Multimodal Analysis of RNA from Circulating Tumor Cells, Circulating Cell-Free DNA and Genomic DNA from a Single Blood Sample Collected into the PAXgene® Blood ccfDNA Tube

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Background

High demand for liquid biopsy tests in cancer research drives the need for combined approaches that allow multimodal testing from a limited blood volume. Although crosslinking-based technologies preserve the circulating cell-free DNA (ccfDNA) profile in whole blood, they hamper the analysis of mRNA from circulating tumor cells (CTCs) [Luk et al., 2017]. Currently, there are no blood collection tubes available that allow RNA analysis from CTCs after prolonged (>48 hours) storage. In this research study, we present a newly developed combined workflow for blood stabilization and subsequent analysis of RNA from CTCs, ccfDNA and leukocyte-derived gDNA from a single blood sample after prolonged (>48 hours) storage.

Materials and Methods

Whole blood from healthy volunteers was collected into PAXgene Blood ccfDNA Tubes*† (PreAnalytiX®) (n = 21) and Cell-Free DNA BCT® (Streck®) (n = 8), aliquoted and manually spiked with tumor cells, 20 LNCaP95 cells/5 ml blood, or 20 µl PBS as a control. Tubes were then stored at 2–8°C (PAXgene samples) and at 25°C (RT) (Streck samples) according to tube manufacturers' instructions before multi-epitope targeting CTC enrichment and analysis with the AdnaTest ProstateCancerPanel AR-V7‡ (QIAGEN®) following the manufacturer's recommendations. CTC-depleted blood from PAXgene Blood ccfDNA Tubes was collected and centrifuged at 1900 × *g* for 15 min. The resulting fractions (plasma and blood cellular fraction) were processed to extract ccfDNA and gDNA using the QIAsymphony® PAXgene Blood ccfDNA Kit* (PreAnalytiX) and the QIAsymphony DSP DNA Mini Kit§ (QIAGEN), respectively, according to the manufacturer's instructions.

The non-crosslinking stabilization reagent in PAXgene Blood ccfDNA Tubes enables RNA-based CTC detection after prolonged storage

RNA from the spiked tumor cells was detected in blood stabilized in PAXgene Blood ccfDNA Tubes at each experimental time point after storage of up to 72 hours at 2–8°C (Figure 1A). In contrast, RNA from spiked tumor cells could not be detected in samples collected and stored in Cell-Free DNA BCTs after 24, 48 or 72 hours of storage (Figure 1B). Control samples stabilized in PAXgene Blood ccfDNA Tubes and spiked with PBS only (no-spike control) were negative for tumor cell-specific RNA at all experimental time points (tested after 48 hours only) (Figure 1C).

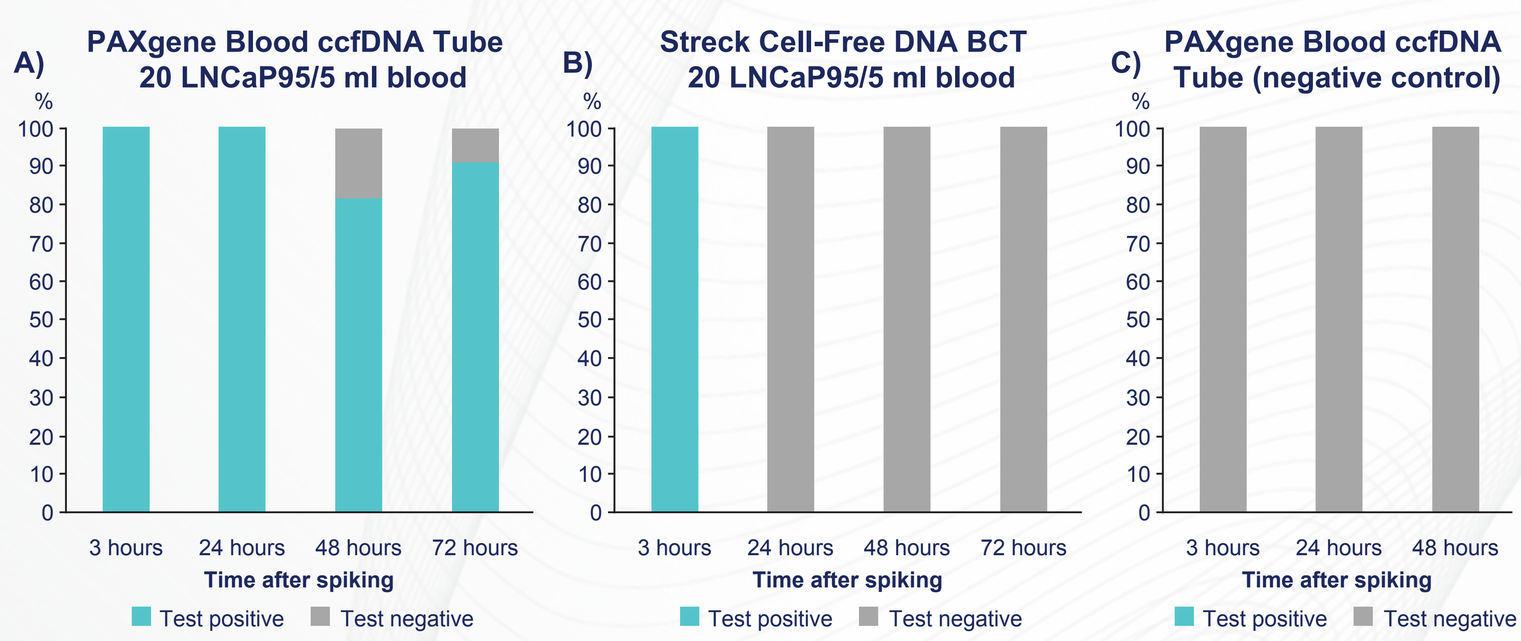


Figure 1. Detection rate of tumor cell-specific RNA in spiked blood samples. Enrichment and detection of tumor cells using the AdnaTest ProstateCancerPanel AR-V7. **(A)** Blood collected into PAXgene Blood ccfDNA Tubes was spiked with 20 LNCaP95 cells/5 ml blood, stored at 2–8°C and analyzed at various experimental time points: 3, 24, 48 and 72 h after spike (n = 21 donors for time points 3, 24 and 48 h and n = 11 for 72 h storage). **(B)** Blood collected into Streck Cell-Free DNA BCTs was spiked with 20 LNCaP95 cells/5 ml blood, stored at RT and analyzed at 3, 24, 48 and 72 h after spike (n = 8 donors). **(C)** Blood collected into PAXgene Blood ccfDNA Tubes was spiked with 20 µl PBS/5 ml blood (no-spike control), stored at 2–8°C and analyzed at 3, 24, and 48 h after spike (n = 10 donors).

Simultaneous analysis of RNA from CTCs, ccfDNA and leukocyte-derived gDNA from a single blood sample is possible using PAXgene Blood ccfDNA Tubes

In the context of the multimodal use of blood stabilized in PAXgene Blood ccfDNA Tubes, the difference in ccfDNA yield from the CTC-depleted blood versus blood used for plasma generation alone was not statistically significant. CTC depletion has no significant impact on ccfDNA extraction in terms of yield or in situ stability (Figure 2A).
Similarly, no statistically significant difference was observed between the yield of gDNA extracted from CTC-depleted blood versus blood used for plasma generation alone (Figure 2B).

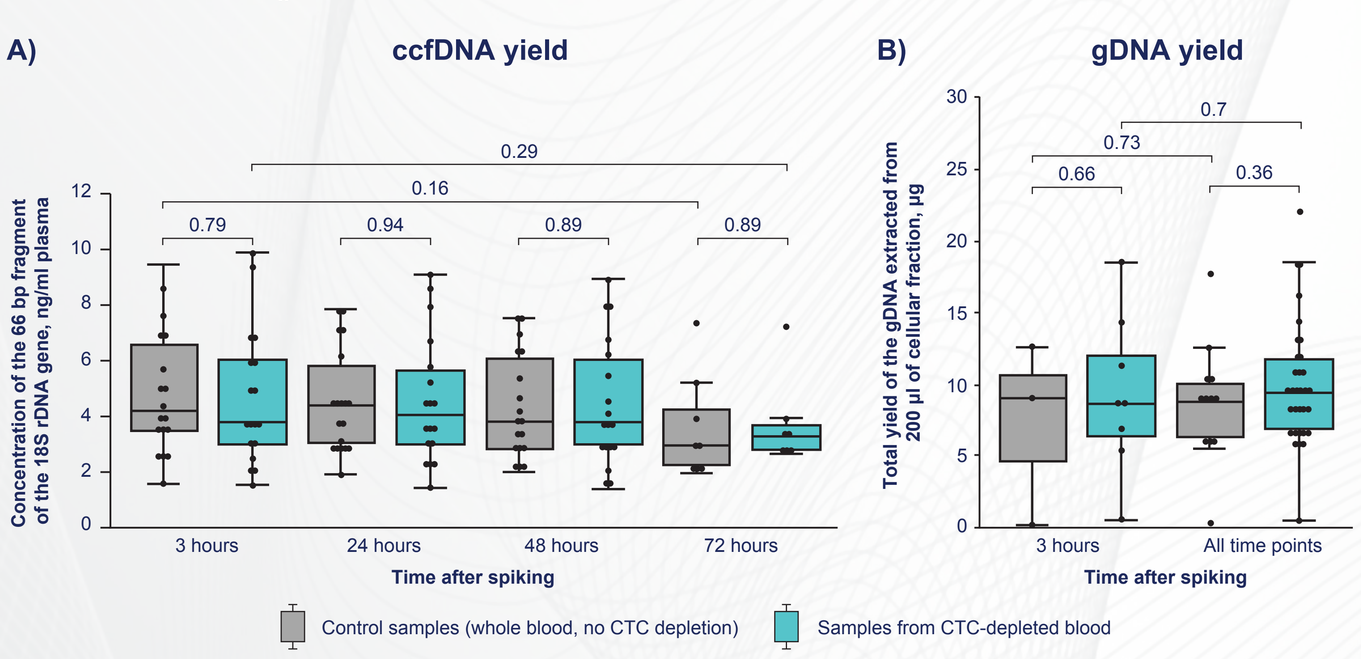
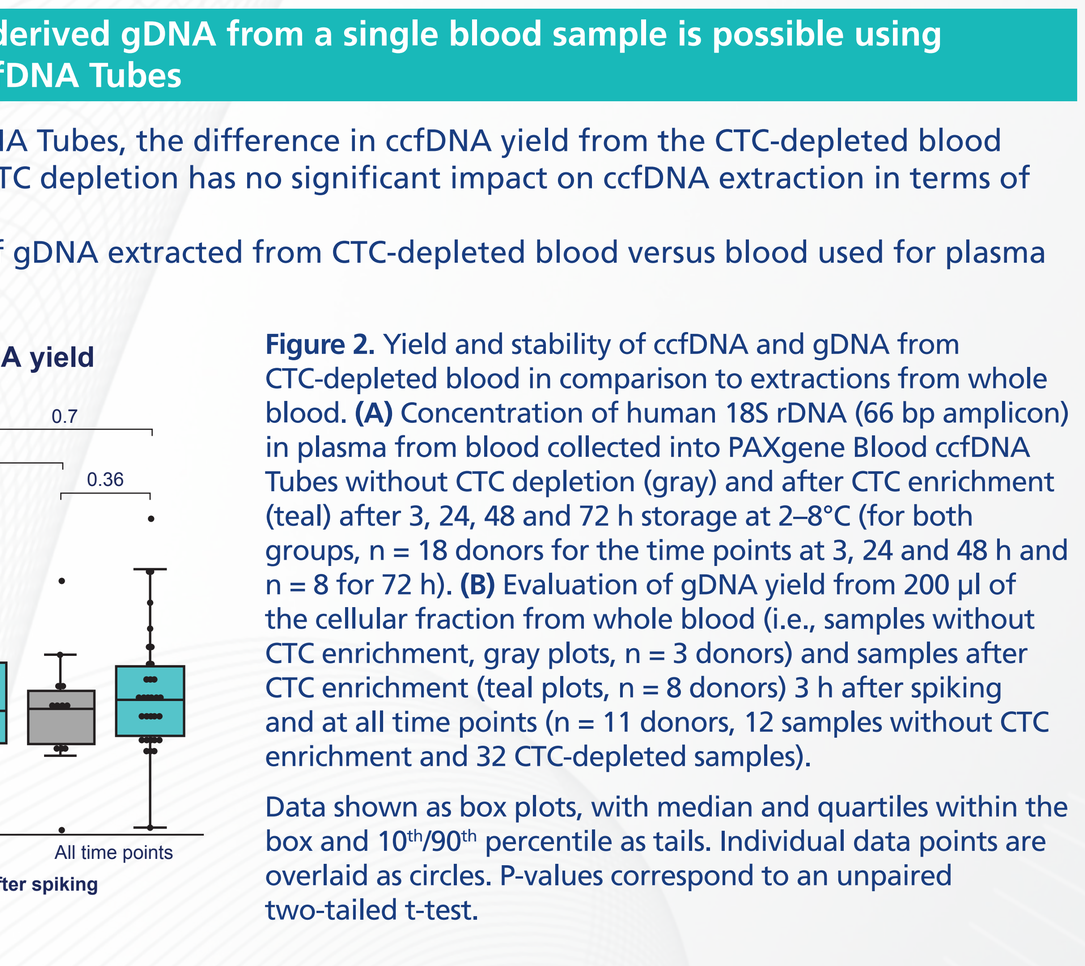


Figure 2. Yield and stability of ccfDNA and gDNA from CTC-depleted blood in comparison to extractions from whole blood. **(A)** Concentration of human 18S rDNA (66 bp amplicon) in plasma from blood collected into PAXgene Blood ccfDNA Tubes without CTC depletion (gray) and after CTC enrichment (teal) after 3, 24, 48 and 72 h storage at 2–8°C (for both groups, n = 18 donors for the time points at 3, 24 and 48 h and n = 8 for 72 h). **(B)** Evaluation of gDNA yield from 200 µl of the cellular fraction from whole blood (i.e., samples without CTC enrichment, gray plots, n = 3 donors) and samples after CTC enrichment (teal plots, n = 8 donors) 3 h after spiking and at all time points (n = 11 donors, 12 samples without CTC enrichment and 32 CTC-depleted samples).

Data shown as box plots, with median and quartiles within the box and 10th/90th percentile as tails. Individual data points are overlaid as circles. P-values correspond to an unpaired two-tailed t-test.

Conclusions

- The non-crosslinking stabilization reagent in PAXgene Blood ccfDNA Tubes is compatible with RNA analysis of isolated CTCs using the AdnaTest ProstateCancerPanel AR-V7.
- In this study, the formaldehyde-releasing, crosslinking fixative in Streck Cell-Free DNA BCTs is not compatible with RNA analysis of isolated CTCs using the AdnaTest after >3 hours storage.
- Blood collected into PAXgene Blood ccfDNA Tubes and stored up to 72 hours at 2–8°C can be used for multimodal analysis of RNA from CTCs, ccfDNA and gDNA in a single blood sample (Figure 3).



- ✓ Blood collected into PAXgene Blood ccfDNA Tubes can be used for CTC analysis with AdnaTest technology within 72 hours after blood draw (storage at 2–8°C)
- ✓ Simultaneous analysis of RNA from CTCs, ccfDNA and leukocyte-derived gDNA from the same blood sample is feasible
- ✓ CTC enrichment has no negative impact on ccfDNA and gDNA yield

Figure 3. A workflow for CTC, ccfDNA and leukocyte-derived gDNA analysis from PAXgene Blood ccfDNA Tubes.

Disclaimer

*For Research Use Only. Not for use in diagnostic procedures.
†This research was conducted using the PAXgene Blood ccfDNA Tube (RUO) which is available in the United States and other parts of the world outside of Europe.
‡For molecular biology applications. Not intended for the diagnosis, prevention or treatment of a disease.
§For in vitro diagnostic use. Presented here for research applications.

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