Enhanced cell-free RNA (cfRNA) recovery for liquid biopsy applications: Comparative analysis using controlled RNA samples for early and advanced cancer detection

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INTRODUCTION

Extracting cfRNA from biological samples is crucial in liquid biopsy applications, particularly for detecting low-frequency mutations like KRAS G12V, which is essential for early cancer diagnosis and minimal residual disease (MRD) monitoring. Traditional cfRNA extraction methods may involve transfer steps, aliquoting, and pooling, leading to cfRNA loss and affecting mutation detection sensitivity and accuracy. The nRichDX Revolution cfTNA Max 20 kit, with its innovative nRicher cartridge, simplifies the extraction process by eliminating these extra steps, potentially enhancing yield and efficiency. This study evaluates the cfRNA recovery efficiency of the nRichDX kit compared to the Qiagen Circulating Total Nucleic Acid kit.

MATERIALS & METHODS

Plasma samples (5 mL each) were spiked with RNA containing KRAS G12V mutation at concentrations ranging from 100 to 2 million copies, mimicking early-stage cancer detection and MRD monitoring, where low copy numbers represent early disease and high copy numbers reflect advanced cancer stages. cfRNA was extracted using the nRichDX and Qiagen kits, with each condition tested in triplicate. cfRNA recovery efficiency was assessed using quantitative reverse transcription PCR (qRT-PCR) to quantify KRAS G12V RNA copies recovered. High-sensitivity RNA ScreenTape Analysis was also used to evaluate cfRNA profile quality.

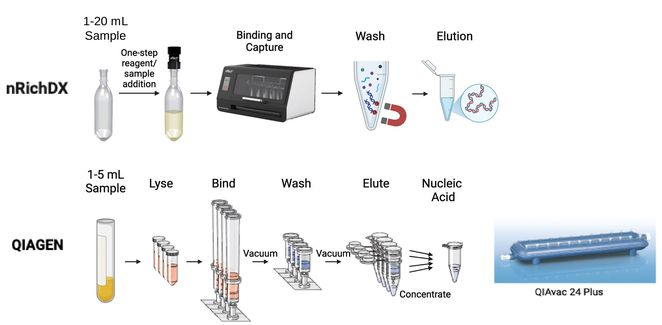

Figure 1. Workflow Comparison: nRichDX cfTNA Extraction Kit (Top) vs. Qiagen Circulating Nucleic Acid Kit (Bottom). The nRichDX Revolution system efficiently extracts larger volumes (up to 20 mL) compared to Qiagen. In contrast, the Qiagen workflow involves more complex steps, including sample transfers and using the QIAvac vacuum system.

RESULTS

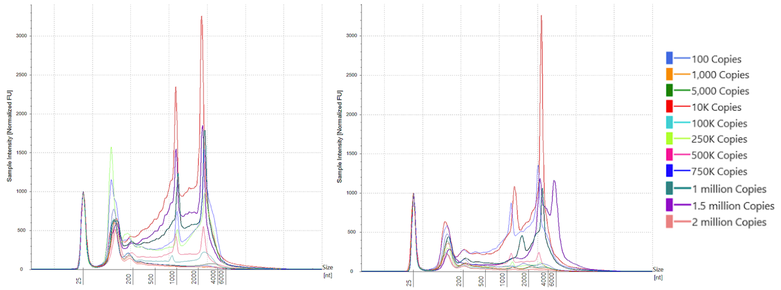

Figure 2. RNA integrity assessment using TapeStation High Sensitivity RNA ScreenTape Analysis. TapeStation electropherogram results revealed linear tracing and higher intensity for samples extracted with nRichDX, whereas Qiagen's samples showed less intensity and consistency.

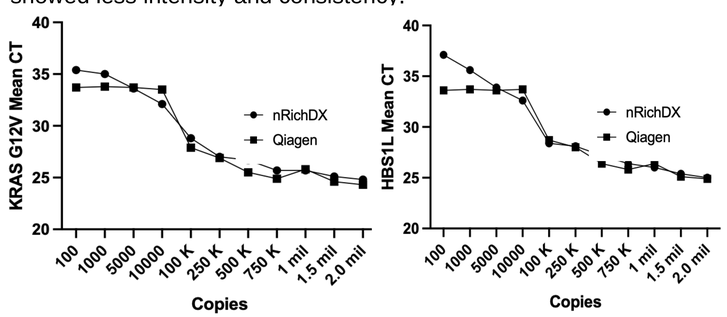

Figure 3. Mean Ct Values for KRAS G12V (Left) and HBS1L (Right) Using a KRAS G12V Mutation Detection Assay. qRT-PCR assay showed similar KRAS G12V and HBS1L mean Ct values for the nRichDX and Qiagen platforms.

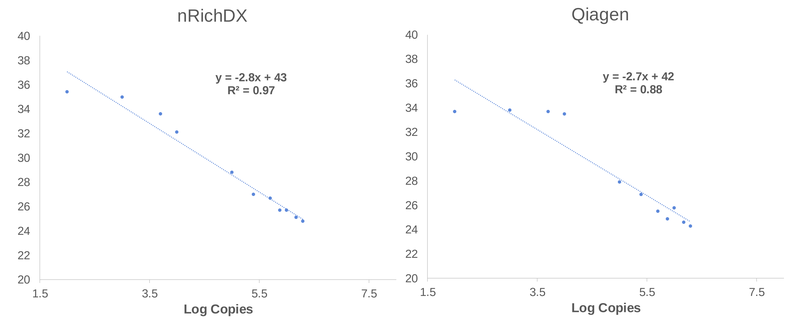

Figure 4. Linearity of KRAS G12V Mutation Copy Titrations from 100 to 2 Million Copies. nRichDX also demonstrated a higher linearity (R^2 = 0.97) for detecting KRAS G12V and HBS1L mean Ct values compared to Qiagen (R^2 = 0.88).

CONCLUSION

The nRichDX cfTNA kit demonstrates exceptional efficiency in extracting cfRNA from plasma across a broad dynamic range, from as few as 100 copies to as many as 2 million copies of the KRAS G12V mutation. Its robust performance makes it a valuable tool for liquid biopsy applications, enabling sensitive and accurate detection of rare mutations. Compared to Qiagen, the nRichDX platform offers a scalable and flexible workflow without using tube extenders, or multiple separate extraction pooling schema for input sample volumes greater than the 5 mL limit for QIAamp. TapeStation electropherogram results showed more consistent linear tracing and higher intensity for nRichDX-extracted samples, while Qiagen's samples were less consistent. qRT-PCR assays revealed similar KRAS G12V and HBS1L mean Ct values for both platforms; however, nRichDX exhibited greater linearity (R^2 = 0.97) compared to Qiagen (R^2 = 0.88). Although the RNA copy numbers were comparable between the two methods, nRichDX produced higher RNA quality and can scale up to 20 mL in a single extraction, which is advantageous for downstream applications requiring superior RNA yield and integrity.