Assessment of the nRichDX Revolution™ instrument and isolation kit for cell-free DNA extraction from liquid biopsy

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INTRODUCTION

Analysis of circulating cell-free DNA (cfDNA) in plasma has emerged as a tool for monitoring cancer patients because of the relative ease, comparative non-invasiveness, and increased frequency to obtain liquid biopsy compared to tissue biopsy. The liquid biopsy market is one of the fastest growing segments (CAGR ~23%) in diagnostics, with a total market estimate of $28.6 billion in 2022. Established criteria for suitable samples for cfDNA analysis are high, with isolation efficiency and total yield essential factors. Several isolation kits are commercially available, yet most of these share a common drawback of limited input volume (0.1mL- 5mL) that they can process, impacting detection of rare or low level targets. The nRichDX product looks to resolve this issue by allowing extraction from volumes of up to 50ml plasma, using a semi-automated system that combines extraction, enrichment, and concentration of cfDNA without the need for transfer steps. Here, we share our experience with the nRichDX Revolution™ instrument and isolation kit, compare its extraction efficacy and yield to the QIAamp Circulating Nucleic Acid Kit (Qiagen), and explore the benefits in the unique feature of extracting increased input plasma volumes.

METHODS

We compared the efficiency and yield (Qubit 3.0) of cfDNA extraction by the nRichDX Revolution cfDNA Isolation Kit to the QIAamp Circulating Nucleic Acid Kit using 5mL normal human plasma. We next compared extractions of 5mL and 15mL normal human plasma and then extractions using 5mL and 15mL cancer patient plasma using the nRichDX method. We performed quality assessment of extracted cfDNA with the LabChip GX Touch Nucleic Acid Analyzer.

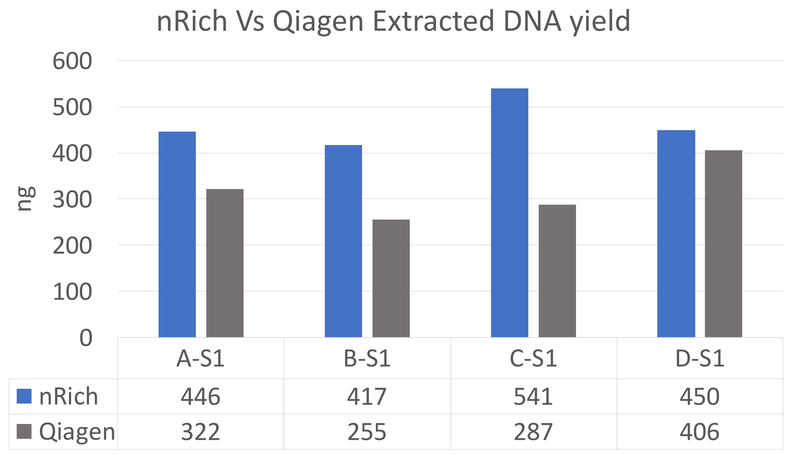
Study1: Cross platform evaluation. Compare nRichDX vs QIAamp extraction protocols. Assessment of DNA concentration yield and quality.

Study 2: Evaluation of extraction from different starting input plasma volumes of 5ml and 15ml. Assessment of DNA concentration yield and quality.

Figure 1. nRichDX Revolution™ instrument

RESULTS

Study1:
Two aliquots of 5ml plasma from 4 plasma pools spiked with a BRAF mutant fragment were extracted for cfDNA and averaged 463.35 ng for nRich and 317.76 ng for Qiagen. qPCR mutant BRAF detection was confirmed in samples from both methods.



	A-S1	B-S1	C-S1	D-S1
nRich	446	417	541	450
Qiagen	322	255	287	406

Figure 2. Bar chart of cfDNA concentration extracted from 5ml of plasma pools A-S1 through D-S1 with either Qiagen or nRichDX.

The cfDNA extracted from 5ml of plasma by nRich and QIAamp was then assessed for purity and quality using the LabChip GX Touch Nucleic Acid Analyzer. The spectrograph traces shown in Figure 3 show similar patterns with a large fragment peak size of 170bp, the expected size of cfDNA, and smaller low level peaks above 300bp, that likely represent non cfDNA fragments. The two extraction methods thus appear comparable in performance in terms of quality of cfDNA product.

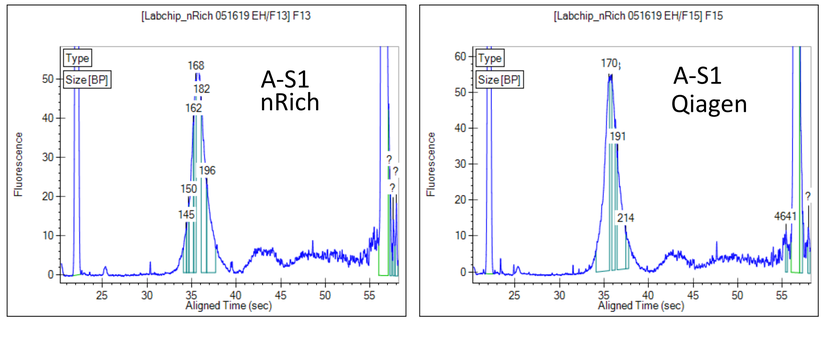
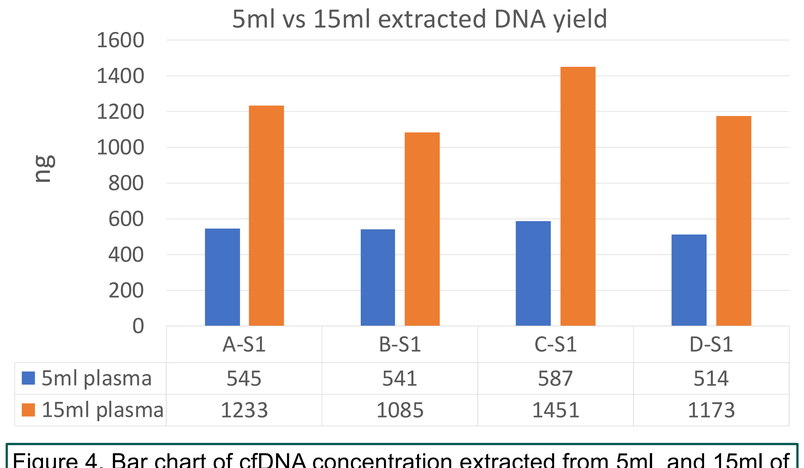


Figure 3. Spectrograph traces showing fragment size distribution of extracted cfDNA from 5ml of plasma pool A-S1 using either nRichDX or Qiagen and analyzed on the LabChip GX Touch Nucleic Acid Analyzer.

Study 2:
Two aliquots of 5ml and 15ml plasma were extracted using the nRichDX and gave an average of 546.78 ng and 1235 ng cfDNA, respectively. cfDNA extracted from 15ml of cancer patient plasma averaged a near 2.5-fold increase in yield over cfDNA extracted from 5ml of plasma. The increased volume of 15ml plasma resulted in an extracted product enriched in the target cfDNA (100-200bp), however an increase of non-cfDNA sized fragments (>300bp) was also seen.



	A-S1	B-S1	C-S1	D-S1
5ml plasma	545	541	587	514
15ml plasma	1233	1085	1451	1173

Figure 4. Bar chart of cfDNA concentration extracted from 5ml and 15ml of plasma pools A-S1 through D-S1

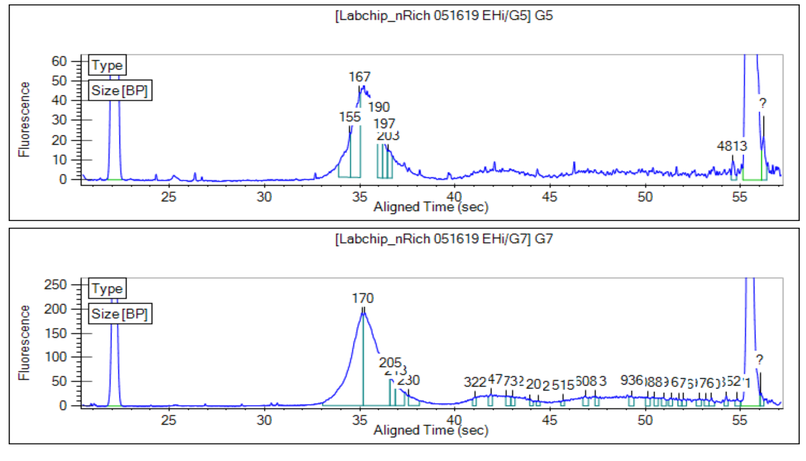


Figure 5. Spectrograph traces showing fragment size distribution of extracted cfDNA from 5ml (top panel) and 15ml (bottom panel) of plasma pool A-S1 using nRichDX and analyzed on the LabChip GX Touch24 Nucleic Acid Analyzer.

CONCLUSIONS

The nRichDX product provides a simple solution to the well-known problem of how to increase the yield of cfDNA extracted from liquid biopsy for downstream analysis by allowing an increased input volume of sample to be extracted.