Unlocking Transrenal DNA: New Methods for Stabilizing and Isolating Small cfDNA in Urine

Daniela Mancarella-Langer,[1] Franziska Kaiser,[1] Daniel Grölz,[1] Moritz Rath,[1] Nguyen van Nhi Le,[2] Eric Provencher,[1] Michelle Walther[1]
[1]PreAnalytiX GmbH, Garstligweg 8, 8634 Hombrechtikon, Switzerland
[2]QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

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Introduction

Urine liquid biopsy is emerging as a non-invasive approach for studying biomarkers in research settings. Transrenal DNA, cell-free DNA that passes from blood through the kidney into urine, contains promising biomarkers that may expand the applications of urine liquid biopsy beyond urological malignancies. Due to the size restriction of the renal barrier, transrenal DNA (<50 bp) is shorter than urological cfDNA (≥150 bp).

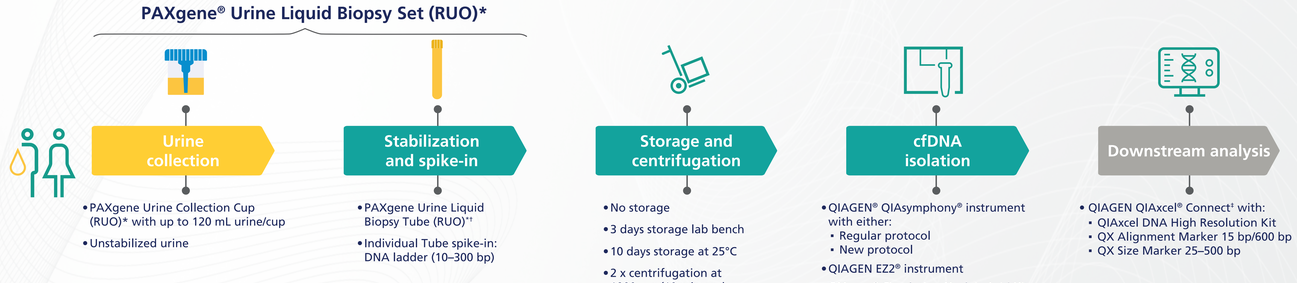
However, the analysis of urine cfDNA is challenging, as it is prone to rapid degradation. Furthermore, isolating short DNA fragments from the complex urine matrix requires optimized extraction protocols.

This study addressed key technical challenges in urine liquid biopsy, presenting optimized workflows for collection, stabilization, isolation, and analysis of urine cfDNA to realize the full potential of transrenal DNA research.

Methods

Urine was collected from apparently healthy, consented individuals and either stabilized or left unstabilized. Urine samples were individually spiked with a DNA ladder of defined size fragments ranging from 10 to 300 bp. DNA was either isolated from urine on the day of collection after spike-in, or after urine storage. DNA spike-in was also performed in separate phosphate-buffered saline (PBS) samples. DNA was isolated either manually or automated using several commercially available cfDNA isolation technologies. The isolated cfDNA was analyzed using capillary gel electrophoresis.

Experimental setup

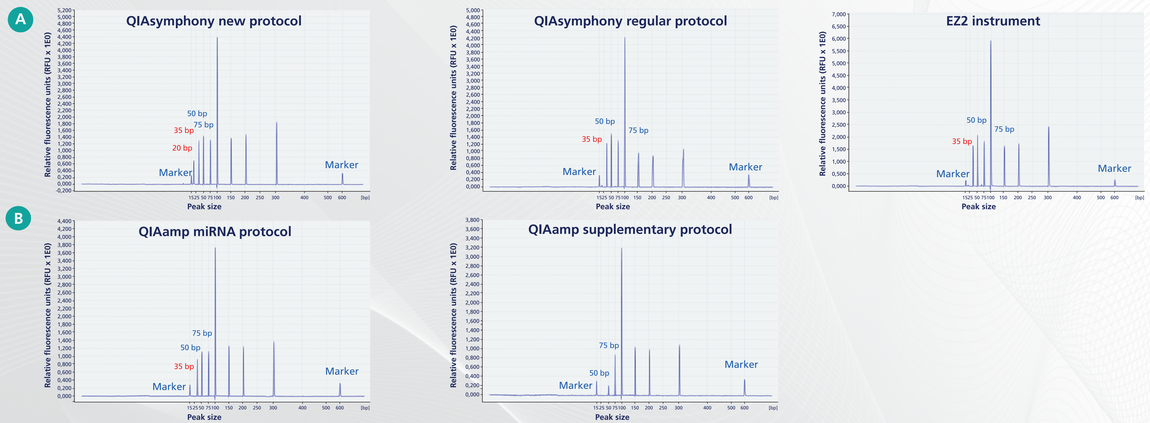


*For Research Use Only. Not for use in diagnostic procedures.
†Sold as part of the PAXgene Urine Liquid Biopsy Set, as well as separately.
‡The QIAxcel Connect and QIAxcel DNA Kits are for Research Use Only. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Results

QIAGEN extraction kits are capable of isolating cfDNA down to 20 bp

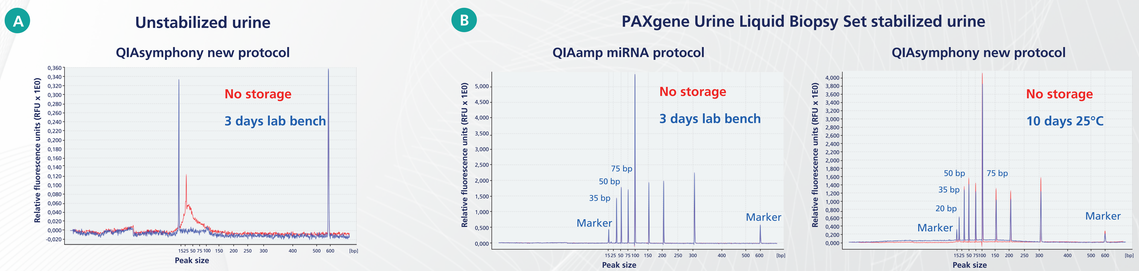
All isolation kits tested were suitable for extraction of cfDNA down to 50 bp. Smaller DNA fragments down to 35 bp were isolated with the miRNA protocol of the manual QIAamp Circulating Nucleic Acid Kit, as well as with automated isolation using the QIAsymphony instrument and the EZ2 instrument. With the new protocol on the QIAsymphony SP instrument, isolation of cfDNA down to 20 bp was possible.



Isolation of cfDNA down to 20 bp. A DNA ladder with defined peak sizes of 10–300 bp was spiked into PBS and isolated **A** automated on the QIAsymphony with a new and a regular protocol or on the EZ2 instrument or **B** manually with the QIAamp Circulating Nucleic Acid Kit either using the protocol 'Purification of circulating microRNA from 1 mL, 2 mL or 3 mL serum, plasma, or urine' or the supplementary protocol, 'Purification of cfDNA from the PAXgene Urine Liquid Biopsy Tube'.
cfDNA eluates were analyzed for fragment size distribution by capillary gel electrophoresis on the QIAxcel Connect instrument using the QIAxcel DNA High Resolution Kit, QX Alignment Marker 15 bp/600 bp and QX Size Marker 25–500 bp. n = 3, one representative image shown.
RFU: Relative fluorescence units.

PAXgene Urine Liquid Biopsy Set is capable of stabilizing small cfDNA

To test the need for urine stabilization for efficient isolation of small cfDNA from urine, the spike-in DNA was isolated from unstabilized urine as well as urine stabilized with the PAXgene Urine Liquid Biopsy Set. For unstabilized samples, the ladder fragments could not be detected even when isolated within hours of urine collection, indicating rapid degradation of the ladder DNA. For stabilized urine samples, the DNA ladder spike-in could be detected even after days of storage, indicating effective stabilization.



Stabilization study of small cfDNA. A DNA ladder with defined peak sizes of 10–300 bp was spiked into **A** unstabilized or **B** PAXgene Urine Liquid Biopsy Set stabilized urine. The cfDNA was either isolated on the day of urine collection, stabilization and spike-in (no storage, cfDNA isolation within 4 hours of urine collection), or after urine storage for 3 or 10 days. cfDNA isolation was performed manually or automated. cfDNA eluates were analyzed for fragment size distribution by capillary gel electrophoresis on the QIAxcel Connect instrument using the QIAxcel DNA High Resolution Kit, QX Alignment Marker 15 bp/600 bp and QX Size Marker 25–500 bp. Representative images are shown. **A** n = 4 individuals; **B1** n = 4 individuals; **B2** n = 4 urine pools, with urine from 3 individuals per pool.

Conclusions

As demonstrated in this study, cfDNA degrades within hours in unstabilized urine. Therefore, urine stabilization is required for efficient isolation of a wide range of cfDNA sizes.

The choice of isolation technology determines the cfDNA size range/cfDNA population available for analysis.

The PAXgene Urine Liquid Biopsy Set, combined with manual or automated isolation technologies, enables recovery of small cfDNA fragments (<50 bp).

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