cfDNA analysis from urine samples using the PAXgene Urine Liquid Biopsy Set for stabilization and standardization of preanalytical workflows

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Introduction

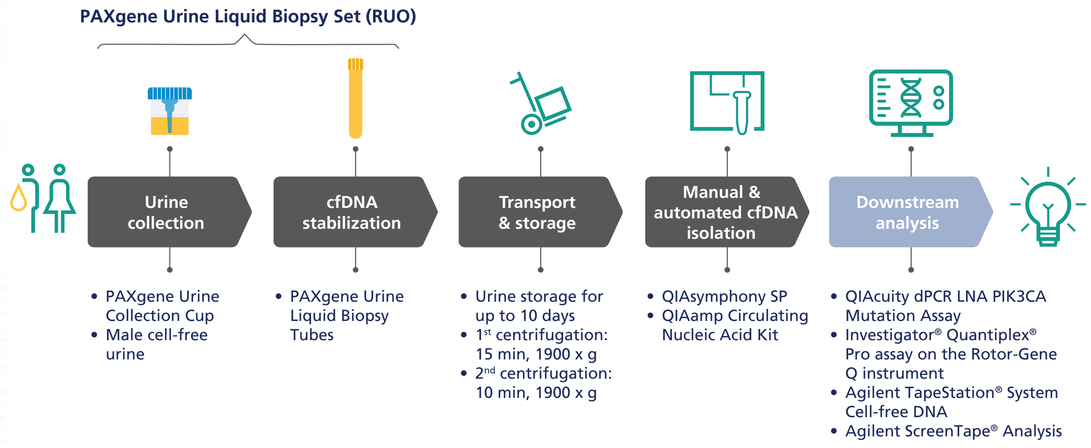
Cell-free DNA (cfDNA) in urine is an avenue for liquid biopsy research. In addition to being a standalone source for biomarkers, urine cfDNA has been shown to provide complementary information to blood cfDNA. However, cfDNA degradation and genomic DNA (gDNA) released from cells and bacteria can impact urine cfDNA research outcomes, indicating the need for urine stabilization and standardization of preanalytical workflows. Preanalytical specifications were only recently published for urine cfDNA (cfDNA) (CEN/TS 17811:2022). In this study, we present the first verified, complete and standardized preanalytical workflow for cfDNA analysis from urine using the PAXgene Urine Liquid Biopsy Set, consisting of the PAXgene Urine Collection Cup and the PAXgene Urine Liquid Biopsy Tube.

Methods

Urine from apparently healthy, consented individuals was collected in the PAXgene Urine Collection Cup. Female urine specimens were spiked with cell-free male urine. Urine was either stabilized with the PAXgene Urine Liquid Biopsy Tube or was left unstabilized.

Urine samples were stored for varying durations at different temperatures or underwent real-world ground and air transportation. After storage and/or transport, urine samples were centrifuged to remove cells and cfDNA was isolated from the supernatant either using QIAGEN® automated or manual cfDNA isolation kits. Autosomal and male-specific targets were quantified using the QIAGEN Rotor-Gene® Q instrument or the QIAGEN QIAcuity® Digital PCR System.

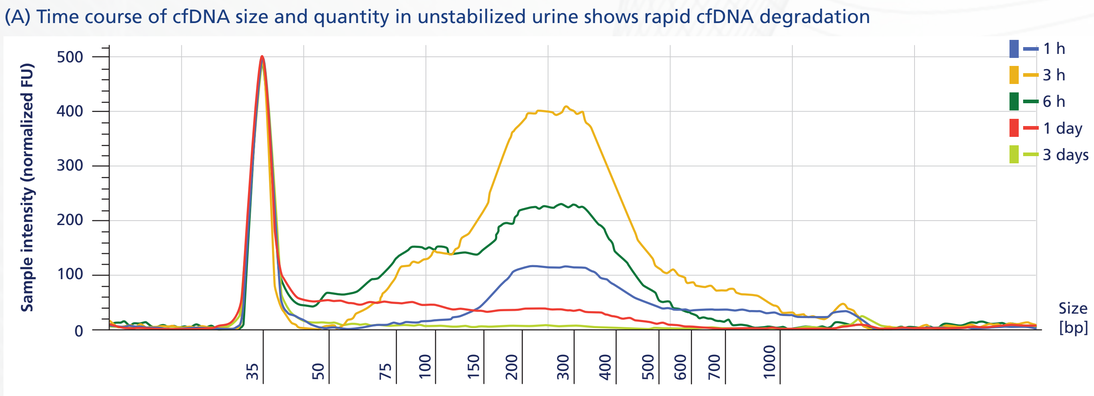
PAXgene Urine Liquid Biopsy Workflow



Results

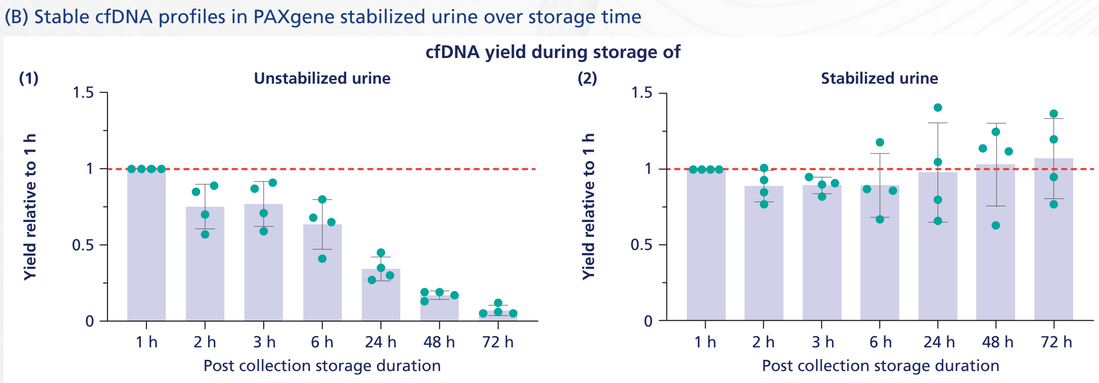
Storage-induced cfDNA profile changes can be prevented by urine stabilization

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



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

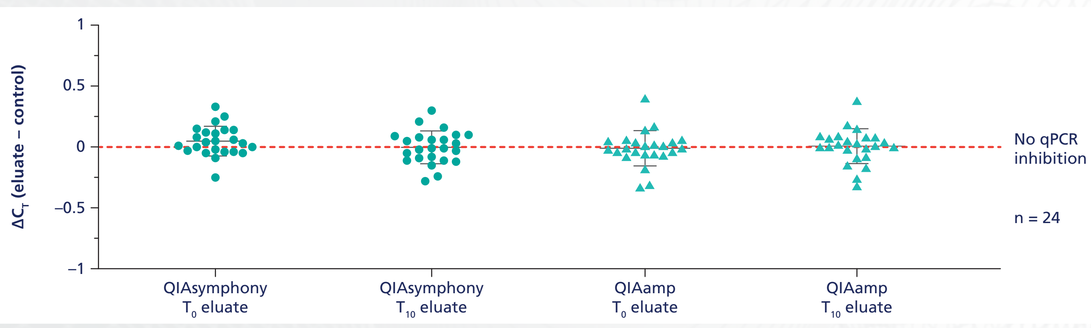
(B) Stable cfDNA profiles in PAXgene stabilized urine over storage time



Yield of spiked male urine cfDNA in female urine, either unstabilized (1) or stabilized with the PAXgene Urine Liquid Biopsy technology* (2) during 15–25°C (including room temperature) storage from 1 h and up to 72 h post urine collection. Data are presented relative to urine processed within 1 h of urine collection (set to 1). cfDNA was isolated with the QIAsymphony SP instrument and analyzed on the QIAGEN RGQ instrument. Data includes urine from 4 individuals. Mean and SD are denoted. Data was generated within a research study of the EASI-Genomics consortium.

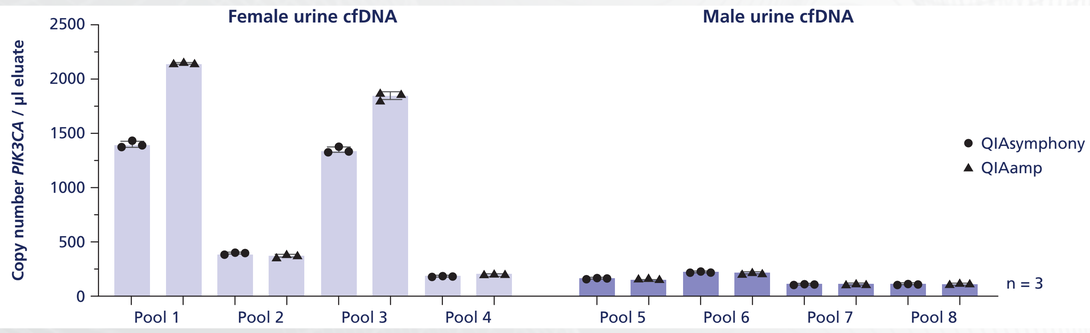
Isolated cfDNA is compatible with qPCR and dPCR

(G) Isolated cfDNA is of high quality as shown by qPCR on the QIAGEN Rotor-Gene Q



ΔC_T calculated between cfDNA eluates and qPCR control samples for the amplification of a qPCR internal control. cfDNA was isolated from urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage for 10 d (T10) or urine processed within 4 h of urine collection (T0).

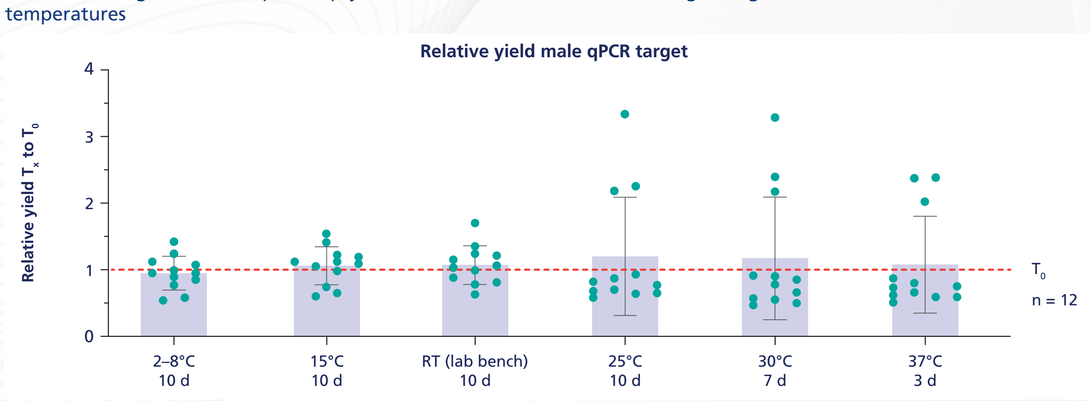
(H) dPCR compatible cfDNA can be isolated automated as well as manually from the PAXgene Urine Liquid Biopsy Tube



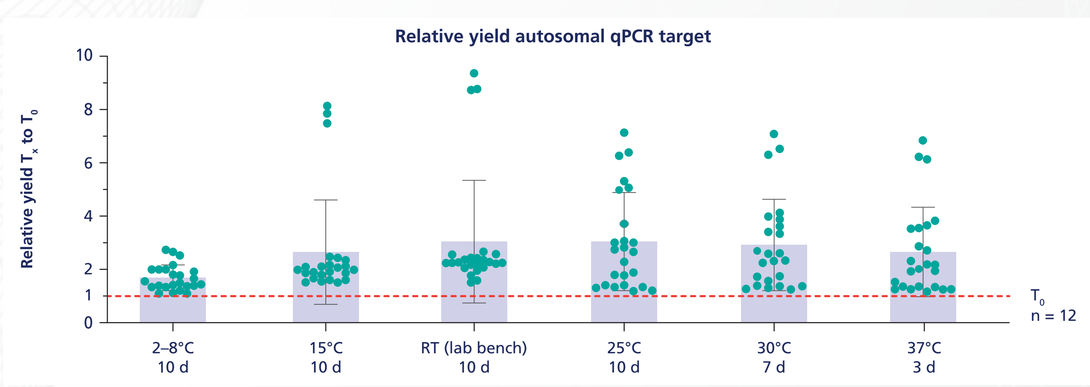
Copy numbers of PIK3CA / µL of cfDNA eluates isolated either with the QIAsymphony DSP Circulating DNA Kit or the QIAamp Circulating Nucleic Acid Kit from PAXgene stabilized urine.

Stabilization minimized cfDNA degradation, gDNA release and bacterial growth during urine sample storage and transport

(C+D) The PAXgene Urine Liquid Biopsy Tube stabilizes cfDNA in urine during storage for various durations and temperatures

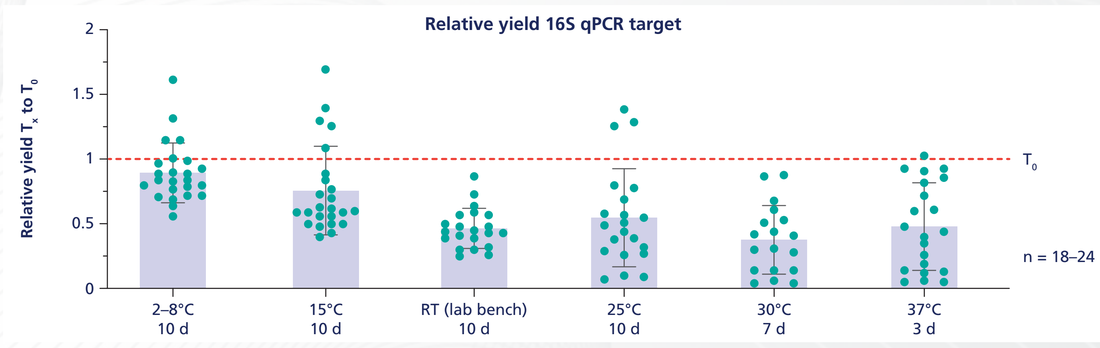


Yield of spiked male urine cfDNA in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage (T3, 7, or 10 days storage) relative to stabilized urine processed within 4 h of urine collection (T0, set to 1). cfDNA was analyzed with the Investigator Quantiplex Pro RGQ assay.



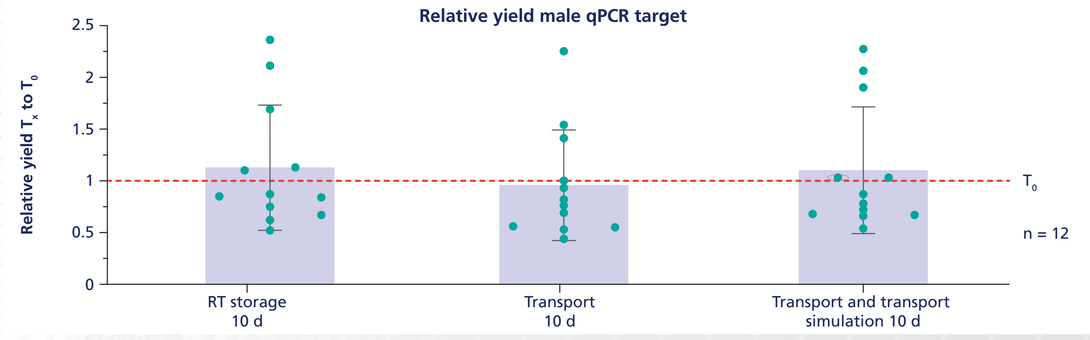
Yield of an autosomal qPCR target in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage (T3, 7, or 10 d storage) relative to stabilized urine processed within 4 h of urine collection (T0, set to 1). cfDNA was analyzed with the Investigator Quantiplex Pro RGQ assay.

(E) cfDNA stabilization by minimization of bacterial growth during whole urine storage



Yield of microbial DNA (16S qPCR target) in urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage (T3, 7, or 10 d) relative to stabilized urine processed within 4 hours of urine collection (T0, set to 1).

(F) The PAXgene Urine Liquid Biopsy Tube stabilizes cfDNA in urine during room temperature storage on the lab bench as well as during real-world transportation



Yield of spiked male urine cfDNA in female PAXgene stabilized urine after urine storage, transport or transport and transport simulation (T10 d) relative to stabilized urine processed within 4 h of urine collection (T0, set to 1).

Conclusion

In this study, urine stabilization with the PAXgene Urine Liquid Biopsy Tube enabled increased total yields of cfDNA in comparison to unstabilized urine, where cfDNA degrades rapidly. Stabilization minimized cfDNA degradation, gDNA release and bacterial growth during urine sample storage and transport. cfDNA isolated from stabilized and stored urine was compatible with qPCR and dPCR. The standardized preanalytical workflow for cfDNA analysis from urine using the PAXgene Urine Liquid Biopsy Set enabled urine storage and transport and allowed consistent analysis of urine cfDNA by qPCR and dPCR.

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