Preservation and characterization of urinary cell-free DNA to facilitate home based sampling

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AIM

The aim of the study was to assess the novel non-lytic UAS™ chemistry for preservation of urinary analytes at room temperatures.

MATERIALS AND METHODS

First-void urine was collected from healthy female and male donors with the Colli-Pee® device, to generate 7-14 pooled samples. For experiment 1, urine samples were incubated at room temperature (RT) without any treatment. For experiments 2 and 3, one aliquot of each pooled urine sample was mixed with UAS™ while the other was used neat. Urine samples were incubated at ambient temperature for 7 (T7) and 14 (T14) days or underwent three freeze/thaw (FT) cycles ranging from -20°C to +40°C to mimic extreme sample transportation conditions.

Total cellular DNA

- QIAamp DNA Mini Kit (Qiagen)
- QIAamp PowerFecal Pro DNA Kit (Qiagen)
- Thymidylate Synthase (TS) gene qPCR assay (~143 bp)
- GAPDH qPCR assay

Cell-free nucleic acids

- QIAamp Circulating Nucleic Acid Kit (Qiagen)
- β-Globin qPCR assay

Bacterial growth

- PowerFecal Pro DNA Kit (Qiagen)
- 16S ribosomal RNA (rRNA) gene qPCR assay (~173 bp)

Cell-free nucleic acids and long-range PCR product profiles were evaluated on Agilent TapeStation. Genomic DNA tape was used for determining total cellular DNA profiles. Calculated ΔC_t values from qPCR assays were used as a measure of stability. Data are represented as median with respective 95% confidence interval. Statistical differences between conditions were calculated with an unpaired t-test (Figure 1B, 3A, 3B) or a Factorial ANOVA for the factor's 'preservative' and 'time' with a Tukey post-hoc test (Figures 2A, B, C).

RESULTS

Impact of not adding UAS™ on human cfDNA

Unpreserved (NA) urine samples incubated at RT for T7 showed loss of cfDNA profile, when compared to T0 (A). The loss of human cfDNA and increased bacterial DNA content, in the β-globin and 16S rRNA gene qPCR assay, respectively are shown in (B).

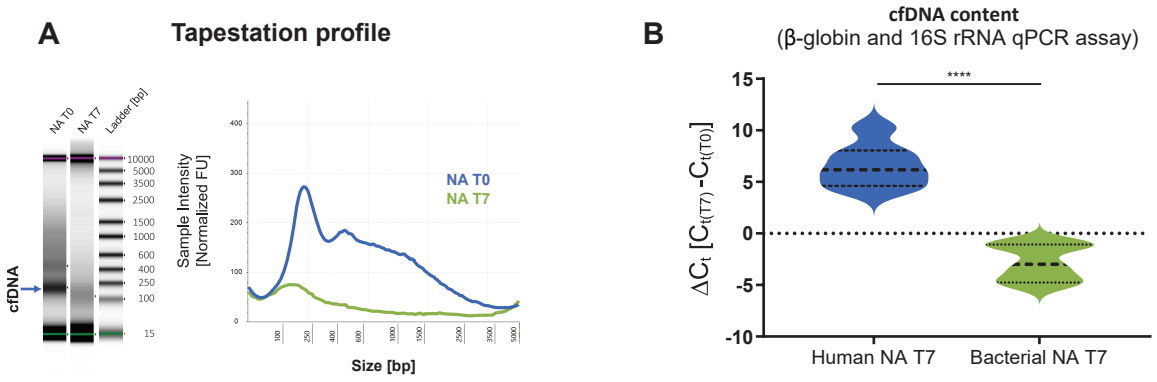

Figure 1: (A) Tapestation and (B) qPCR assay on human and bacterial cfDNA content

Unpreserved urine samples incubated at room temperature for up to 7 days showed loss in quantity and profile of human cfDNA

Impact of UAS™ on microbial growth, cellular DNA and cfDNA

Unpreserved urine samples incubated at RT showed significant differences compared to baseline conditions (T0) at T7 and T14 in bacterial (A) and human cfDNA (B) and human cellular DNA (C). The UAS™ chemistry, on the other hand, effectively preserved bacterial (A) and human cfDNA (B) and human cellular DNA (C) in urine samples incubated at RT for T7 and T14 compared to T0. Overall, a significant improvement in microbial growth prevention and the preservation of human cellular and cfDNA was demonstrated.

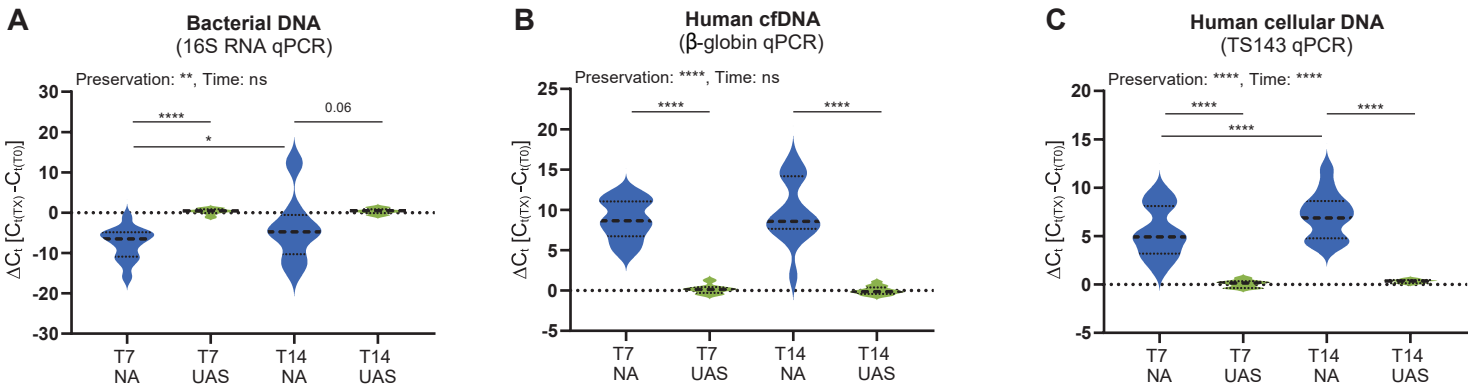

Figure 2: qPCR assays for the detection of (A) bacterial DNA, (B) human cfDNA and (C) human cellular DNA in unpreserved (NA) and UAS™ preserved urine samples at T7 and T14. TX: T7 or T14 respectively.

UAS™ prevents microbial growth and preserves cellular DNA and cfDNA in the urine samples incubated at room temperature for up to 14 days

Impact of UAS™ on cellular DNA, cfDNA and cellular integrity

A significant loss in human cfDNA (A) and cellular DNA (B) was seen in NA under simulated transport conditions in contrast to UAS™ preserved samples, which showed efficient preservation of human cfDNA (A) and cellular DNA (B) under similar conditions. In addition, changes in total cellular DNA profiles (C) as well as marked loss of amplifiable genomic DNA (~1 kb) (D) were observed in NA samples incubated at RT for T7. In contrast, the total cellular DNA profiles (C) and total amplifiable genomic DNA (D) were unchanged in UAS™ preserved samples incubated at RT for T7 implying maintenance of cellular integrity.

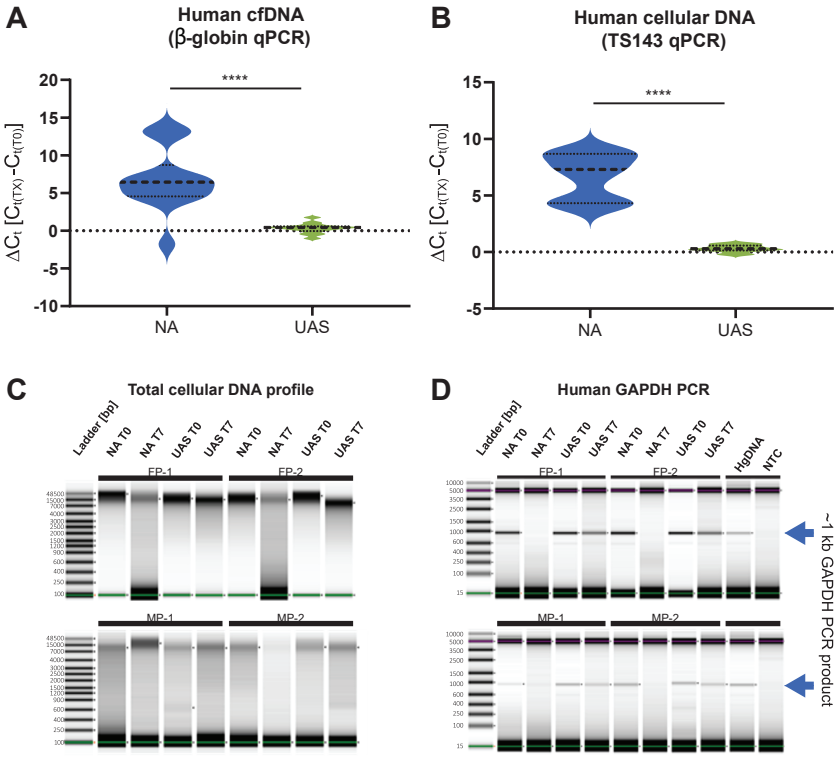

Figure 3: qPCR assays for the detection of (A) human cfDNA and (B) human cellular DNA as well as gel profiles of total cellular DNA (C) and a human housekeeping gene (D) in unpreserved (NA) and UAS™ preserved urine samples at T7. MP: male pooled urine sample, FP: female pooled urine sample, HgDNA: human genomic DNA, NTC: non-template control.

UAS™ preserves both cellular DNA, cfDNA and maintains human cellular integrity under wide range of temperature storage conditions

CONCLUSION

Colli-Pee® UAS™ (RUO) device offers a user-friendly method for home-based collection and preservation of urine samples. In addition, Colli-Pee® allows for standardized and volumetric first-void urine collection, while UAS™ preserves urinary analytes up to 14 days at RT and after freeze-thaw cycles for oncology research.

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