# Impact of Specimen Storage and Transport on ccfRNA Multiplex Analysis in Dedicated Blood Collection Tubes

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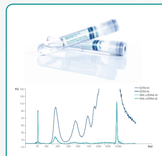
## Introduction

In addition to circulating cell-free DNA from blood, circulating cell-free RNA (ccfRNA) has gained relevance for liquid biopsy biomarker studies. The combination of insights from both analytes provides increased understanding of molecular processes, such as in tumor biology. There are still challenges to overcome in the preanalytical workflow, to preserve the valuable information of the original ccfRNA profile.

In this research study, we investigated an exemplary workflow with blood collection, transport and ccfRNA isolation from plasma of different tubes. The samples were then analyzed with the NanoString PanCancer Panel. The workflow was performed in accordance with ISO 20186-3:2019 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma.

### PAXgene® Blood ccfDNA Tube (RUO)*



**The PAXgene Blood ccfDNA Tube (RUO)* 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 collection, stabilization, transport and storage
- Standardized preanalytical sample processing

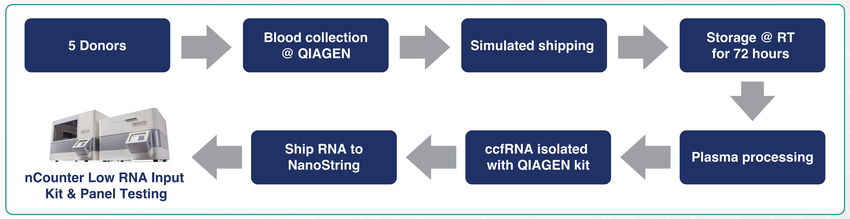
*PAXgene Blood ccfDNA stabilization reagent helps prevent release of gDNA into plasma*

Data from www.preanalytix.com

## Methods

Paired whole blood specimens from 5 healthy consented donors were collected into PAXgene Blood ccfDNA Tubes (RUO)* (PreAnalytiX), RNA Complete BCT™ (Streck), cf-DNA/cf-RNA Preservative Tubes (Norgen) and BD Vacutainer® K$_2$EDTA tubes (BD). Transport was simulated by storing the tubes for 72 hours at 25°C and by performing a drop test according to ASTM D4169-14, Standard Practice for Performance Testing of Shipping Containers and Systems and ISO 11607-1:2019, Packaging for terminally sterilized medical devices — Part 1: Requirements for materials, sterile barrier systems and packaging systems. Plasma was generated with the tube supplier's protocol and ccfRNA was extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). Quality control for ccfRNA was carried out with the Bioanalyzer 2100 instrument and RNA 6000 Pico Kit (Agilent Technologies). RNA was analyzed with the nCounter Low RNA Input Kit and nCounter PanCancer Pathways Panel (NanoString Technologies).

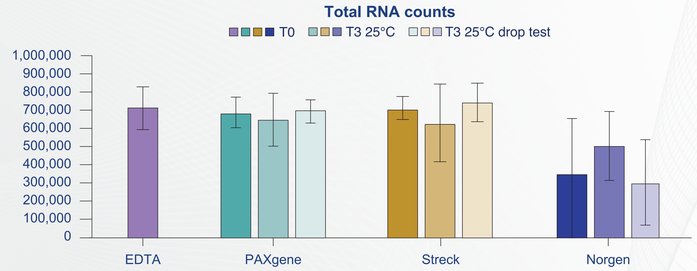
### Workflow for ccfRNA multiplex analysis in dedicated blood collection tubes

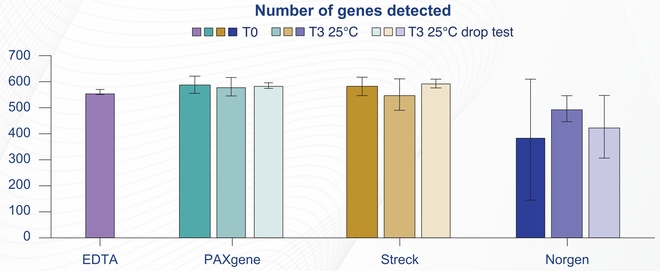


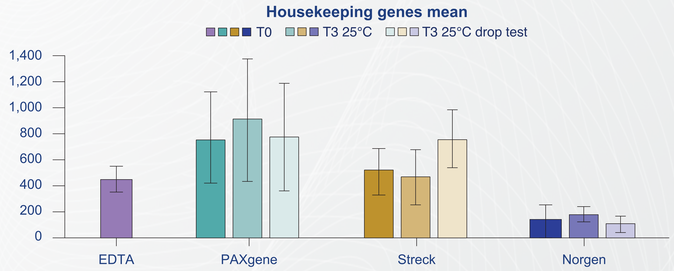

## Results

### NanoString nCounter™ Low RNA Input Kit and nCounter PanCancer Pathways Panel (QC count & detection)

- Yield and quality of ccfRNA was comparable between samples collected and stored in PAXgene Blood ccfDNA Tubes* and Streck RNA tubes.
- RNA extracted from PAXgene Blood ccfDNA Tubes* and Streck RNA tubes had higher average counts on the NanoString panel compared to Norgen (PAXgene 720,300; Streck RNA 727,500; Norgen 419,500).
- This resulted in higher number of transcripts detected in PAXgene Blood ccfDNA Tubes* and Streck RNA tubes (PAXgene 581; Streck RNA 562; Norgen 391).



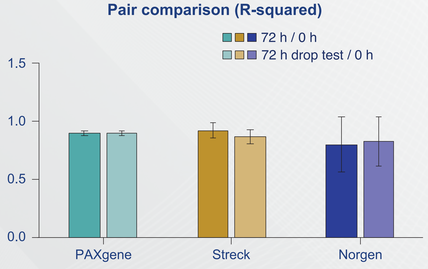



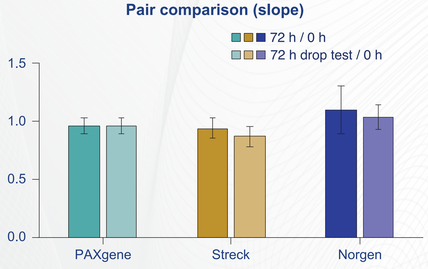




### NanoString nCounter Low RNA Input Kit and nCounter PanCancer Pathways Panel (correlation)

**Pairwise comparison between T0 and T 72 h or T 72 h with drop test within each group:**

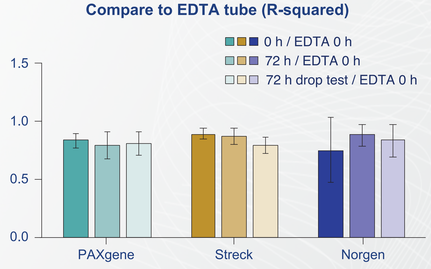
- T 72 h Norgen performed the least favorably with lower R-squared value with higher variation between samples.
- Streck RNA T 72 h with drop test is lower than the control group without drop test.
- PAXgene performance is consistent at T 72 h with or without drop test.

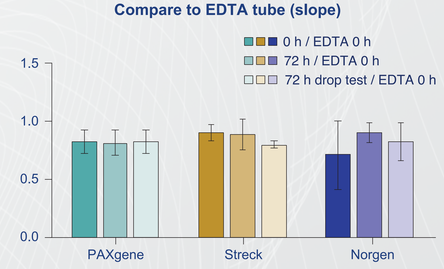



**Comparison to EDTA (T0):**

- Norgen performed the least favorably with lower R-squared value with higher variation between samples with or without drop test.
- Streck RNA with drop test is lower than 0 h and 72 h testing without drop test.
- PAXgene performance at T 72 h with or without drop test is consistent with T0 results.




## Conclusions

- PAXgene Blood ccfDNA Tubes effectively preserved ccfRNA during simulated transport conditions.
- The Streck RNA tube showed an increase in mean ccfRNA counts following drop testing, suggesting ccfRNA profile change during shipping.
- Samples from the Norgen tube show large variations and inconsistent results.
- The nCounter Low Input RNA Pan Cancer Pathway Panel was compatible with all samples used in this study.

## Disclaimers