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

Mayer Saidian, Jason Saenz, Cory Arnold, Jackson Uriu, Lauren Lee, Tom Curtis, and Nafiseh Jafari
nRichDX Inc., 15339 Barranca Parkway, Irvine, California, 92618

INTRODUCTION

Liquid biopsies are emerging as an alternative to conventional tissue biopsy. This is a relatively minimally invasive procedure for detecting and monitoring cancer by analyzing biomarkers such as cell-free DNA (cfDNA), cell-free RNA (cfRNA), and circulating tumor cells (CTC).

Tumor cells can secrete RNAs into the circulatory system, and these secreted RNAs are closely related to tumor proliferation and metastasis. The exact role of RNAs in circulation remains unclear. However, it is becoming a thriving method for early cancer detection and identification of cancer's origins. Isolation of cfRNA from biofluids is challenging due to its scarcity in samples, specifically in urine.

Urine is becoming one of the most favorable sample types for liquid biopsy because it can be easily collected in large volumes.
Many RNA extraction kits are limited to small sample volumes (≤5mL), making it challenging to extract high enough yields needed for downstream testing. The nRichDX Total Nucleic Acid Kit can extract high-quality cfRNA from large urine samples (up to 20mL) without losing cfRNA yield. This workflow has no transfer steps or eluant pooling, and eluants are ready for downstream applications such as RT-qPCR, TapeStation®, and RNA-seq.

MATERIALS & METHODS

cfRNA extractions were performed using the nRichDX Total Nucleic Acid Kit and Qiagen® QIAamp® Circulating Nucleic Acid Kit. Urine samples were collected and then processed at 16,000 x g for 10 minutes at 4˚C. cfRNA was extracted from eight 16mL urine samples using the nRichDX Total Nucleic Acid Kit. Four samples were spiked with RNA standard containing the KRAS G12V mutation at a concentration of 10ng/mL. All eight urine samples were eluted in 80μL.

The QIAamp Circulating Nucleic Acid Kit can extract RNA from up to 4mL urine samples. Therefore, eluates from four 4mL urine samples were pooled together to emulate a 16mL urine sample extraction. Twelve samples were spiked with a cfRNA standard at a concentration of 10ng/mL. All samples were eluted in 20μL and then pooled together to bring the eluants to 80μL to match the elution volumes of the nRichDX urine samples.

A one-step multiplex RT-qPCR assay determined the log copies per reaction of the HBS1L gene and the percent recovery of the KRAS G12V mutation. The quality of extracted cfRNA was determined using the Agilent High Sensitivity RNA ScreenTape®.

RESULTS

Figure 1. The log copies per reaction of the housekeeping gene HBS1L from the nRichDX Total Nucleic Acid Kit and the QIAamp Circulating Nucleic Acid Kit. The two-tailed t-test P-value = 0.2; by conventional criteria, this difference was not statistically significant.

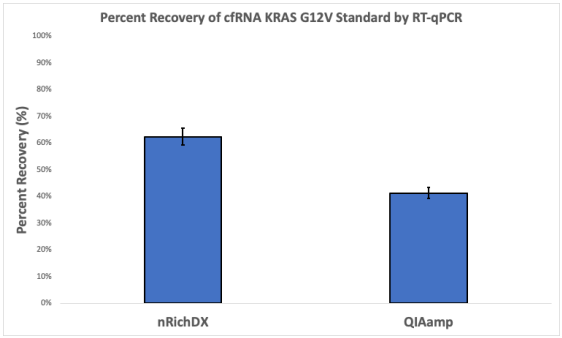

Figure 2. The percent recovery of cfRNA standard calculated by KRAS G12V mutation detection assay was 55% - 68% for the nRichDX Total Nucleic Acid Kit and 39% - 42% for the QIAamp Circulating Nucleic Acid Kit, respectively. The two-tailed t-test P-value = 0.0004; by conventional criteria, this difference was found to be statistically significant.

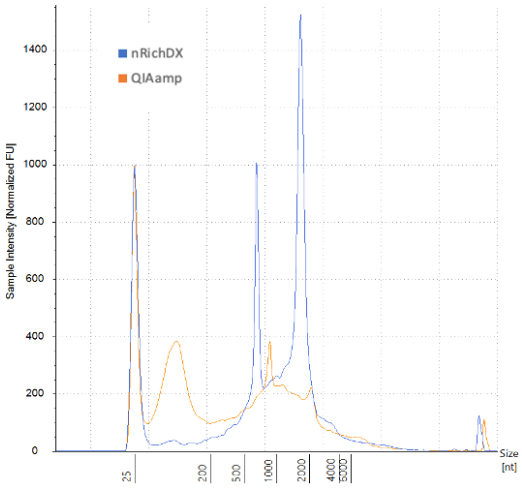

Figure 4. TapeStation electropherogram tracings of 16mL samples from the nRichDX and QIAamp extraction kits. The nRichDX extraction kit shows concise peaks and more than double the sample intensity compared to the QIAamp extraction kit. The peak at ~150bp in the QIAamp tracing is likely due to the RNA carrier used in the extraction process.

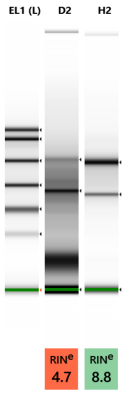

Figure 5. Gel electrophoresis indicates the cfRNA standards size (bp) tracings and RIN score. Column EL1 is the electronic ladder, D2 is the QIAamp extraction kit, and H2 is the nRichDX extraction kit.

CONCLUSION

RNA was extracted using the nRichDX Total Nucleic Acid Kit and QIAamp Circulating Nucleic Acid Kit. The two kits were comparable when extracting endogenous RNA (HBS1L). However, nRichDX recovered significantly more spiked RNA (KRAS G12V mutation) than QIAamp. TapeStation tracings showed that the nRichDX recovered more than double that of QIAamp extraction kit. The quality of the RNA, calculated by the RIN score, was 8.8 for nRichDX and 4.7 for QIAamp. These data indicates that the nRichDX Total Nucleic Acid Kit can extract more RNA with higher quality, and therefore, more mutations per sample volume can be detected. This will lead to more accurate data obtained from patients' samples using the nRichDX Total Nucleic Acid Kit and assist the physician in making better patient decisions.