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

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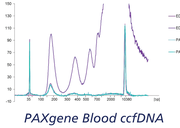
Background and Methodology

Analysis of mRNA from circulating tumor cells (CTCs) and circulating cell-free DNA (ccfDNA) is challenging and sensitive to preanalytical parameters, namely in multimodal testing from a limited blood volume. A workflow was recently developed that enables blood stabilization and subsequent isolation and analysis of CTC mRNA, ccfDNA and genomic DNA (gDNA) after prolonged storage. The PAXgene Blood ccfDNA Tubes* that were used to stabilize whole blood are For Research Use Only (RUO). Not for use in diagnostic procedures.

PAXgene Blood ccfDNA Tube

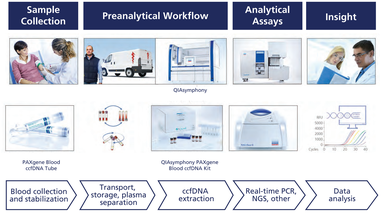
The PAXgene Blood ccfDNA Tube enables:

- 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 blood collection, stabilization, transport and storage
- Standardized preanalytical sample processing

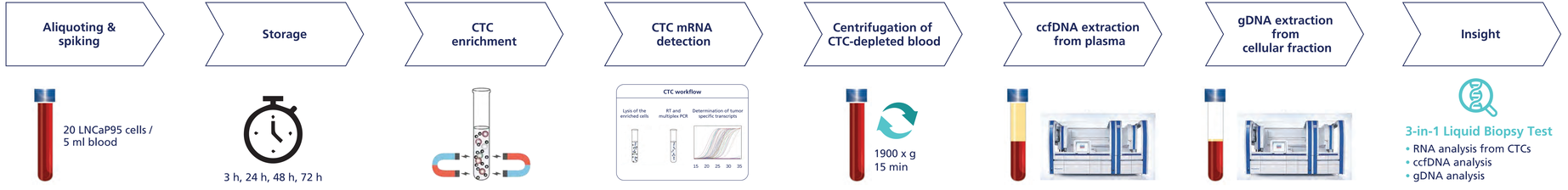


PAXgene Blood ccfDNA stabilization reagent helps prevent release of gDNA into plasma

PAXgene Blood ccfDNA System Workflow



Multimodal workflow for the analysis of CTC RNA, ccfDNA, and gDNA



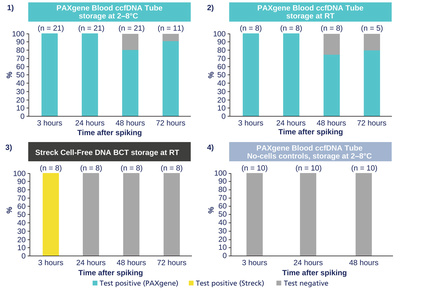
To model CTCs, blood samples from healthy donors were manually spiked with 20 LNCaP95 cells / 5 ml blood or 20 µl PBS as a control.

Blood was collected into PAXgene Blood ccfDNA Tubes or Streck® Cell-Free DNA BCTs®, aliquoted, spiked and stored at either 2–8°C or 25°C (RT) until being processed 3, 24, 48, and 72 hours after spiking. To establish the multimodal workflow, CTC-depleted blood from PAXgene Blood ccfDNA Tubes stored at 2–8°C was collected and centrifuged at 1,900 × 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.

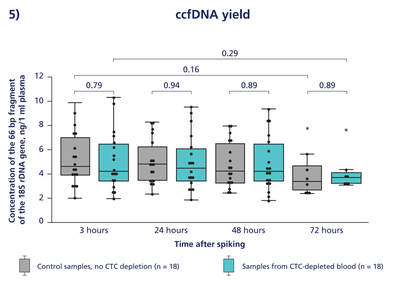
To confirm that the CTC depletion step did not influence the yield of ccfDNA and gDNA, control samples that did not undergo CTC depletion / enrichment were also prepared. CTCs were enriched and detected using the AdnaTest ProstateCancerPanel AR-V7‡ (QIAGEN).

Results

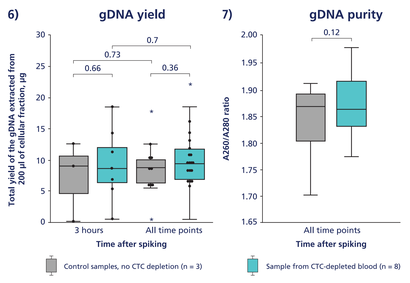
CTC RNA



ccfDNA



gDNA



Disclaimer

*For Research Use Only. Not for use in diagnostic procedures.

†For in vitro diagnostic use. Presented here for research applications.

‡For molecular biology applications. Not intended for the diagnosis, prevention, or treatment of a disease.

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Key Findings

- The non-crosslinking reagent in the PAXgene Blood ccfDNA Tube* is compatible with mRNA analysis of enriched CTCs within 72 hours storage at 2–8°C (Fig. 1) and at room temperature (Fig. 2)
- The formaldehyde-releasing, crosslinking fixative in Streck Cell-Free DNA BCTs is not compatible with mRNA analysis of isolated CTCs if samples are stored for >3 hours and analyzed with the AdnaTest ProstateCancerPanel AR-V7 (Fig. 3)
- The stabilization reagent in PAXgene Blood ccfDNA Tubes does not cause false-positive results in unspiked no-cells control samples (Fig. 4)
- CTC depletion has no negative impact on ccfDNA yield and in situ stability (Fig. 5)
- **The non-crosslinking reagent in PAXgene Blood ccfDNA Tubes allows for 3-in-1 simultaneous isolation and subsequent analysis of CTC RNA, ccfDNA and gDNA from a single blood sample***
- CTC depletion has no negative impact on gDNA yield and in situ stability (Fig. 6) as well as on gDNA purity (Fig. 7)

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