High-Volume (50 mL) Urine Input Outperforms Lower Volumes in Liquid Biopsy cfDNA Detection of Low-Frequency Mutations

Nafiseh Jafari, Jason Saenz, Carlos Hernandez, Daniel Cedeno, Cameron Van Dieren and Mayer Saidian nRich^{DX} Inc., 15339 Barranca Parkway, Irvine, CA 92618

nRich^{DX} POWERING LIQUID BIOPSY

INTRODUCTION

Urine represents a non-invasive and easily obtainable specimen type for molecular diagnostics, offering greater convenience and lower patient burden compared to blood-based collection. However, its utility for mutation detection has historically been constrained by the low abundance of circulating cell-free DNA (cfDNA) present in urine. This limitation poses challenges, particularly in applications requiring high analytical sensitivity such as early-stage cancer detection and molecular residual disease (MRD) surveillance. One potential strategy to overcome this sensitivity barrier is to increase urine input volume during the cfDNA extraction process.

MATERIALS & METHODS

To investigate the impact of urine input volume on the sensitivity of mutation detection, contrived samples were generated by spiking a KRAS G12V cfDNA reference standard into pooled urine from healthy donors. Input volumes of 10 mL, 20 mL, and 50 mL were tested in triplicate. Each volume was spiked with the same concentration—approximately 4 mutant copies per mL—yielding total expected mutant loads of 40, 80, and 200 copies, respectively. Samples were preserved using EDTA-Tris buffer (pH 8.0), centrifuged to remove debris, and processed for cfDNA extraction using the nRichDX Revolution cfDNA Max 50 Kit. Total cfDNA concentration was quantified using Qubit, and mutation copy number was measured using KRAS G12V mutation-specific qPCR.

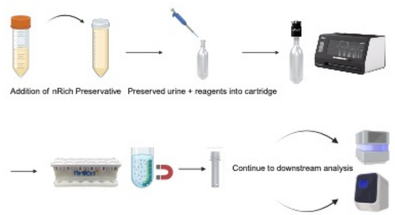

Figure 1. nRichDX Revolution cfDNA Kit Max 50 workflow

RESULTS

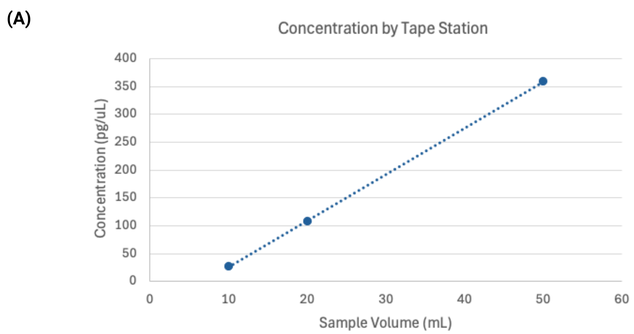

Figure 2. cfDNA yield scales with input volume. (A) Agilent TapeStation concentrations (pg/µL) for 10, 20, and 50 mL inputs show strong linearity (R^2 = 0.90), demonstrating scalable performance of the Revolution cfDNA Max 50 kit without loss of sensitivity. (B) Qubit concentrations (ng/µL), converted to total yield per sample, also scale with input (R^2 = 0.90). Mean total yield: 10 mL = 11.0 ng (SD 3.17); 20 mL = 28.1 ng (SD 2.89); 50 mL = 63.5 ng (SD 1.32).

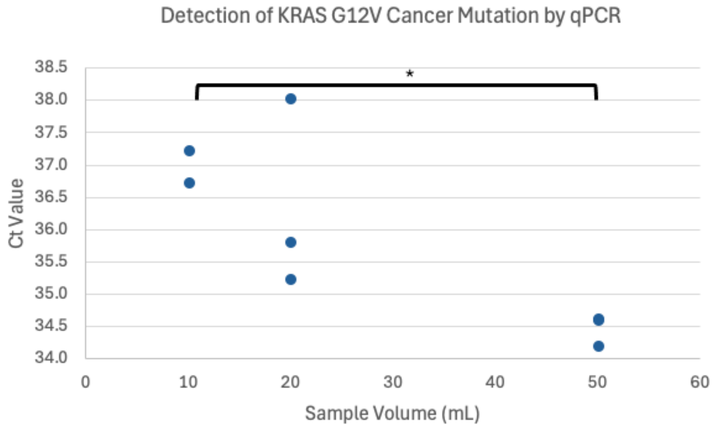

Figure 4. KRAS G12V qPCR performance by input volume. At matched near-LoD VAFs, the 50 mL input yields earlier Cts and tighter replicate spread than 10–20 mL, indicating higher sensitivity and precision. The 10 mL vs 50 mL comparison is statistically significant (P = 0.006); 20 mL vs 50 mL is not (P = 0.08). One 10 mL replicate failed to amplify (undetermined Ct).

CONCLUSION

Increasing urine input proportionally increased cfDNA yield and improved low-frequency mutation detection. Across all metrics, 50 mL outperformed 10–20 mL: KRAS G12V mean recoveries were 39, 72, and 199 copies for 10, 20, and 50 mL, respectively, with one of three 10 mL replicates failing to amplify (stochastic dropout). Ct values decreased with input—36.99 (10 mL), 36.36 (20 mL), and 34.48 (50 mL)—with 10 vs 50 mL reaching statistical significance (P = 0.006) and 20 vs 50 mL trending (P = 0.08). Total cfDNA scaled with volume (R^2 ≈ 0.90) while variability fell—0.2 ng/µL (CV 29%) at 10 mL, 0.6 ng/µL (CV 10%) at 20 mL, and 1.3 ng/µL (CV 2%) at 50 mL—and no qPCR inhibition was observed. These results support 50 mL as the most sensitive and consistent input for urine cfDNA workflows, well suited to clinical liquid biopsy applications, particularly early detection and MRD.