Development of a preanalytical workflow for liquid biopsy research from urine

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Abstract

Background

Liquid biopsy from blood has emerged as a valuable diagnostic tool and is established in clinical routine testing. In contrast, the use of urine, a truly non-invasive sample type for liquid biopsy, is still less common. Urine cfDNA is of interest as it derives from the urological and also from non-urological organs. The information obtained from urine samples can be complementary to insights from blood samples.

For adoption of urine liquid biopsy into laboratory routine, standardized and optimized workflows for collection, stabilization, and automated cfDNA extraction are needed.

Objective

To develop a standardized preanalytical workflow for urine liquid biopsy research including collection and cfDNA stabilization with automated cfDNA extraction on the QIAsymphony SP from high input volumes, to obtain sufficient and high-quality cfDNA yield for downstream analyses, Further, to demonstrate that analysis by capillary electrophoresis on the QIAxcel Connect instrument for fragment length and concentration can be used for quality control of isolated cfDNA.

Methods

Urine samples were collected and stabilized in the PAXgene Urine Liquid Biopsy Set (RUO; PreAnalytiX). Unstabilized urine was used as comparator. Samples were stored at different conditions before centrifugation and cfDNA extraction from urine supernatant. cfDNA was purified with automated extraction methods and analyzed on the QIAxcel Connect instrument and by qPCR.

Results

The PAXgene Urine Liquid Biopsy Tube stabilized cfDNA in urine . Increased input volumes (6–10 ml) for cfDNA extraction showed good linearity to smaller volumes (1–4 ml) in terms of cfDNA yield. Fragment size analysis of urine cfDNA revealed different size patterns. While cfDNA in blood shows clear nucleosome-length peaks (170 bp), the fragments of urine cfDNA were more heterogenous.

Conclusion

With the presented workflow, urine samples can be collected and cfDNA can be stabilized in a standardized way. The cfDNA yield was proportional to higher input volumes for extraction , which provides sufficient yield for sensitive downstream analyses. Capillary electrophoresis was a well-suited method for quality control of urine cfDNA extraction.

The provided preanalytical workflow for cfDNA from urine, combined with high volume extraction approach, has the potential to improve and standardize clinical research.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

All authors are employees of QIAGEN GmbH, Hilden, Germany.