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 (ccfDNA) 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 Tube 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 T799M 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 QIAasympson 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 GeneReader™ QAuct 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 increased 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 Streek tubes, decreased mutation call rates were found in samples stored for 14 days at 25°C.

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Conclusions


PreAnalytiX ccfDNA Workflow

Sample Collection
Preanalytical Workflow
Analytical Analyses

Hemolysis, Prevention of RBC Lysis

Detection of Formaldehyde

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

ccfDNA Yield and In Situ Stability

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