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First-void urine as a non-invasive proxy for plasma in prostate cancer-related liquid biopsy applications.

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INTRODUCTION

Prostate cancer ranks as the second leading cause of cancer-related mortality in men. Despite introducing various therapies, effective prediction or monitoring methods remain challenging. While liquid biopsy from blood requires invasive procedures, first-void urine (FVU) has emerged as a promising, less invasive alternative. The nRichDX Revolution cell-free DNA (cfDNA) isolation method enables higher cfDNA yield and recovery rates from larger urine volumes, making it a valuable non-invasive diagnostic tool. This study aims to investigate the potential of FVU-derived cfDNA for diagnostics, advancing liquid biopsy research.

MATERIALS & METHODS

Paired male FVU and venous blood samples were collected from healthy donors (n=16) using the Colli-Pee UAS devices (Novosanis) and BD K2EDTA Vacutainer tubes, respectively. From these, six paired FVU and blood samples were spiked with 10 ng/mL of cfDNA reference standard containing the KRAS p.G12V mutation. Approximately 35 mL of urinary cell-free (CF) supernatant and 3.5 mL of plasma were obtained after FVU and blood sample centrifugation, respectively. CF supernatant was split into two 17.5 mL samples and 2.5 mL of DPBS, used as input material for cell-free nucleic acid extraction using the nRichDX Revolution Max20 cfDNA Isolation Kit for 20 mL sample volume. After extraction, eluant (25 µL) from two CF samples were pooled together to form a total eluant volume of 50 µL similar to the total eluant (50 µL) volume for cfDNA extracted from plasma samples. The extracted cfDNA profile was assessed on the 4200 TapeStation System using HSD5000 and cfDNA ScreenTape (Agilent). Extracted nucleic acids from FVU samples were subjected to β-globin qPCR assay to quantify endogenous human cfDNA content using 2X iTaq Universal SYBR Mastermix (Bio-Rad). Human cfDNA quantification (g/mL) was performed by incorporating C_t values in the standard curve obtained for β-globin qPCR assay. The recovery of actionable cfDNA molecules was determined by a KRAS mutation detection qPCR assay using TaqMan Genotyping.

RESULTS

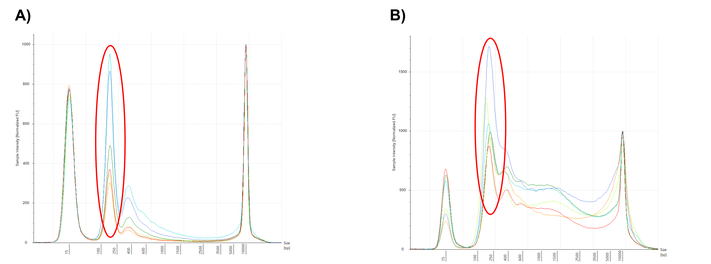

Figure 1. Representative images of TapeStation electropherogram tracings of cfDNA extracted from 3.5-mL Plasma (**A**) and 35-mL CF-urine (**B**) show the presence of a 100-250 bp peak (highlighted in red circles) in paired plasma and CF-urine samples collected from healthy donors.

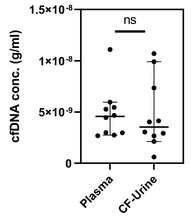

Figure 2. cfDNA qPCR-based quantification (g/mL) of endogenous β-globin demonstrates that the average cfDNA concentration (g/mL) for Plasma samples is 4.83E-09 ± 2.52E-09 and 4.75E-09 ± 3.4E-09 for CF-Urine samples. The two-tailed p-value = 0.9; conventional criteria consider this difference statistically non-significant (ns).

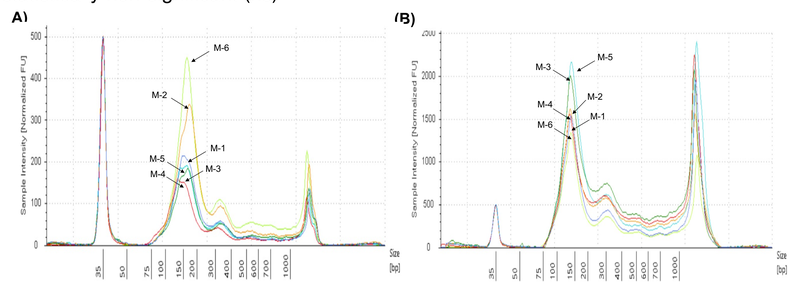

Figure 3. TapeStation electropherogram tracings showing extracted cfDNA from 3.5 mL Plasma (**A**) and 35 mL CF-Urine (**B**) spiked samples. Recovery of cell-free DNA spike was similar in plasma and CF-urine.

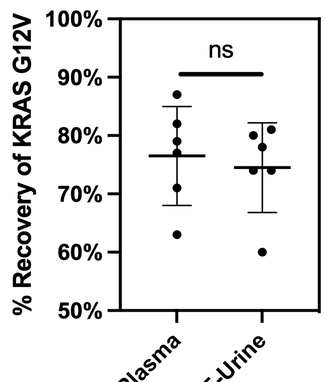

Figure 4. The percent recovery of the KRAS p.G12V mutation spike was calculated using the TaqMan Genotyping Assay for 35-mL CF-Urine and 3.5-mL Plasma spiked samples**.** The average KRAS percent recovery for CF-Urine and plasma samples is 75% ± 8.0% and 77% ± 8.4%, respectively. The two-tailed p-value = 0.68; conventional criteria consider this difference statistically non-significant (ns).

CONCLUSION

This study explored the correlation between cfDNA extracted from 3.5 mL plasma and 35 mL CF-urine. TapeStation electropherogram tracing profiles of extracted cfDNA showed the presence of 100-250 bp peak in paired plasma and CF-urine samples (**Figure 1**). These results and similar mean cfDNA content (g/mL) based on the β-globin qPCR assay (**Figure 2**) indicated similar cfDNA recovery rates between extracted CF-urine and plasma samples. Comparing CF-urine and plasma spiked with cell-free DNA reference standards showed consistent results (**Figure 3**). qPCR KRAS mutation detection results demonstrate consistent KRAS percent recovery from spiked CF-Urine and Plasma samples (**Figure 4**). This study suggests that male FVU samples can serve as a non-invasive alternative for prostate cancer-related liquid biopsy applications. Further investigations are needed to evaluate clinical samples and biomarkers to establish a robust correlation between cfDNA levels and cancer types and stages. This study highlights the potential of first-void urine samples as a reliable proxy for blood-based liquid biopsy applications.