High-Volume cfDNA Extraction Enables Rare Variant Recovery in Liquid Biopsy for Early Cancer Detection

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nRichDX POWERING LIQUID BIOPSY

INTRODUCTION

The ability to detect rare circulating tumor DNA (ctDNA) variants in cell-free DNA (cfDNA) is a cornerstone of liquid biopsy approaches for early cancer detection, treatment monitoring, and minimal residual disease (MRD) assessment. Sensitivity, however, is limited when working with low-yielding biofluids such as urine, pleural fluid, and peritoneal fluid, where nucleic acid concentrations are inherently low. Clinical studies increasingly highlight that maximizing input volume can mitigate these challenges, yet many extraction workflows are constrained by low-volume capacity and variability in recovery when scaled. Optimizing recovery across large volumes is therefore essential to extend liquid biopsy into sample types and clinical contexts that would otherwise be inaccessible.

MATERIALS & METHODS

To model low-yield scenarios, healthy human plasma was spiked with 0.01 ng/mL (10 copies/mL) of a cfDNA reference standard carrying the KRAS G12V mutation. Volumes of 1 mL, 5 mL, 10 mL, 20 mL, and 50 mL were processed, and recovered cfDNA was quantified by qPCR. Although plasma was used here as a surrogate matrix due to availability, the study design reflects the conditions faced when analyzing low-abundance clinical samples such as urine.

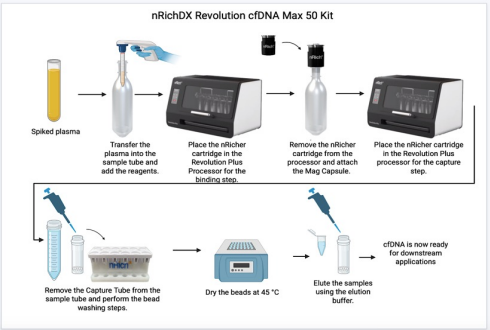

Figure 1. nRichDX Revolution cfDNA Max 50 Kit workflow.

RESULTS

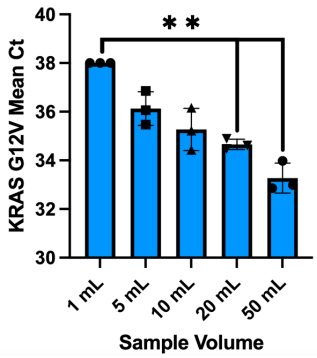

Figure 2. KRAS G12V qPCR mean Ct values as a function of plasma input volume (1, 5, 10, 20, and 50 mL), each spiked at 10 copies/mL. Increasing input volume consistently lowers Ct, with 50 mL producing the strongest signal (significantly lower Ct) compared to smaller input volumes. Error bars represent replicate variation.

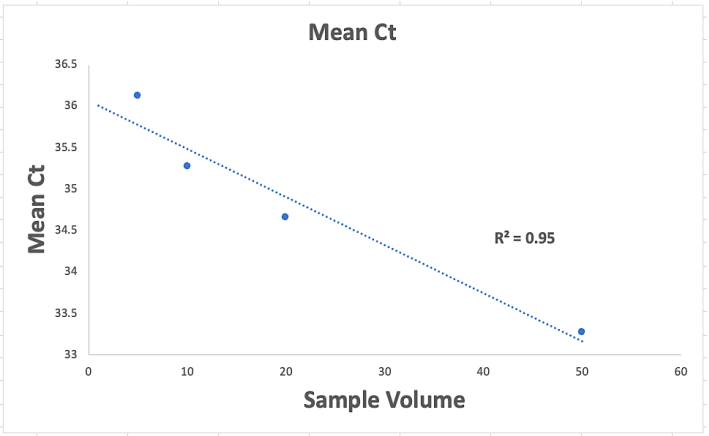

Figure 3. Mean Ct for KRAS G12V vs Plasma input volume (5-50 mL). A linear fit gives R^2= 0.95, indicating a strong, predictable scaling of detected signal with increasing sample volume.

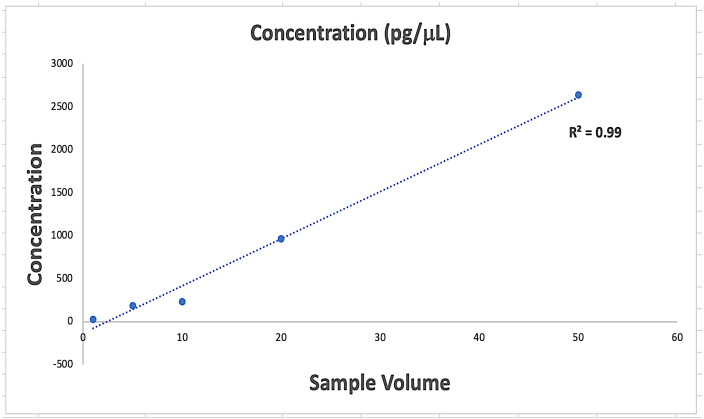

Figure 4. Qubit-measured cfDNA concentration (pg/µL) versus input volume. Linear regression gives R^2= 0.99, indicating near-perfect proportionality of measured concentration with increasing sample volume.

CONCLUSION

KRAS G12V was not detected at 1 mL and was only marginally detected at 5 mL (Ct=36.12). Sensitivity improved with larger inputs: 10 mL (Ct =35.27), 20 mL (Ct =34.51), and 50 mL (Ct =33.27). The ~2.85-cycle shift from 5 to 50 mL corresponds to roughly 7-8x higher detected copies ($2^{\Delta CT}$), indicating greater recovery of rare alleles. Recovery efficiency quantified both by Qubit and qPCR was consistent across volumes, demonstrating scalability without loss of performance (R^2= 0.99).

Our findings demonstrate that high-volume cfDNA extraction significantly improves recovery of rare ctDNA variants, particularly in contexts where input material is limited. These results have direct clinical implications for early cancer detection and MRD monitoring in challenging fluids such as urine, pleural, and peritoneal effusions. High-volume liquid biopsy enables the analysis of low-yield samples, thereby broadening the reach of noninvasive cancer diagnostics to a diverse range of patients.