Preanalytical workflow enabling cfDNA analysis from urine samples

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Introduction

Urine has become an important source of information in the liquid biopsy field. In contrast to blood, specifications for the collection, storage, transport and processing of urine intended for molecular examination are not widely established. Preanalytical specifications were published for urine cell-free DNA (cfDNA) only recently (CEN/TS 17811:2022).

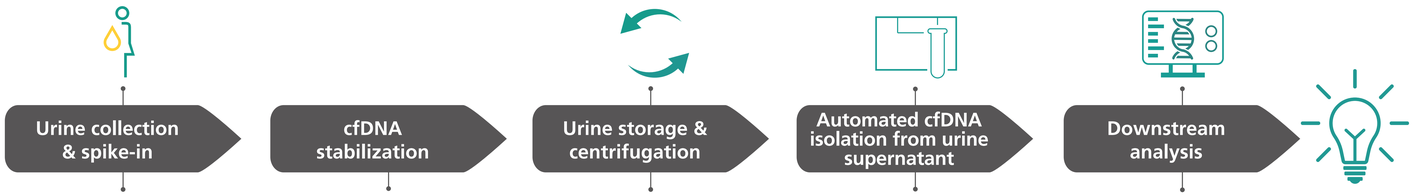
In this study, we investigated post-collection changes to cfDNA profiles in urine samples and present the performance of an optimized preanalytical workflow for cfDNA analysis.

Methods

Urine from apparently healthy, consented female individuals was collected. Where indicated in the figures, urine specimens were pooled (urine of at least 3 individuals). Urine was spiked with cell-free supernatant from male urine or with a cfDNA standard containing *PIK3CA* mutations (Multiplex 5% allele frequency (AF) cfDNA standard, SensID). The urine was stabilized with a urine collection/stabilization solution under development at PreAnalytiX or was left unstabilized. Urine samples were stored for varying durations at different temperatures in time course experiments to simulate research settings. After storage, urine samples were centrifuged to remove cells and cfDNA was isolated from the supernatant via the QIAsymphony® platform (QIAGEN).

Autosomal (cfDNA target 1) and male-specific targets (cfDNA target 2) were quantified using the Rotor-Gene® Q instrument (QIAGEN) and the Investigator® Quantiplex® Pro RGQ assay (QIAGEN). *PIK3CA* H1047R mutation was analyzed by a *PIK3CA* qPCR assay on the Rotor-Gene Q instrument or by dPCR on the QIAcuity® Digital PCR System using the dPCR LNA *PIK3CA* Mutation Assay (QIAGEN). Fragment size distribution was determined by TapeStation® Cell-free DNA ScreenTape® (Agilent Technologies).

Experimental setup for cfDNA analysis using stabilized urine

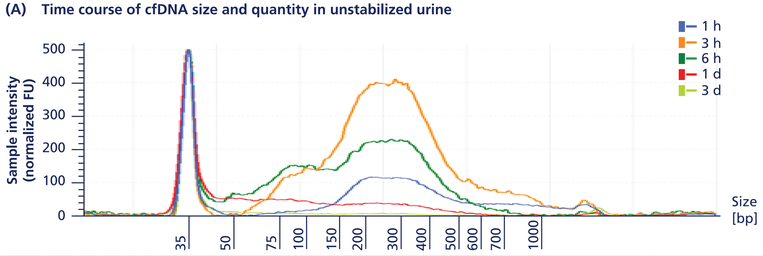


Results

Storage-induced cfDNA profile changes can be prevented by urine stabilization

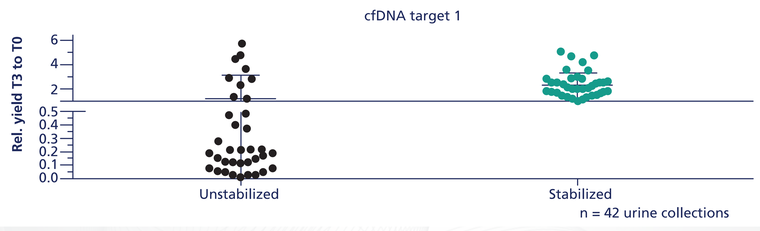
Degradation of cfDNA and release of genomic DNA is minimized by urine stabilization

(A) Time course of cfDNA size and quantity in unstabilized urine

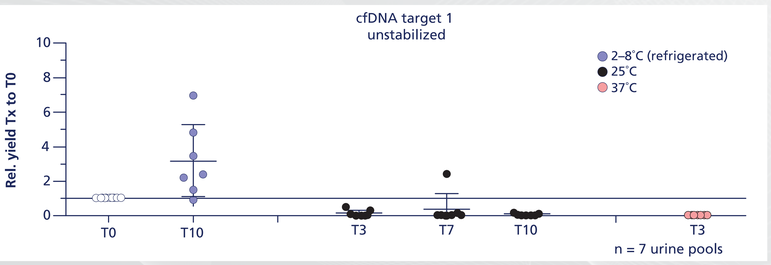


Fragment size profiles of cfDNA isolated from unstabilized urine after storage for 1–72 hours (3 days) at room temperature (18–25°C). Fragment size of isolated cfDNA profiled using Agilent TapeStation Cell-free DNA ScreenTape.

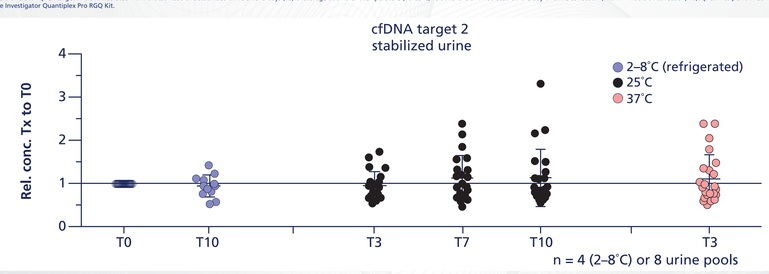
(B) Time course of cfDNA spike-in in unstabilized and stabilized urine over 10 days of storage



Yield of cfDNA qPCR target in cfDNA isolated from unstabilized and stabilized urine after 3 days (T3) of storage at ambient temperature (18–25°C) relative to cfDNA isolated on the day of urine collection (within 4 hours of collection, T0). qPCR was performed with the Investigator Quantiplex Pro RGQ Kit.



Yield of qPCR target in cfDNA isolated from unstabilized urine after storage for 3, 7 or 10 days (T3, T7, T10) refrigerated (2–8°C), at 25°C or at 37°C relative to cfDNA isolated on the day of urine collection (cfDNA isolation within 4 hours of collection, T0). qPCR was performed with the Investigator Quantiplex Pro RGQ Kit.

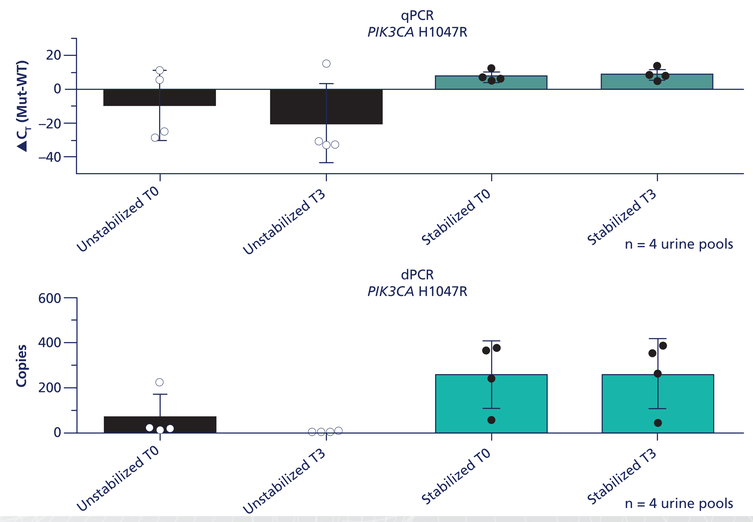


Yield of qPCR target in cfDNA isolated from stabilized urine after storage for 3, 7 or 10 days (T3, T7, T10) refrigerated (2–8°C), at 25°C or at 37°C relative to cfDNA isolated on the day of urine collection (cfDNA isolation within 4 hours of collection, T0). qPCR was performed with the Investigator Quantiplex Pro RGQ Kit in triplicates.

Preservation of qPCR- and dPCR- detectable cfDNA targets

cfDNA from urine stored after stabilization can be detected with qPCR and dPCR

(C) qPCR- and dPCR-based *PIK3CA* H1047R mutation detection after urine storage



PIK3CA H1047R mutation detection in samples spiked with a multiplex 5% AF cfDNA standard. cfDNA was either isolated on the day of urine collection (within 4 hours of the urine collection, (T0) or after 3 days of storage (T3) at room temperature (18–25°C). *PIK3CA* was analyzed by qPCR with an assay where positive ΔC$_T$ of mutated (Mut) and wildtype (WT) targets indicates detection and by dPCR with the dPCR LNA PIK3CA Mutation Assay on the QIAcuity Digital PCR System.

Conclusion

This study showed that post-collection changes in unstabilized urine result in an artificial cfDNA profile and loss of target of interest leading to failed outcomes. Urine stabilization with a collection/stabilization solution under development at PreAnalytiX minimized DNA degradation and gDNA release. It enables urine storage, allowed analysis of urine cfDNA profile and target detection by qPCR and dPCR.

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