# Stabilization of urinary cfDNA with novel UAS chemistry to facilitate post-collection sample transportation and storage

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## Introduction

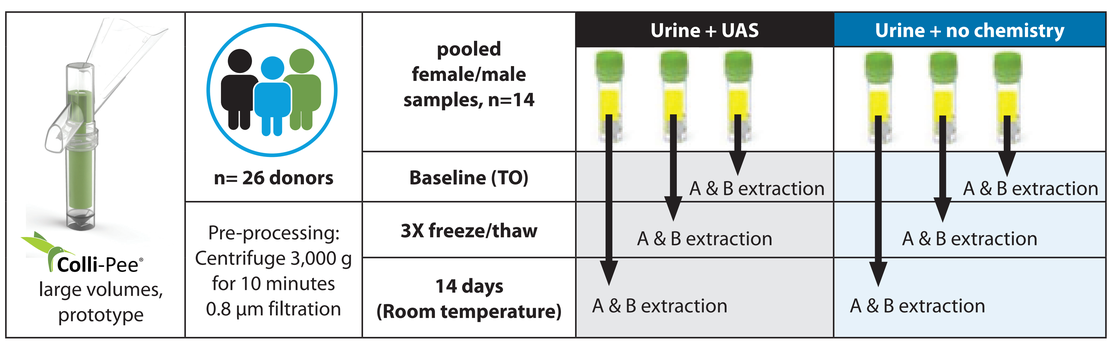
Urine as a liquid biopsy offers many advantageous clinical applications, as its collection is non-invasive, large volumes can be obtained, and contains multiple analytes (including cell-free DNA (cfDNA), extracellular vesicles, and proteins) which may act as biomarkers for local and systemic diseases. However, proper understanding of how pre-analytical conditions can influence urinary liquid biopsies is critical.

## Objective

Evaluate the ability of the **UAS** liquid chemistry to preserve cfDNA in urine samples under common sample transportation and storage conditions for at home collections.

## Study design and methodology

First void urine was collected from healthy female and male donors (n=26) with the 45 mL, Colli-Pee® Large Volumes device, Novosanis, Belgium. Pooled female and male samples (n=14) had one aliquot mixed with UAS chemistry, and the other left with no chemistry addition. Urine samples underwent either **three freeze/thaw cycles** (-20°C to 40°C; minimum 3 hours at each temperature) to mimic sample transportation conditions, or were stored at **room temperature for up to 14 days**. cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit **(A)** and cellular pellet DNA extracted using the QIAamp Powerfecal Pro Kit **(B)** from all samples, with cfDNA stability evaluated by β-globin qPCR, and host cell lysis inferred by human-DNA specific TS143 qPCR.




## Results

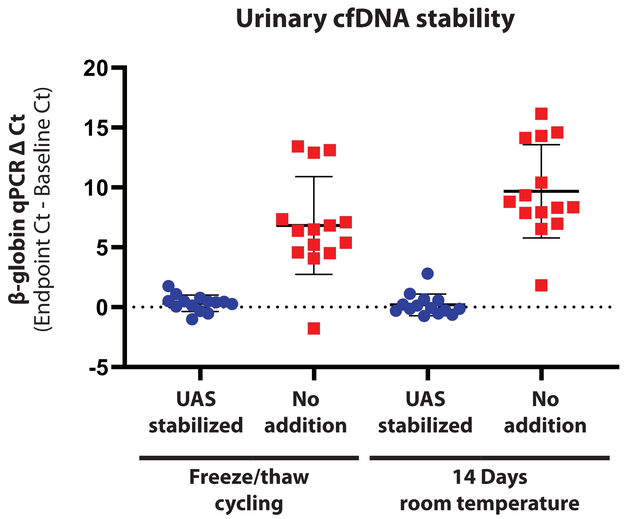



**Figure 1.** Urinary cfDNA stability following three freeze/thaw cycles (left) or 14 days at room temperature (right). Error bars representing the average Δ Ct ± SD. Dotted line through zero represents no change and ideal stability conditions.

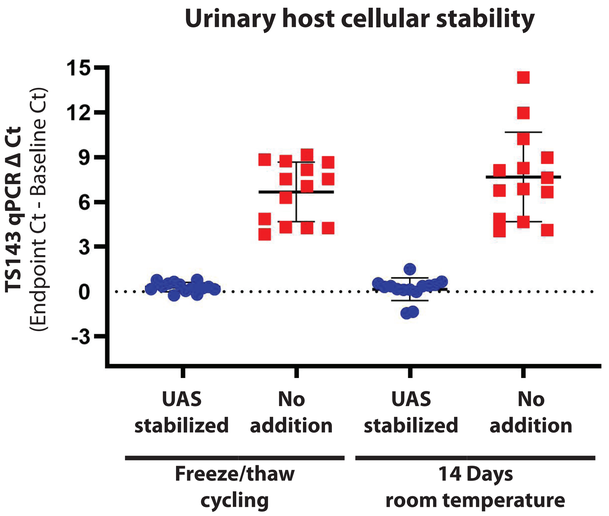



**Figure 2.** Urinary host cellular stability following three freeze/thaw cycles (left) or 14 days at room temperature (right). Error bars representing the average Δ Ct ± SD. Dotted line through zero represents no change and ideal stability conditions.

A significant reduction in cfDNA detection was observed after freeze/thaw cycling in samples without chemistry addition, compared to baseline (average β-globin qPCR Δ Ct = 6.82 ± 4.09; **Figure 1**). UAS chemistry successfully stabilized urinary cfDNA after both freeze/thaw cycling (average Δ Ct = 0.32 ± 0.68), and for up to 14 days storage at room temperature (average Δ Ct = 0.19 ± 0.91) compared to baseline UAS samples. Additionally, the UAS chemistry prevented host cellular lysis under both freeze/thaw cycling (average Δ Ct =0.17 ±0.76) and room temperature storage conditions (average Δ Ct =0.31 ±0.32) **(Figure 2)**.

## Conclusions

Without proper preservation, urinary cfDNA detection significantly decreased under conditions commonly experienced during primary sample transportation. The addition of UAS chemistry to urine samples effectively stabilized cfDNA, while preventing host cellular lysis capable of introducing undesirable cellular genomic DNA into the cell-free sample. Pairing UAS chemistry with the Colli-Pee® urine collection device could offer a simple and convenient approach to collect and stabilize molecular biomarkers of interest in first void urine.

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