Higher cfDNA Recovery and Precision from Large Urine Sample Volumes Using the nRichDX® Revolution Sample Prep System™

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INTRODUCTION

Liquid biopsies can detect, analyze, and monitor cancer in various biofluids (blood, urine, plasma, etc.) while having a minimally invasive collection procedure. Multiple biomarkers can be identified using liquid biopsies, such as cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), and circulating tumor cells (CTC).

Cell-free DNA has become very sought after in this field due to its specificity in early cancer detection. However, isolating cfDNA from biological fluids is challenging due to their low concentration in the sample, especially in urine. Urine is the least invasive biofluid as it can be relatively easily collected in large amounts quickly and efficiently.

One of the main challenges of extracting cfDNA from urine is that many urine cfDNA extraction kits are limited to small sample volume inputs (≤5mL), leading to low cfDNA yields. The nRichDX cfDNA Isolation Kit can extract cfDNA from large urine sample volumes (up to 20mL) without losing cfDNA yield while keeping genomic DNA (gDNA) to a minimum. This workflow has no transfer steps and extracted sample eluates are ready for downstream applications such as qPCR, TapeStation, and NGS.

MATERIALS & METHODS

cfDNA extractions were performed using the nRichDX cfDNA Max 20XL Isolation Kit and the QIAamp® Circulating Nucleic Acid Kit. Urine samples were collected using the Colli-Pee® UAS™ device. A urine preservative from Novosanis® (UAS) was added to all urine samples. cfDNA was extracted from four 20mL urine samples using the nRichDX cfDNA Isolation Kit. All samples were spiked with a cfDNA standard containing the KRAS G12V mutation at a concentration of 20ng/mL. Samples were eluted in 100μL.

The sample volume capacity for QIAamp's Circulating Nucleic Acid Kit is only 4mL for urine samples. Therefore, the eluates were pooled together to emulate a 20mL urine sample extraction. Samples were spiked with a cfDNA standard at a concentration of 20ng/mL. All samples were eluted in 20μL and then were pooled together to bring the eluants to 100μL to match the elution volume from the nRichDX extraction kit.

The cfDNA yield was determined by fluorescence on the Qubit™ 4 Fluorometer. The percent recovery of cfDNA was determined by a KRAS G12V mutation detection assay on the QuantStudio® 3 Real-Time PCR System. The quality of extracted cfDNA was determined using the Agilent® High Sensitivity D1000 ScreenTape.

RESULTS

Sample Number	Extraction Kit	Total DNA Yield (ng/mL)	Mean Total DNA Yield (ng/mL)	Standard Deviation (ng/mL)	%CV
1	nRich^{DX} cfDNA Isolation Kit	14.8	15.3	0.5	4%
2		14.8			
3		15.9			
4		15.5			
1	QIAamp Ciculating Nucleic Acid Kit	17.7	16.1	1.4	9%
2		16.8			
3		15.1			
4		14.7			

Table 1. Qubit analysis shows similar DNA yields between the nRichDX and Qiagen® kits. The percent coefficient of variation of the nRichDX and Qiagen kits was 4% and 9%, respectively. The two-tailed t-test P-value = 0.3232; by conventional criteria, this difference was not statistically significant.

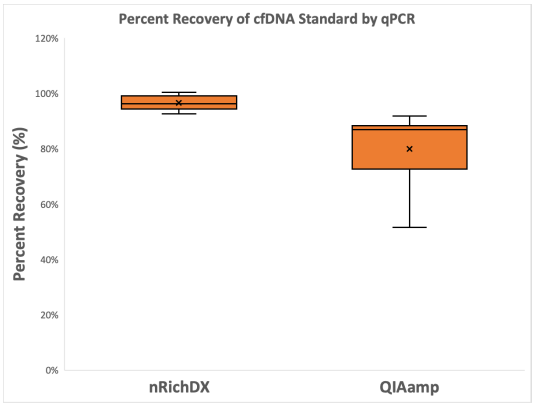
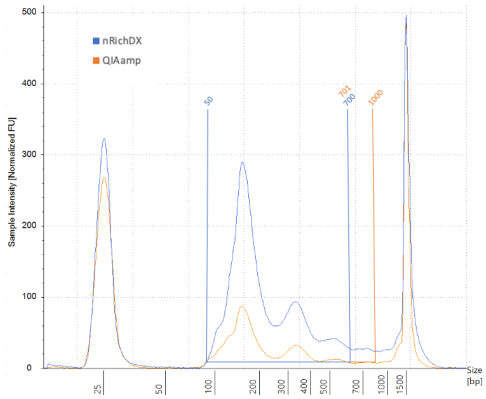


Figure 1. The yield of cfDNA calculated by KRAS G12V mutation detection assay was 90% - 100% for the nRichDX cfDNA Isolation Kit and 52% - 92% for the QIAamp Circulating Nucleic Acid Kit, respectively. Each kit is the result of 4 samples performed in duplicate. A two-tailed t-test gave a P-value = 0.0050; the difference was statistically significant by conventional criteria.



cfDNA Extraction Kit	Base Pair Range (bp)	Concentration (pg/µL)
nRichDX	50-700 (cfDNA)	963
	701 - 1000 (gDNA)	47.3
QIAamp	50 - 700 (cfDNA)	371
	701 - 1000 (gDNA)	18.5

Figure 4. TapeStation electropherogram tracings of 20mL samples from the nRichDX and QIAamp extraction kits. The concentration of the nRichDX extraction kit was about three times higher than the QIAamp extraction kit.

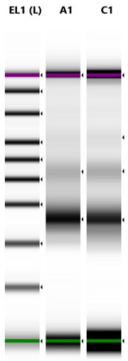


Figure 5. Gel electrophoresis indicates the tracings of the cfDNA standard monomer, dimer, and trimer. Column EL1 is the electronic ladder, A1 is the nRichDX 20mL urine sample, and C1 is the QIAamp 20mL urine sample.

CONCLUSION

The nRichDX cfDNA Isolation Kit and QIAamp Circulating Nucleic Acid Kit successfully extracted cfDNA from urine samples. The nRichDX cfDNA Isolation Kit extracted cfDNA from large urine volumes (20mL) with percent recoveries of ~95% while maintaining high precision. Due to the maximum sample volume constraint, the QIAamp Circulating Nucleic Acid Kit was able to extract from smaller urine volumes (4mL) with percent recoveries of ~80% but lost precision and yield compared to extractions from the nRichDX method. Loss in precision and yield could be due to transfer steps and eluant pooling. Both kits showed minimal genomic DNA contamination, but the concentration (between 50bp – 700bp) via TapeStation of the nRichDX cfDNA extraction kit was almost three times higher than the QIAamp extraction kit. Although these two kits are comparable in total DNA yield by Qubit, the nRichDX cfDNA Isolation Kit outperforms the QIAamp Circulating Nucleic Acid Kit in sample volume input, percent recovery, precision, and concentration of cfDNA in the region of 50bp-700bp.