EGFR Mutation Analysis – Performance Evaluation of Blood Collection Tubes for ccfDNA Stabilization

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Background

Standardized preanalytical workflows for circulating tumor DNA (ctDNA) analysis from blood are key to obtaining reliable results for cancer research. Some blood collection tubes can decrease assay sensitivity or accuracy by diluting ctDNA with genomic DNA released from white blood cells. Others chemically modify DNA, impairing quality and recovery.

This research study compared how different stabilizing blood collection tubes containing either non-crosslinking or formaldehyde-releasing substances perform in a quantitative PCR (qPCR) assay workflow for detecting EGFR mutations.

Methods

Blood from healthy consented donors was collected into PAXgene® Blood ccfDNA Tubes*† (PreAnalytiX®, n = 20 + 10), Cell-Free DNA BCT® (Streck®, n = 20) and LBgard® Blood Tubes (Biomatrica®, n = 10). Sheared DNA with 500 copies of different EGFR mutations (T790M, L858R and Exon 19 deletion (DEL)) or 500 µl male EDTA plasma was spiked into subsets of samples. Blood was processed either directly after phlebotomy or stored for 7 days at 30°C.

Manual extraction of ccfDNA was performed using spin column-based QIAGEN® extraction kits and automated extraction was performed on the QIAsymphony® SP instrument (QIAGEN) with tube-specific kits and protocols.

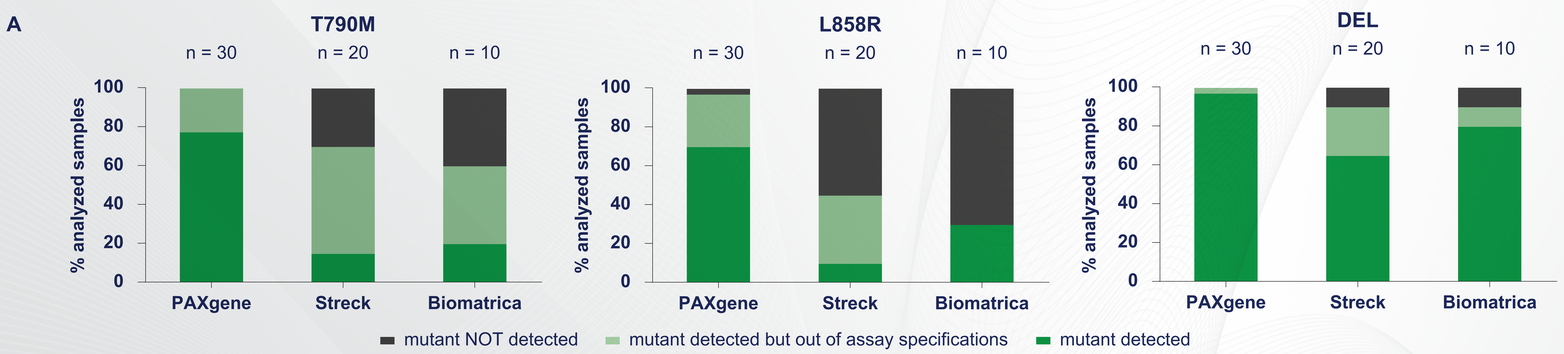
Yield and stability of ccfDNA as well as EGFR mutations were analyzed by qPCR. For monitoring changes in yield and stability, relative copy number changes of 18S rDNA or male specific DYS14 fragments were analyzed in relation to sample replicates processed directly after blood draw. EGFR mutation occurrences were determined by building ΔC_T values subtracting C_T of the wildtype assay from C_T for the respective mutation. For each mutation, the assay supplier defined a ΔC_T threshold which defines a valid assay result.

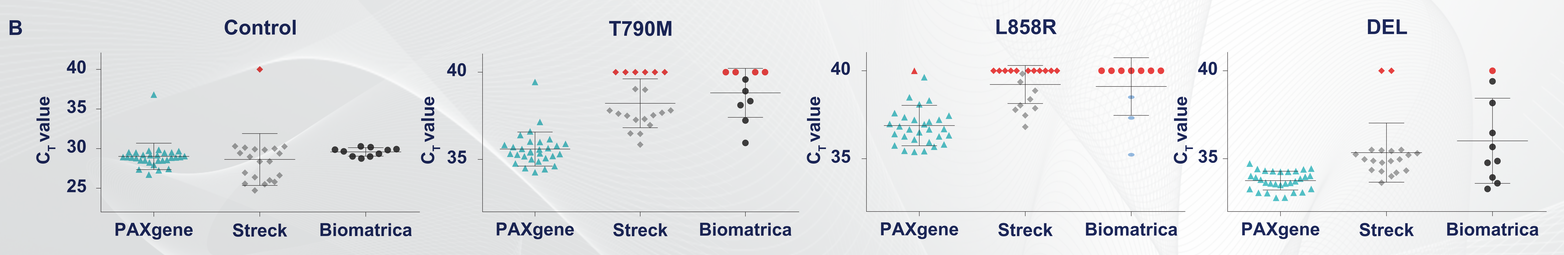
PAXgene Blood ccfDNA Workflow

Results

High analytical sensitivity in EGFR mutation detection

- Higher analytical sensitivity for EGFR mutation detection after storage with the PAXgene Blood ccfDNA Tube compared to alternative tubes
  - Stable control C_T values for PAXgene samples while control C_T values in many Streck tubes are lower
  - Overall stable detection of spiked target molecules in PAXgene samples while samples generated from both alternative tubes showed an increased number of detection failures for all three tested EGFR mutations

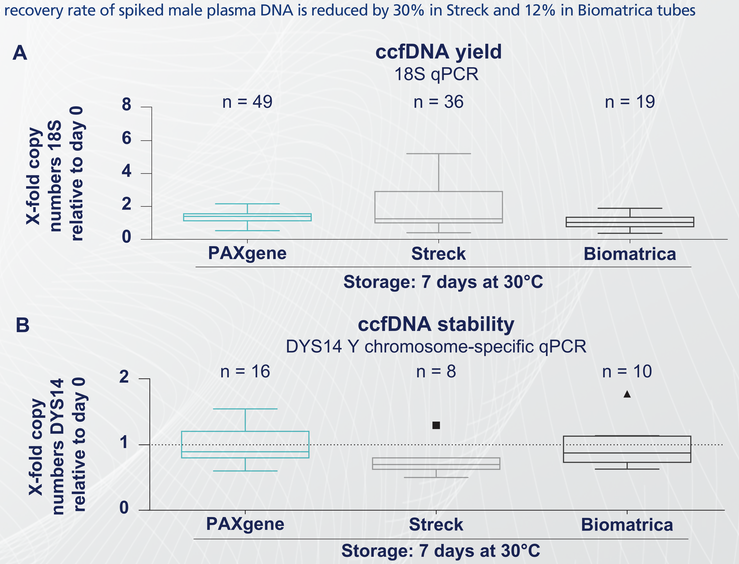




DNA containing a low copy number (500 copies) of different EGFR mutations (T790M, L858R and Exon 19 deletion (DEL)) was spiked into PAXgene Blood ccfDNA Tubes, Streck Cell-Free DNA BCT, and Biomatrica LBgard Blood Tubes directly after blood draw. Plasma was processed after blood storage for **7 days at 30°C**. EGFR mutation variants were 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 mutant was detected. No signal in the qPCR reaction is indicated with a red symbol.

ccfDNA yield and stability

- PAXgene Blood ccfDNA Tubes efficiently stabilized native ccfDNA yield and enabled improved retrieval of spiked-in ccfDNA (90% ccfDNA stability) after 7 days blood storage at 30°C
- Storage of blood in Streck tubes for 7 days at 30°C increased the variance of total DNA released into plasma
- The recovery rate of spiked male plasma DNA is reduced by 30% in Streck and 12% in Biomatrica tubes



Whole blood was collected into PAXgene Blood ccfDNA Tubes, Streck Cell-Free DNA BCT, and Biomatrica LBgard Blood Tubes. For 18 female donors, 500 µl male EDTA plasma was spiked into the tubes directly after draw. ccfDNA was extracted after storage for 7 days at 30°C.

A 18S rDNA gene copies were determined in all samples by qPCR.

B DYS14 Y chromosome-specific DNA gene copies were determined in samples from female donors by qPCR.

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 and extremes were excluded from analysis.

Conclusions

- The PAXgene Blood ccfDNA Tube outperforms alternative tubes in detection of low EGFR mutant copy numbers in ccfDNA generated from stabilized blood.
- Recovery of spiked male ccfDNA in female blood confirmed the performance of the PAXgene Blood ccfDNA Tube.
- Storage of blood in Streck tubes resulted in an increased variance of total DNA release into plasma.
- The non-crosslinking stabilization reagent in the PAXgene Blood ccfDNA Tube enables detection of low abundant target molecules and ensures detection sensitivity in downstream reactions.

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