

INTRODUCTION

Urine represents a promising, truly noninvasive biofluid for kidney cancer detection and disease monitoring. However, clinical adoption of urine cfRNA has been limited by low RNA abundance and the small sample volumes processed in standard extraction workflows. We evaluated a high-volume urine cfRNA extraction method (up to 20 mL) designed to improve analytical sensitivity for renal tumor-associated transcripts.

MATERIALS & METHODS

Fresh and preserved urine samples were contrived with both quantitative fragmented PRCC-TFE3 RNA and gene-specific synthetic RNA transcripts obtained from Anchor Molecular to evaluate extraction and detection performance under clinically relevant conditions. The study assessed the performance of the nRichDX Revolution cfTNA Max20 Kit in comparison with commonly used low-volume cfRNA extraction kits. Key analytical performance parameters included overall cfRNA recovery and downstream RT-qPCR sensitivity for detection of the clinically relevant renal cancer fusion biomarker PRCC-TFE3.



Figure 1. cfRNA Max 20 cfTNA workflow.

RESULTS

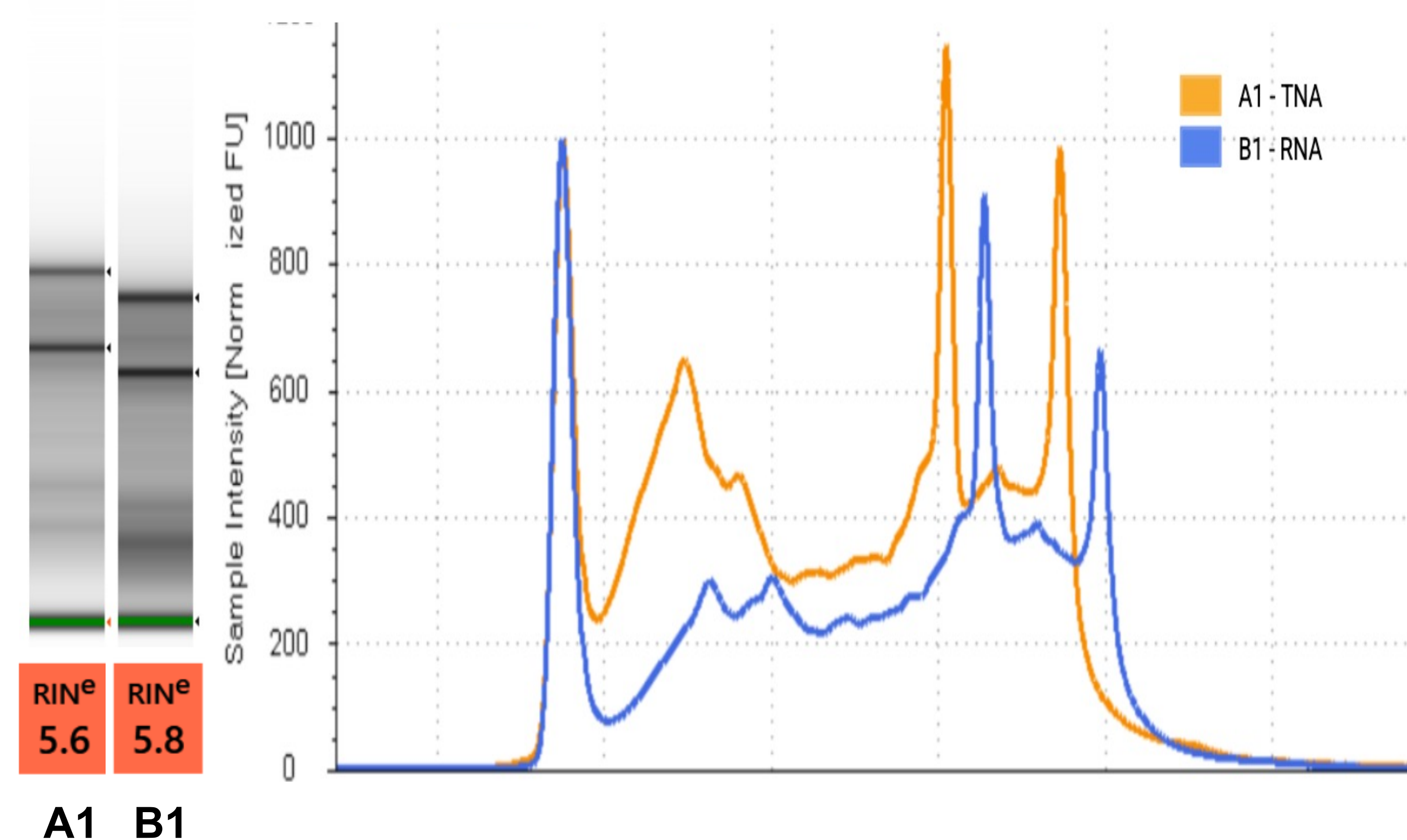


Figure 2. TapeStation analysis of urine samples spiked with PRCC-TFE3 and preserved with the nRichDX urine preservative showed clear and prominent 18S and 28S ribosomal RNA peaks, indicating effective RNA preservation and extraction.

Target	Mean Measured Copy Number
PRCC-TFE3	12,001 ± 2,425 copies/μL

Figure 3. Recovery of PRCC-TFE3 RNA spiked into urine at 10 ng/mL. Extraction and RT-qPCR analysis showed a mean measured concentration of 12,001 ± 2,425 copies/μL. These results demonstrate reproducible recovery of fragmented PRCC-TFE3 RNA from urine and support the suitability of the workflow for detection of low-abundance renal cancer-associated cfRNA targets.

CONCLUSION

Higher-volume urine processing, up to 20 mL, increased cfRNA yield and improved RT-qPCR detection of renal cancer-associated biomarkers relative to lower-volume workflows. PRCC-TFE3 spike-in experiments demonstrated reproducible and quantitative recovery, with a mean measured concentration of 12,001 ± 2,425 copies/μL supporting the robustness of the workflow for recovery of fragmented RNA targets in urine. In parallel, TapeStation analysis of preserved urine showed clear 18S and 28S peaks, indicating effective RNA preservation and supporting sample stability in the nRichDX urine preservative. Together, these data show that the workflow can support both target recovery and maintenance of RNA quality in preserved urine samples.

Overall, these findings demonstrate that the workflow provides sensitive and reproducible recovery of fragmented urinary cfRNA relevant to renal cancer biology. By improving recovery from larger urine inputs while maintaining RNA integrity, this approach may help overcome a key technical barrier in urine-based liquid biopsy, where low analyte abundance and RNA fragmentation often limit assay sensitivity. Enhanced recovery of urinary cfRNA may improve the reliability of renal biomarker detection and support future noninvasive applications in kidney cancer detection, treatment monitoring, and longitudinal disease surveillance.

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