by stable DNA integrity index
- gDNA yield and integrity were reduced by collection and storage in Streck RNA and Biomatrica tubes



Concentration and DNA integrity index (DIN) assessment of gDNA extracted from whole blood in PAXgene Blood ccfDNA Tubes*, Streck cfDNA, Streck RNA and Biomatrica tubes. DNA was extracted from cellular fraction after first plasma centrifugation step using the QIAamp Blood DNA Kit and analyzed with the Agilent Genomic DNA ScreenTape® on the TapeStation System.

Disclaimer

*The PAXgene Blood ccfDNA Tube is For Research Use Only in the US. Not for use in diagnostic procedures.
**Babayan et al. 2020 (Poster shown at AACR Meeting: Advances in Liquid Biopsies, Miami, FL)
[†]For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN or PreAnalytiX handbook or user manual. QIAGEN and PreAnalytiX handbooks and user manuals are available at www.qiagen.com or www.preanalytix.com or can be requested from QIAGEN Technical Services or your local distributor.

Conclusions

- The non-crosslinking technology of the PAXgene Blood ccfDNA Tube* enables the isolation and analyses of cell-free miRNA, mRNA, ccfDNA and cellular gDNA.
- Along with recent data on CTC enrichment and detection**, RNA data from this study show that the PAXgene Blood ccfDNA Tube* can be used as part of a multimodal workflow in liquid biopsy research.
- Other blood collection tubes for ccfDNA stabilization showed impaired analysis efficiency after whole blood storage for the tested miRNA targets and gDNA.