Verification of Liquid Biopsy Blood Collection, ccfDNA Stabilization and Purification Systems for Liquid Biopsy Cancer Biomarker Applications

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Abstract

Introduction: There is a growing need for collection devices that stabilize circulating tumor DNA (ctDNA) in liquid biopsies. Often these devices can contain reagents that can have negative effects on sensitive downstream assays. In this study, blood samples stabilized with either non-crosslinking or crosslinking (formaldehyde releasing substances) were evaluated using quantitative PCR (qPCR) and NGS.

Methods: Formaldehyde concentration in PAXgene® Blood ccfDNA Tubes* and Streck Cell Free DNA BCT® was determined using the MQuant™ Formaldehyde Test (Merck).

Blood samples from healthy donors were collected into EDTA, PAXgene and Streck tubes. Fragmented DNA, equivalent to 500 copies of EGFR mutations T790M and L858R was spiked-in after phlebotomy. Paired tubes were stored for up to 14 days at temperatures ranging from 4 to 30°C. Automated ccfDNA extraction was performed on the QIAsymphony instrument (QIAGEN) using dedicated kits and protocols.

Hemolysis was measured as absorbance at 414 nm in plasma. ccfDNA yield and in situ stability were determined by qPCR for the 18S gene (18S rDNA). EGFR mutations were detected by qPCR using the *therascreen*® EGFR Plasma RGQ PCR Kit (QIAGEN) and by NGS using the GeneReader™ instrument (QIAGEN) with the GeneRead™ QIAact Actionable Insights Tumor (AIT) Panel (QIAGEN).

Results: The PAXgene Blood ccfDNA Tube was shown to have no formaldehyde and not to modify the ccfDNA profile or cause gross hemolysis.

ccfDNA yield directly after phlebotomy was similar in stabilized and unstabilized blood. Within 7 days of storage, PAXgene and Streck tubes prevented increase in genomic DNA at ambient temperatures. In contrast to PAXgene, a significant yield increase was observed in Streck tubes after 14 days storage at 25°C.

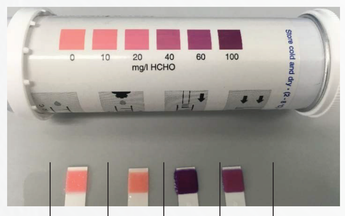
Reliable EGFR mutation detection was achieved with the PAXgene system for samples stored up to 14 days at 25°C. In Streck tubes, decreased mutation call rates were found in samples stored for 14 days at 25°C.

*For Research Use Only. Not for use in diagnostic procedures.
Current stability claims for the PAXgene Blood ccfDNA Tube are 2–25°C for up to 7 days and 35°C for up to 1 day. These studies represent ongoing data generation to explore the performance limits of stabilization and purification technologies.

Detection of Formaldehyde

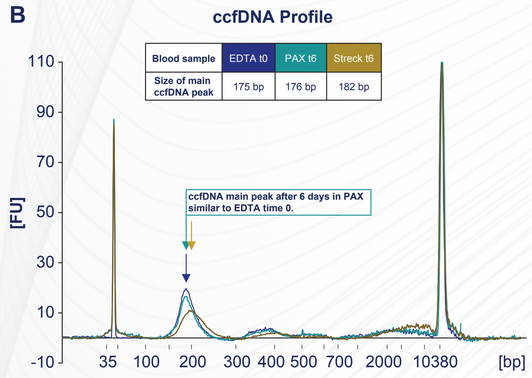
- The PAXgene Blood ccfDNA Tube reagent is free of formaldehyde or formaldehyde releasing substances.

A



Reagent:	H_2O	PAXgene	Streck	Formalin
Dilution:	0	0	1:100	1:1000
Formaldehyde conc.:	0 g/L	0 g/L	10 g/L ~ 1%	40 g/L ~ 4%

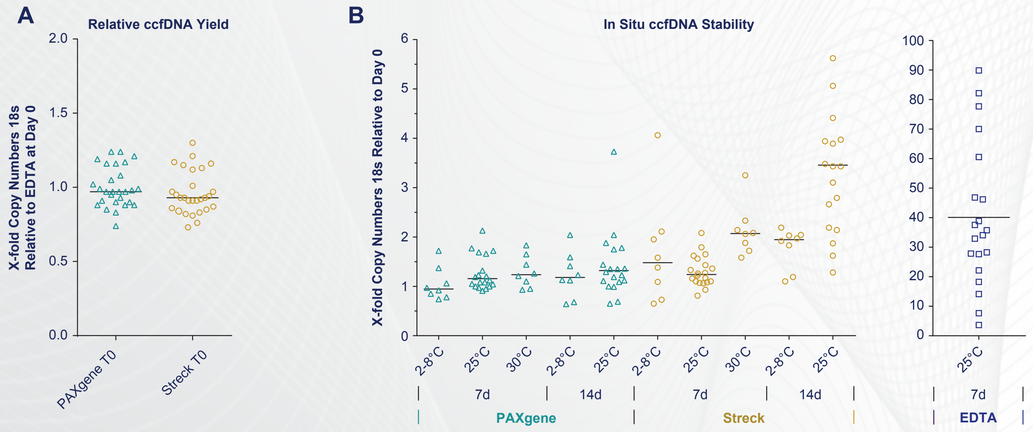
B



A: Formaldehyde concentration measured with MQuant Formaldehyde Test (Merck, Product number 110036). Formaldehyde (HCHO) reacts with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole to form a purple-red tetrazine. The formaldehyde concentration is measured semiquantitatively by visual comparison of the reaction zone of the test strip with the fields of a color scale. Formalin = Formalin Solution, 10% Neutral Buffered, containing 4% formaldehyde (w/v) (Sigma-Aldrich). **B:** ccfDNA profile from EDTA blood directly after blood draw (t0) and from PAXgene Blood ccfDNA Tube or Streck Cell-Free DNA BCT after blood storage for 6 days at 25°C (t6), analyzed on the Agilent Bioanalyzer using the Agilent High Sensitivity DNA Kit.

ccfDNA Yield and In Situ Stability

- ccfDNA yield obtained with the PAXgene Blood ccfDNA System is comparable to EDTA time 0 yield.



Relative copy number change of 18S rDNA 66bp fragments in EDTA, PAXgene and Streck blood collection tubes, **A:** Directly after blood collection (T0) compared to EDTA blood; **B:** After storage compared to T0; Copy numbers were determined with a Primer/Probe qPCR assay; Data are shown as scatter plots with median; day 0 n = 28, storage at 25°C n = 20, at 4–8°C and 30°C n = 8.

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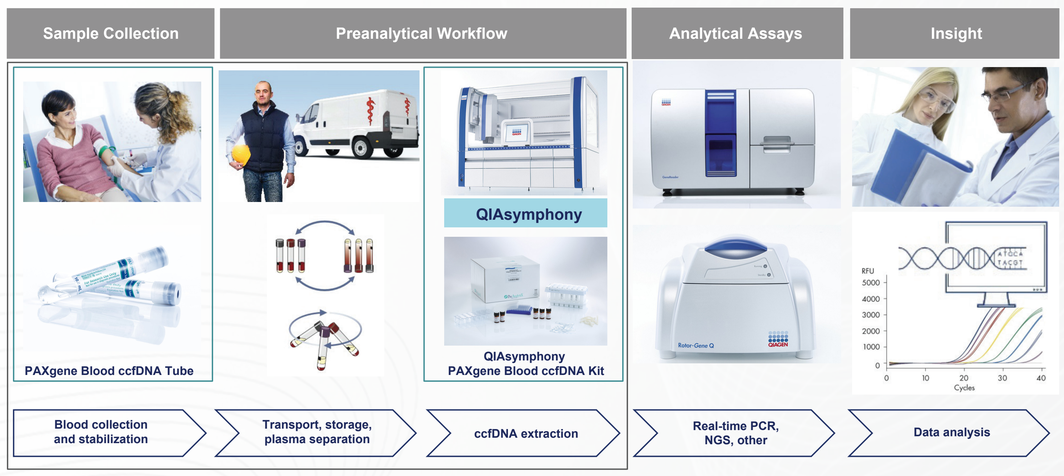
www.gov.uk/government/organisations/innovate-uk; http://gtr.rcuk.ac.uk/projects?ref=101867

PreAnalytiX ccfDNA Workflow

- PAXgene Blood ccfDNA Tube and automated QIAsymphony PAXgene Blood ccfDNA Kit: Integrated collection-stabilization-preparation system.



Hemolysis, Prevention of RBC Lysis

- The PAXgene Blood ccfDNA Tube reagent helps prevent red blood cell (RBC) lysis in blood samples stored for up to 14 days at 25°C.

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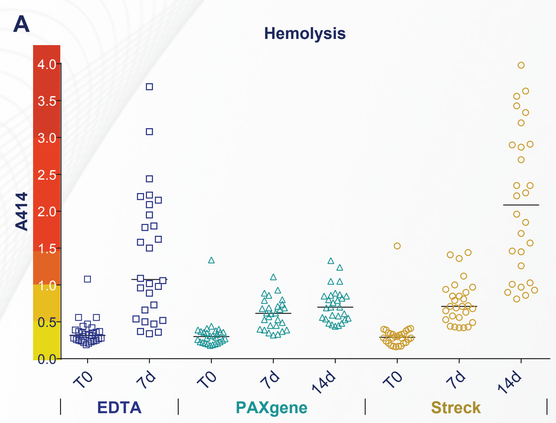


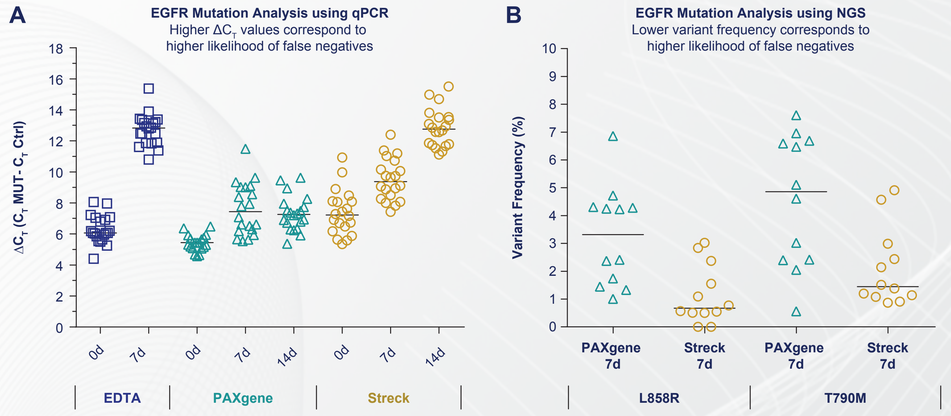
B

% RBC (v/v)	A414
4.00%	3.67
2.00%	3.61
1.00%	3.02
0.50%	1.81
0.25%	1.11
0.125%	0.75
0.063%	0.55
0.031%	0.46
0.016%	0.41
0.008%	0.39
0.004%	0.38
0.000%	0.36

A: Hemolysis measured as absorbance at 414 nm in plasma from blood collected in EDTA tubes, PAXgene Blood ccfDNA Tubes and Streck Cell-Free DNA BCT directly after blood collection (T0) or after incubation at 25°C. Show as scatter plot with median; n = 28. **B:** Relation between RBCs concentration (v/v) and absorbance at 414 nm. Serial dilution of lysed RBCs with plasma from one donor.

EGFR Mutation Detection with qPCR and NGS

- Improved sensitivity with the PAXgene Blood ccfDNA System for EGFR mutation detection using
  - The *therascreen* EGFR Plasma RGQ PCR Kit.
  - The GeneReader NGS workflow.



DNA containing 500 copies of the EGFR mutation was spiked in blood tubes directly after blood draw. ccfDNA was extracted after storage at 25°C. **A:** Mutation analysis for EGFR T790M variant with the *therascreen* EGFR Plasma RGQ PCR Kit (CE-IVD); change of delta C_T (ΔC_T) from EDTA, PAXgene and Streck tubes stored for 0, 7 and 14 days. ΔC_T values were calculated by subtracting C_T of the wildtype assay from C_T for the mutation, n = 20. **B:** Variant frequencies for EGFR mutations L858R and T790M detected with the GeneReader NGS AIT Panel after storage in PAXgene and Streck tubes for 7 days. Data is presented as percentage variant calls in wildtype background reported from QIAGEN QCI™ Interpret analysis, n = 12. Data are shown as scatter plots with median.

Conclusions

- The PAXgene Blood ccfDNA reagent does not contain formaldehyde and the ccfDNA profile is similar to EDTA.
- The PAXgene Blood ccfDNA Tube reagent minimizes red blood cell (RBC) lysis in blood samples.
- The PAXgene Blood ccfDNA Tube minimizes release of genomic DNA at ambient temperature.
- The PAXgene Blood ccfDNA System allows more sensitive detection of cancer-relevant mutations using PCR and NGS applications compared to alternative supplier.
- **Stabilization of whole blood for sensitive ctDNA applications is mandatory but reagents which chemically modify ccfDNA may lead to decreased sensitivity in downstream reactions.**