Enhancing Sensitivity of ctDNA Copy Number Detection by Increasing Plasma Sample Volume

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nRich[DX] POWERING LIQUID BIOPSY

INTRODUCTION

Liquid biopsies are pivotal in oncology for detecting actionable genetic mutations, including KRAS G12V. Detecting these mutations at low concentrations is often challenging, necessitating improved methods to enhance sensitivity. Early detection and monitoring of minimal residual disease (MRD) are particularly critical when ctDNA is not abundant. This study explores the impact of increasing plasma sample volume on the sensitivity of detecting KRAS G12V mutations using a PCR assay and the Revolution cfDNA Max 20 kit from nRichDX. The nRichDX cfDNA Max 20 kit can process four tubes of blood or 20 mL plasma sample volume in one extraction due to its novel engineered nRicher cartridge solution. The nRicher cartridge streamlines the process, ensuring higher yield and efficiency, unlike other cfDNA extraction methods that require additional transfer steps, aliquoting, fluid volume concentration, and sample pooling, which leads to ctDNA loss.

MATERIALS & METHODS

Blood was collected in K2EDTA tubes and processed following the nRichDX centrifugation protocol for obtaining cell-free plasma from whole blood. Using the nRichDX Revolution cfDNA Max 20 kit, cell-free plasma samples were extracted at volumes ranging from 1 mL to 20 mL (1 mL, 3 mL, 5 mL, 8 mL, 10 mL, 13 mL, 15 mL, 17 mL, and 20 mL). Each extracted sample volume had four technical replicates. All samples were spiked with a cfDNA Reference Standard containing the KRAS G12V mutation to a concentration of 10 copies/mL and eluted in 50µL. The quality of extracted cfDNA was determined using the Agilent cfDNA ScreenTape. Total DNA yield (ng) was determined via dsDNA High Sensitivity Qubit and the extracted actionable cfDNA copies analyzed using a PCR assay to quantify the number of KRAS G12V copies accurately extracted.

RESULTS

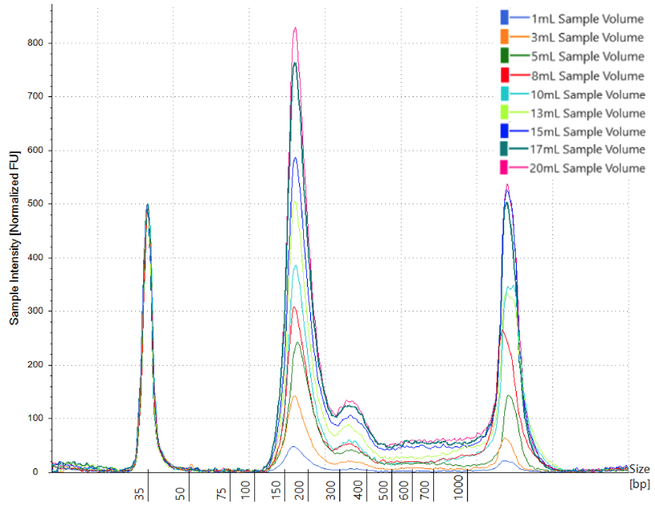

Figure 1. TapeStation overlay displaying electropherogram traces, highlighting the cfDNA intensity and base-pair length for each extracted sample volume. The overlay shows a linear increase in cfDNA intensity, providing clear insights into sample quality and yield across different volumes.

#Blood Tubes				
Plasma Vol (mL)	1 - 5	8 - 13	15 - 17	20
Copies Spiked	10 - 80	100 - 130	150 - 170	200
Mean Ct	Undetermined - 35.2 ± 0.6	34.4 - 34.3 ± 0.1	34.2 - 34.0 ± 0.4	33.7 ± 0.7

Table 1. The mean Ct values corresponding to plasma input demonstrate that increasing plasma volume enhances detection sensitivity. This highlights the significance of plasma volume, as copy detection via qPCR is not possible with volumes under 3mL.

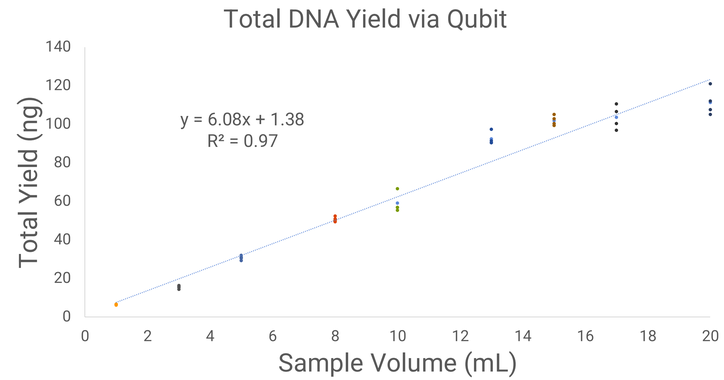

Figure 2. The total DNA yield from each sample volume was measured using the dsDNA High Sensitivity Qubit assay. The graph points represent each technical replicate and their respective mean total yield, indicating that cfDNA yield increases with higher sample volume input, potentially enhancing assay sensitivity.

CONCLUSION

Extracting cfDNA from small-volume plasma samples poses challenges in achieving sufficient actionable cfDNA. Increasing the initial sample volume significantly enhances the capture of total and actionable cfDNA. TapeStation analysis showed that larger sample volumes correlate with increased sample intensity while maintaining cfDNA quality. qPCR results revealed that actionable cfDNA was undetectable in samples below 5 mL, with larger volumes leading to significant increases in detection. Qubit analysis demonstrated a strong correlation ($R^2 = 0.97$) between total DNA recovery and sample volume, indicating linear extraction efficiency as sample volume increases.

The nRichDX Revolution cfDNA Max 20 kit effectively captures more actionable cfDNA molecules by accommodating larger sample volumes, unlike competitor kits often limited to 5 mL or less. Drawing more blood from patients enhances cfDNA yield, thereby improving diagnosis and treatment response and potentially reducing assay failures due to insufficient analyte quantity, while reducing the need for multiple blood draws, minimizing discomfort, and improving the patient experience.