The nRich^{DX} Revolution Sample Prep System™ enables recovery of more amplifiable copies and more cancer fraction detection from cfDNA as evaluated on the MassARRAY® System

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Nafiseh Jafari[1], Mayer Saidian[1] and Darryl Irwin[2]

[1] nRichDX | [2] Agena Bioscience

njafari@nrichdx.com, Darryl.Irwin@agenabio.com

INTRODUCTION

Liquid biopsies utilize cell-free DNA (cfDNA) from plasma or urine across various applications, including cancer detection, treatment selection, and minimal residual disease monitoring. The plasma of early-stage cancer patients contains meager amounts of cfDNA, which is challenging for correct diagnosis. These low yields can lead to the inability to detect amplifiable copies of cancerous biomarkers in downstream applications such as NGS and qPCR. Agena® Bioscience utilizes the MassARRAY system that can detect cancer variants as low as 0.1% allele frequency allowing much higher sensitivity for detecting rare cancer biomarkers.

The nRich^{DX} Revolution system is designed to isolate cell-free DNA from 1mL to 20mL of human plasma. Extracting cfDNA from large sample volumes may increase the amplifiable copies of cancerous biomarkers, leading to higher sensitivity in downstream applications.

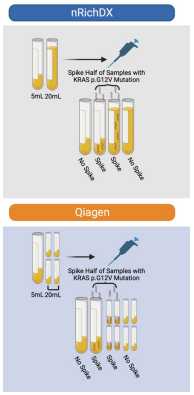

Figure 1. Design of experiment for cfDNA extraction from 5 mL and 20 mL of plasma using nRich^{DX} and Qiagen kits.

METHODS

Whole blood from healthy donors was collected in K2EDTA tubes, and plasma was isolated and then pooled in preparation for cfDNA extraction. 5mL and 20mL plasma samples were extracted using the nRich^{DX} Revolution Max20 cfDNA isolation kit and the Qiagen QIAamp Circulating Nucleic Acid kit. Half of all samples were spiked with a cfDNA reference standard containing KRAS p.G12V mutation to a final concentration of 20ng/mL. The nRich^{DX} system followed the Revolution Max20 cfDNA isolation kit IFU for 5mL and 20mL plasma samples and was eluted in 50µL. The QIAamp kit is limited by its max sample volume of 5mL. To replicate the 20mL sample using the QIAamp Circulating Nucleic Acid kit, we extracted 5mL samples following their protocol, eluted in 50µL, then pooled the eluants together and concentrated the pooled eluant down to 50µL using the Amicon Ultra Centrifugal filter Device.

The amplifiable cfDNA copies, high/low molecular weight dynamic, and the overall cfDNA quantity and quality were assessed using the LiquidIQ® panel on the MassARRAY system. Recovery and frequency of the cfDNA Reference Standard were determined with the UltraSEEK™ Lung V2 Panel.

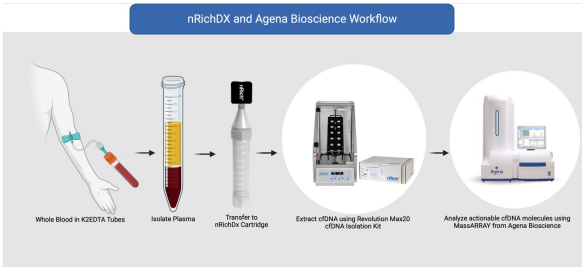

Figure 2. nRich^{DX} and Agena's workflow to isolate cfDNA and determine amplifiable copies and the overall cfDNA quantity and quality.

RESULTS

The amplifiable copies from the nRich^{DX} and Qiagen extraction kits, respectively, were: 5mL non-spiked 517±36 vs. 398 ±17, 5mL spiked 946±99 vs. 732±73; 20mL non-spiked 1526±26 vs. 1071±55, and 20mL spiked 3004±278 vs. 1777±207. All results were statistically significantly different via a two-sample t-test. KRAS p.G12V mutation was recovered consistently from all samples with a mutation frequency of 5.3±0.3 and a mutation significance z-score of 296±19.

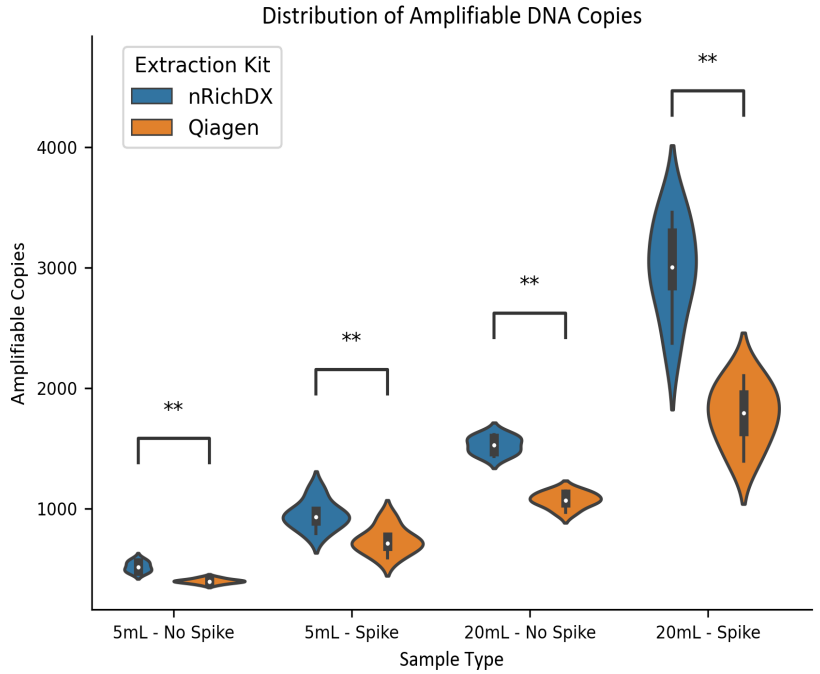

Figure 3. Total amplifiable copies were analyzed by LiquidIQ panel separated by extraction kit and sample volume. The distribution of the amplifiable DNA copies extracted from samples using the Revolution Max20 cfDNA isolation kit and Qiagen QIAamp Circulating Nucleic Acid kit was statistically significantly different at all sample volumes (p-value <0.05).

CONCLUSION

This study evaluated the difference between the quality of cfDNA extracted with two extraction kits while testing the amplifiable DNA copies. cfDNA extracted with nRichDX demonstrated statistically significantly higher amplifiable copies than Qiagen in all conditions (p-value < 0.05). This indicates that the nRich^{DX} system can extract more actionable DNA, which is the main factor for cancer diagnosis. The MassARRAY system's specificity and high sensitivity allow for earlier cancer detection, leading to more precise diagnoses for early-stage cancer patients.

The combination of the nRichDX system's ability to efficiently extract cfDNA from large sample volumes and Agena Bioscience's MassARRAY system can lead to the very early detection of various cancer biomarkers.