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

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AIM

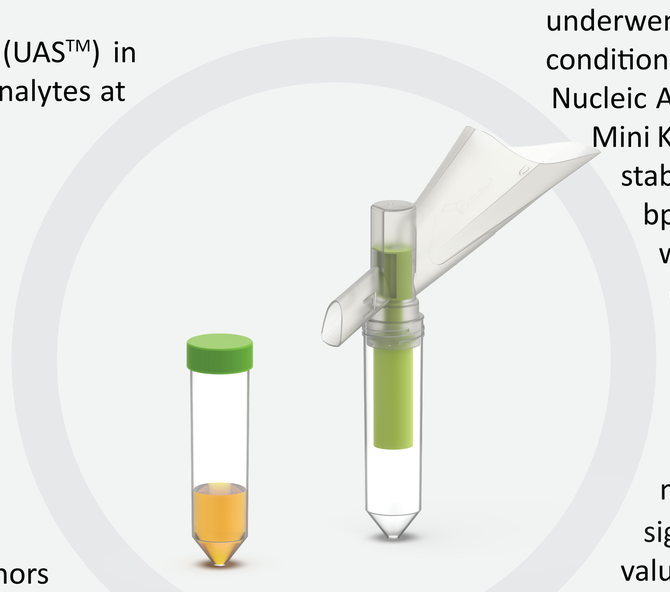
Highlight the performance of our novel non-lytic formulation (UAS™) in sample preservation for detection and stabilization of urinary analytes at ambient temperatures.

OUTCOME

A combination of Colli-Pee® device and UAS™ buffer offers a standarized and user-friendly method for the home based volumetric collection and preservation of urine samples for the stabilization of analytes involved in liquid biopsy applications.

MATERIALS AND METHODS

First-void urine was collected from healthy female and male donors with the Colli-Pee® devices. Forced-failure urine samples were incubated at ambient temperature without any treatment. Pooled urine samples had one aliquot mixed with UAS™ chemistry, and the other left unstabilized with no chemistry addition.



Urine samples were stored at ambient temperature for 7-14 days or underwent freeze/thaw (FT) cycles to mimic sample transportation conditions. Cell-free nucleic acids were extracted using QIAamp Circulating Nucleic Acid Kit. Total cellular DNA was extracted using either QIAamp DNA Mini Kit or QIAamp PowerFecal Pro DNA Kit. Human cell-free DNA (cfDNA) stability and bacterial growth was quantified using β-globin and (~100 bp) and 16S rRNA gene qPCR assay (~173 bp). Human cellular integrity was determined by quantifying Thymidylate Synthase (TS) gene qPCR assay (~143 bp), also referred as TS143 and/or detection of GAPDH PCR product (~1 kb). Cell-free nucleic acids and long range PCR product profiles were evaluated on Agilent 2100 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. Two-tailed p-values ($p \leq 0.05$) from Wilcoxon signed rank test were used as statistical measure. Actual median ΔC_t values obtained were compared to a hypothetical median value of $\Delta C_t = 0$ to determine the efficiency of UAS™ based stabilization of urinary analytes. In the figures, horizontal bar representing median with 95% confidence interval, dotted lines (...) is $\Delta C_t = 0$, which reflects absolute stability, and time-points for day 0, day 7 and day 14 are indicated as T0, T7 and T14.

RESULTS AND CONCLUSION

Unpreserved urine samples held at room temperature showed loss in quantity and profile of human cfDNA

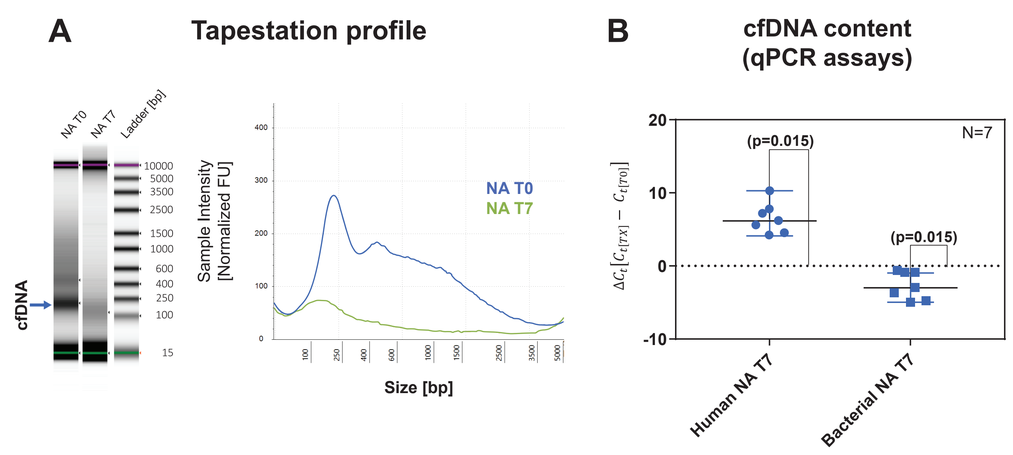


Figure 1: (A) Representative gel and electropherogram traces of extracted cell-free nucleic acids. Unpreserved (NA) urine samples incubated at room temperature (RT) for T7 showed loss of cfDNA profile, when compared to T0. (**B**) β-globin and 16S rRNA gene qPCR assay showed loss of human cfDNA (median ΔC_t: +6.2; p=0.015) and increased bacterial cell-free DNA content (median ΔC_t: -3.0; p=0.015), respectively.

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

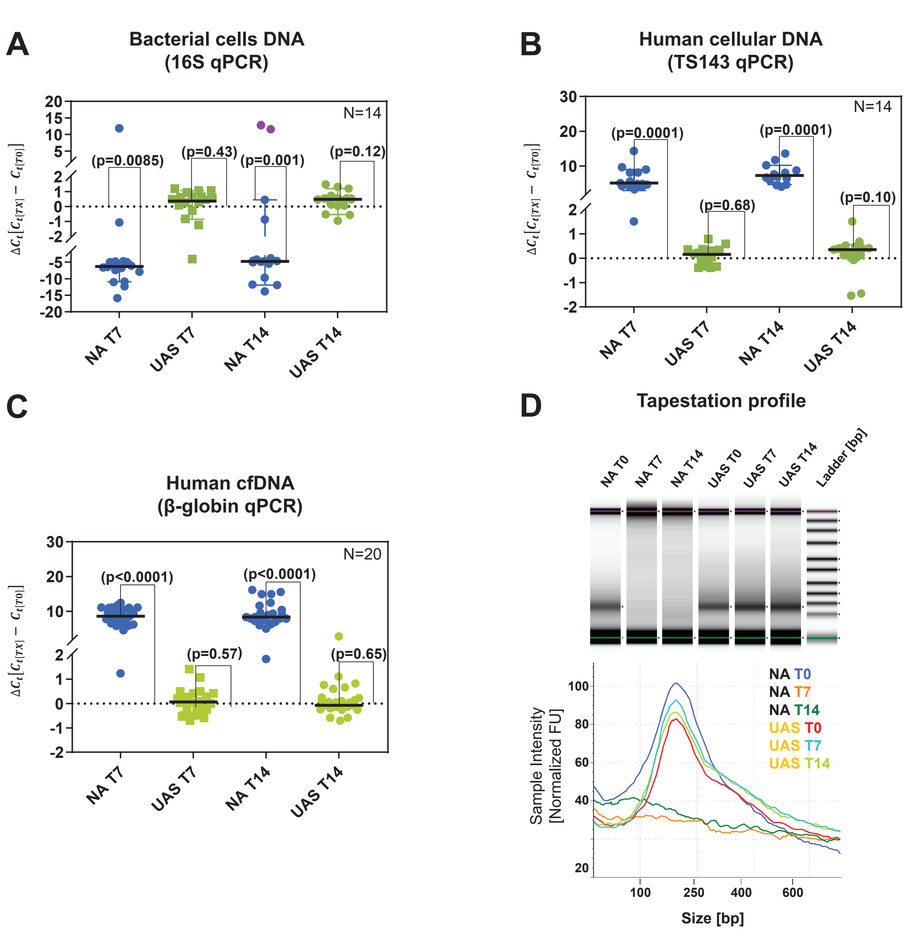


Figure 2: In unpreserved (NA) urine samples held at RT for T7 and T14, (**A**) 16S rRNA gene and (**B**) TS143 qPCR assay demonstrated significant increase in bacterial DNA [median ΔC_t: -4.8 to -6.3; p= 0.0085(T7) and 0.001*(T14)] and loss of human cellular DNA content (median ΔC_t: +8.3 to +9.3; p=0.001), respectively. UAS™ stabilized samples under similar storage conditions showed no significant change (median ΔC_t: ≤ +0.5; p=0.1 to 0.7) for both 16S rRNA and TS143 gene, demonstrating prevention of microbial growth and stabilization of cellular DNA. *p-value of 0.001 was calculated after the identification and removal of outliers (●). (**C**) β-globin qPCR assay showed significant loss of human cfDNA content (median ΔC_t: +8.5; p<0.0001) for both T7 and T14 time points in unstabilized samples unlike UAS™ stabilized samples, which showed effective cfDNA preservation (median ΔC_t: -0.07 to 0.07; p=0.6 to 0.7) On y-axis, TX stands for T7 or T14. (**D**) Representative gel and electropherogram traces of extracted urinary cell-free nucleic acids. Unpreserved (NA) urine samples incubated at RT for T7 and T14 showed loss of cfDNA profile, when compared to T0 samples. UAS™ stabilized samples showed preservation of cfDNA profiles at both T7 and T14 time points.

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

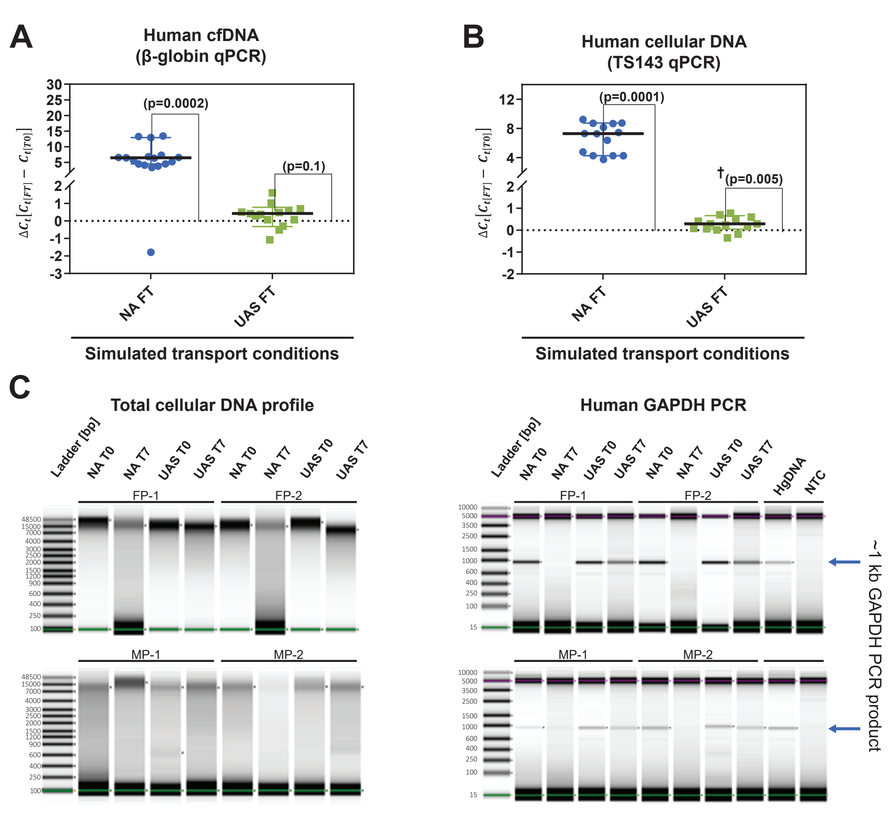


Figure 3: (**A**) β-globin and (**B**) TS143 qPCR assay demonstrated significant loss of human cfDNA (median ΔC_t: +6.4; p=0.0002) and cellular DNA (median ΔC_t: +7.3; p=0.0001) in the unpreserved samples (NA) under simulated transport conditions unlike UAS stabilized samples, which showed efficient preservation of cfDNA (median ΔC_t: +0.4; p=0.1) and cellular DNA (median ΔC_t: +0.3; p=0.005*) under similar conditions. †Despite showing a significant deviation from the median $\Delta C_t = 0$, this result is not relevant, with only minimal change in cellular DNA stability (median ΔC_t: +0.3) in UAS™ stabilized samples. (**C**) Gel profiles of total cellular DNA extracted from both unpreserved and UAS™ stabilized samples held at RT for T7. When stored at RT for T7, unpreserved samples showed significant changes in the total cellular DNA profile as well as marked loss of amplifiable genomic DNA (gDNA). Presence of long amplifiable gDNA (~1 kb) and total cellular DNA profile preservation in UAS™ stabilized samples suggest maintenance of cellular integrity under similar conditions. MP and FP stands for male and female pooled urine samples, respectively. HgDNA and NTC stand for human genomic DNA (positive PCR control) and non-template control (negative PCR control), respectively.

EACR European Association for Cancer Research

EACR Liquid Biopsies conference, Bergamo, Italy, May 24-26, 2022
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