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 Tube and Streck Cell Free DNA BCT was determined using the MiQuam™ 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, 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 DNA). EGR mutation detection was performed by qPCR using the therascreen® EGR Flame Plasma RQ PC Kit (Qiagen) and by ngs using the GeneRead™ NGS AIT Panel with the GeneRead™ NGS AIT Panel.

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 is 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 EGR 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.

Conclusion: The PAXgene Blood ccfDNA Tube minimizes release of genomic DNA at ambient temperature. 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.

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.

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 suppliers.
- Stabilization of whole blood for sensitive ctDNA applications is mandatory but reagents which chemically modify ccfDNA may lead to decreased sensitivity in downstream reactions.