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Enhanced sensitivity and scalability in liquid biopsy through integrated high-volume cfDNA extraction and droplet digital PCR mutation detection

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INTRODUCTION

Detecting rare tumor-derived variants in plasma requires both efficient recovery of cell-free DNA (cfDNA) and highly quantitative mutation analysis. Conventional extraction methods are limited by low input capacity and potential bias toward longer fragments, which can compromise sensitivity for low-frequency alleles.

MATERIALS & METHODS

Healthy human plasma was thawed at room temperature and centrifuged; the supernatant was combined and thoroughly mixed prior to aliquoting. cfDNA was extracted from plasma volumes of 1 mL, 5 mL, 10 mL, and 15 mL (each in triplicate) using the nRichDX Revolution cfDNA Max20 Kit. The Qiagen QIAamp Circulating Nucleic Acid Kit was used in parallel following the manufacturer's instructions, including the addition of 1 µg carrier RNA. DNA concentration was quantified by Qubit fluorometry, and ddPCR. Contrived cfDNA samples were prepared using RMD plasma spiked with sheared wild-type DNA (200 bp) and mutant gBlocks (EGFR E746_A750delELREA (COSM6223) and KRAS G12C (COSM516), 500 bp) at a nominal variant allele frequency (VAF) of 1% (30,000 wild-type and 300 mutant copies/mL plasma). Extracted aliquots of 5 µL or 10 µL were used in ddPCR assays for EGFR and KRAS targets.

RESULTS

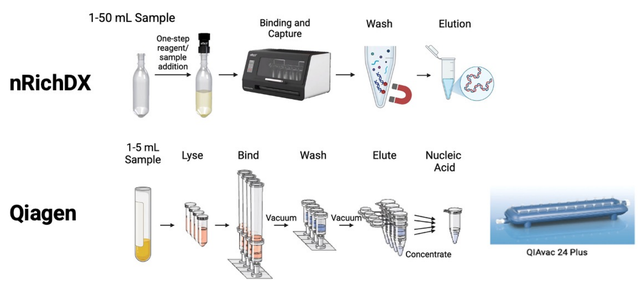

Figure 1. Comparison of cfDNA extraction workflows. The nRichDX platform supports a streamlined, high-volume workflow with minimal hands-on time and no transfer steps. In contrast, the Qiagen workflow requires multiple manual handling and transfer steps and is limited to lower sample input volumes.

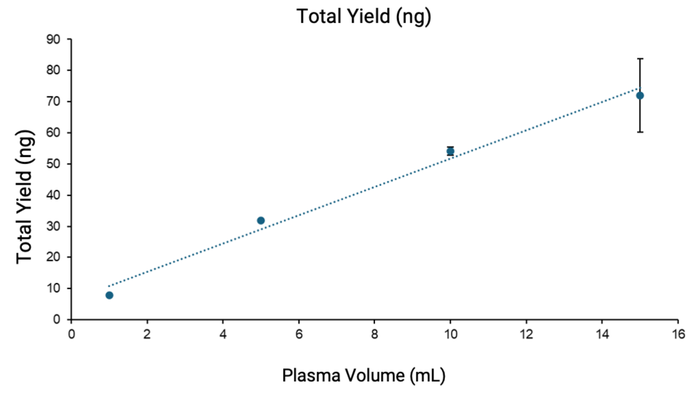

Figure 2. Total yield (ng) vs plasma volume. Qubit fluorometric quantification was performed on human plasma samples processed at 1 mL, 5 mL, 10 mL, and 15 mL input volumes following extraction with the nRichDX Revolution cfDNA Max20 Kit. The results show strong linearity across input volumes ($R^2 = 0.98$).

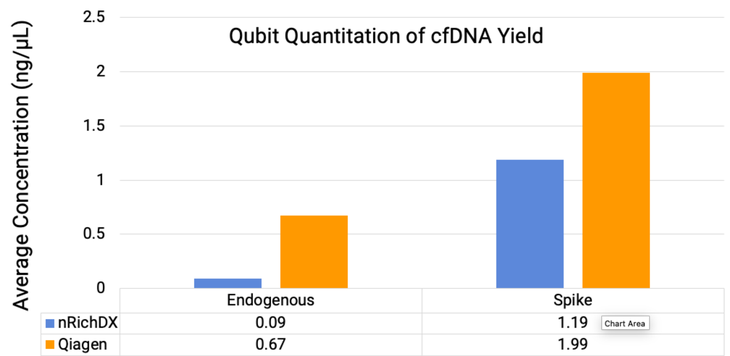

Figure 3. Mean cfDNA concentrations (± SD, n = 3) for both extraction methods. Qubit fluorometric quantification show higher overall nucleic acid readings in Qiagen eluates. Similar ddPCR concentrations of the mutant gBlocks indicate the increased nucleic acid readings are due to the inclusion of carrier RNA during extraction.

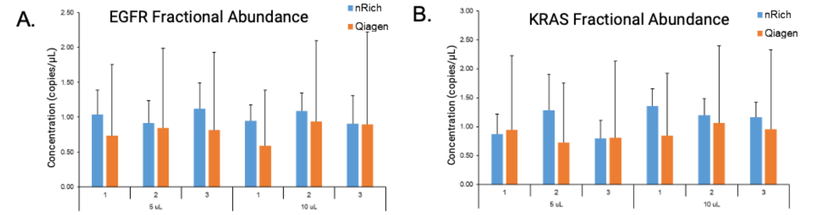

Figure 4. Improved mutation detection sensitivity with nRichDX. Contrived plasma samples containing 1% KRAS and EGFR mutant gBlocks were analyzed by Bio-Rad ddPCR using 5 µL and 10 µL template inputs. For both (A) EGFR and (B) KRAS, samples extracted with nRichDX tended to be closer to the nominal fractional abundance of 1% with smaller error bars compared with samples extracted using Qiagen.

CONCLUSION

The combined workflow of the nRichDX Revolution Sample Prep System and the Bio-Rad Droplet Digital™ PCR (ddPCR) platform delivers a powerful, scalable, and highly reproducible solution for cfDNA extraction and mutation detection in liquid biopsy applications. Across multiple plasma volumes, the nRichDX system demonstrated linear cfDNA recovery ($R^2 = 0.98$), enhanced detection sensitivity for EGFR and KRAS mutations, and accurate representation of actionable cfDNA molecules compared with Qiagen. The simplified one-step reagent addition and automated magnetic capture technology minimizes manual handling and enables processing of up to 50 mL of plasma in a single cartridge—eliminating the need for multiple parallel extractions or reconcentration steps associated with Qiagen. The nRichDX workflow consistently provided cfDNA that translated into more reliable ddPCR quantification than Qiagen. Together, these results establish the nRichDX–Bio-Rad integrated workflow as an advanced, end-to-end solution that improves analytical sensitivity, scalability, and workflow efficiency for translational and clinical research in liquid biopsy.