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Enhancing urine-based cfDNA detection for prostate cancer diagnosis through increased input volume

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INTRODUCTION

Prostate cancer is the second most common cancer in men; about 1:8 will be diagnosed with prostate cancer during their lifetime. The currently used prostate-specific antigen blood test is not ideal for early detection. It can often lead to false negatives or detect cancers that may otherwise not need to be treated. Cell-free DNA (cfDNA) extracted from urine has opened new horizons in non-invasive cancer diagnostics, particularly for prostate cancer. Detecting cfDNA in urine can be challenging due to its inherently low concentration. The nRichDX Revolution cfDNA Extraction Kit can increase the input volume of urine to address this limitation, enhancing the sensitivity of cfDNA detection. This study explores the benefit of collecting larger volumes of urine spiked with a PIK3CA mutation and the corresponding increase in assay sensitivity for early prostate cancer diagnosis and monitoring.

MATERIALS & METHODS

Pooled urine from healthy donors was collected, preserved, and processed using the nRichDX centrifugation protocol. 10, 20, 30, 40, and 50 mL of pooled urine were extracted using the Revolution Max20 cfDNA Extraction kit. 10 mL and 20 mL sample inputs were extracted and pooled to produce the 30-, 40-, and 50-mL inputs. Replicate samples were extracted using the MagMAX cfDNA Isolation Kit from ThermoFisher Scientific. 10 mL samples were pooled to produce the 20-, 30-, 40-, and 50-mL inputs. All samples were spiked with a cfDNA standard containing the PIK3CA mutation. All samples were eluted to a total of 50µL.
Recovery of the prostate cancer mutation was assessed using Ct values obtained through a PIK3CA mutation detection assay on the QuantStudio 3 Real-Time PCR system. The quality of the extracted cfDNA was determined using the Agilent Cell-free DNA ScreenTape assay for TapeStation systems.*

RESULTS

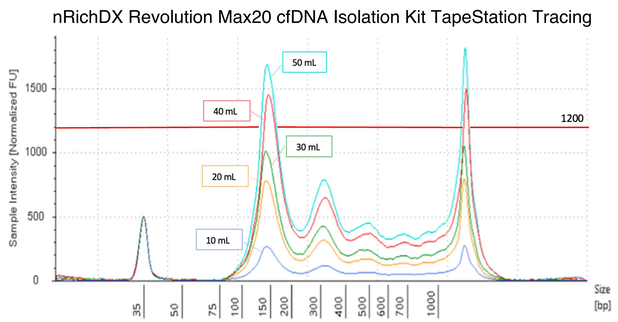

Figure 1. TapeStation electropherogram tracings show cfDNA extracted from 10, 20-, 30-, 40-, and 50-mL urine samples using the nRichDX Revolution Max20 cfDNA Kit. The samples show proportional recovery of cfDNA across sample volumes.

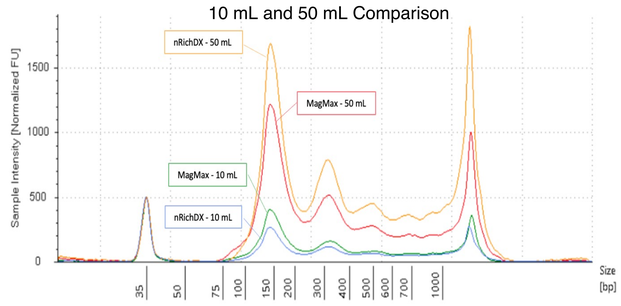

Figure 3. TapeStation electropherogram tracings comparing the cfDNA extracted from the 10 mL and 50 mL urine samples from the nRichDX and MagMax kits. The 10 mL samples show similar recovery between kits, while the 50 mL sample volume shows increased recovery from the nRichDX kit.

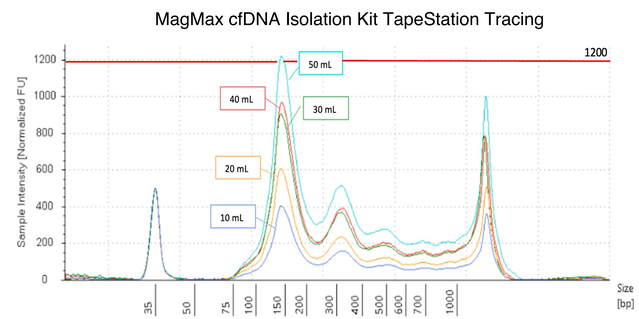

Figure 2. TapeStation electropherogram tracings show cfDNA extracted from 10, 20, 30, 40, and 50-mL urine samples using the ThermoFisher MagMax cfDNA Isolation Kit. The 10, 20, and 50-mL sample volumes show proportional recovery, while the 30 and 40- mL volumes show similar recovery.

	PIK3CA Ct		Agilent TapeStation cell-free DNA ScreenTape cfDNA Concentration (pg/uL, 50-700 bp)	
Sample Volume	nRichDX Revolution Max20 cfDNA Kit	Thermofisher MagMax cfDNA Isolation Kit	nRichDX Revolution Max20 cfDNA Kit	Thermofisher MagMax cfDNA Isolation Kit
10 mL	33.2	Undetermined	716	1100
20 mL	31.4	Undetermined	1990	1660
30 mL	31.0	Undetermined	2770	2480
40 mL	30.1	Undetermined	4110	2700
50 mL	30.0	Undetermined	4900	3580

Table 1. Ct values from the PIK3CA mutation qPCR assay and cfDNA concentration between 50 and 700 base pairs calculated using the Agilent cell-free DNA ScreenTape assay across all sample volumes for both extraction kits. The nRichDX Revolution Max 20 cfDNA Kit shows detection of the PIK3CA mutation across all sample volumes and higher cfDNA concentration for volumes 20 mL and above.

CONCLUSION

This study compared cfDNA recovery from increasing urine sample volumes using the nRichDX Revolution Max 20 cfDNA kit and MagMAX cfDNA Isolation Kit. TapeStation tracings demonstrated proportionally increasing cfDNA yield with increasing sample volume when using the nRichDX Revolution system. qPCR confirmed the detection of the PIK3CA prostate cancer mutation in all cfDNA eluates extracted using the nRichDX Revolution system. The mutation was not detected in any eluates extracted with the MagMax cfDNA Isolation kit.

The Revolution Max 20 cfDNA kit outperforms the MagMax Kit in extracting cfDNA with high efficiency, as shown by the Agilent Cell-free DNA ScreenTape and the qPCR assay. As input volume increases, it consistently produces high cfDNA yields while maintaining quality. The Revolution Max20 kit's enhanced capability to yield more significant quantities of high-quality cfDNA from urine samples may profoundly impact liquid biopsy sensitivity and early detection of prostate cancer, potentially revolutionizing the diagnostic process.

*Agilent TapeStation systems are for Research Use Only (RUO). Not for use in diagnostic procedures.