

INTRODUCTION

Cell-free urine (cfUrine) is a valuable non-invasive medium for detecting biomarkers associated with various cancers such as prostate cancer. The challenge lies in efficiently extracting and preserving high-quality RNA from large volumes of cfUrine, such as 50 mL, which is crucial to reliable downstream analysis and assay sensitivity. This study highlights nRichDX's extraction methodology and unique nRicher Cartridge, which are tailored to address these challenges. Central to our analysis is using the Agilent TapeStation system with the High Sensitivity RNA ScreenTape assay, a powerful tool for assessing RNA integrity and quality, ensuring that the extracted RNA meets the stringent requirements for accurate testing.

MATERIALS & METHODS

Twelve 50 mL cfUrine samples were spiked with free RNA extracted from LnCAP cells containing the PTEN mutation. Extractions were performed using the nRichDX Revolution cfTNA Max 50 Kit. We also performed parallel extractions using the Zymo ZR Urine RNA Isolation Kit and Norgen Urine Cell-free Circulating and Viral Nucleic Acid Purification Maxi Kit for comparative purposes. To evaluate the quality and integrity of the extracted RNA, we employed the Agilent TapeStation system with the High Sensitivity RNA ScreenTape assay. The extracted RNA was further analyzed using a qPCR assay to confirm the presence of the PTEN mutation.

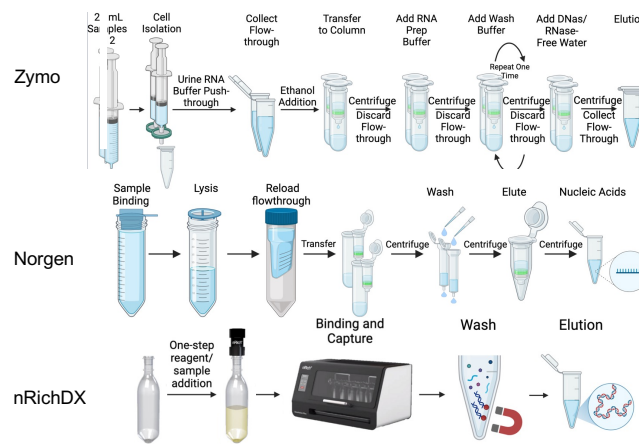


Figure 1. Workflow comparison: The nRichDX system offers a more streamlined and efficient workflow than Zymo and Norgen.

RESULTS

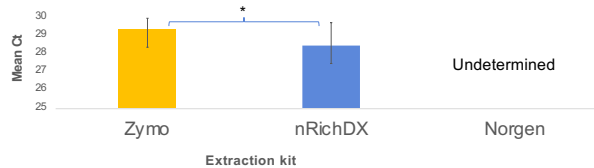


Figure 2. PTEN Mutation Detection: The nRichDX kit yielded a Ct value of 28.6 ± 1.3 , indicating effective recovery of the PTEN mutation. The Zymo kit showed a slightly higher Ct value at 29.4 ± 0.6 , while the Norgen kit did not produce a detectable Ct value, suggesting minimal or no recovery of the PTEN mutation.

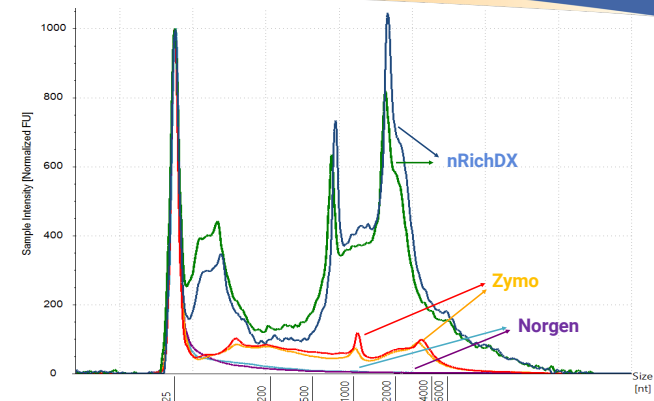


Figure 3. TapeStation electropherograms: nRichDX demonstrates strong 18S and 28S peaks with a RIN score above 7, indicating high RNA quality. Zymo shows weaker 18S and 28S peaks, while Norgen lacks visible peaks, illustrating nRichDX's superior RNA integrity.

CONCLUSION

This study demonstrates the superior performance of the nRichDX Revolution cfTNA Max 50 Kit as compared to the Zymo ZR Urine RNA Isolation Kit and the Norgen Urine Cell-Free Nucleic Acid Purification Maxi Kit. Uniquely, nRichDX can extract cfTNA from 50 ml of urine in a single extraction, unlike competing kits. The nRichDX kit achieved RIN scores of 7.6 indicating higher RNA integrity than Zymo (RIN 4.4) and Norgen (undetermined). Additionally, nRichDX's mean qPCR Ct value of 28.5 ± 1.3 is lower than Zymo's 29.4 ± 0.6 , demonstrating greater sensitivity in detecting clinically relevant mutations. In summary, nRichDX's kit provides efficient RNA extraction from larger urine volumes, superior RNA quality, and enhanced mutation detection, making it a valuable tool for non-invasive diagnostics.