

INTRODUCTION

Urine cfDNA offers a promising, non-invasive option for molecular profiling in breast cancer. However, challenges with cfDNA stability, yield, and compatibility with preservatives have limited its widespread clinical adoption. This study evaluated the recovery and mutation detection performance of large-volume (50 mL) urine samples preserved with three common urine stabilizers and analyzed after storage for 0 and 3 days at room temperature.

MATERIALS & METHODS

Pooled healthy donor urine was contrived with Anchor Molecular's cfDNA reference standards containing four clinically actionable breast cancer mutations: *IDH1* (R132H), *PIK3CA* (E545K), *PTEN* (R130G), and *TP53* (R248Q). Three preservatives—Streck Urine preservative, UAS urine analyte stabilizer, and Tris-EDTA (TE) buffer pH (8.0)—were evaluated at time zero (T0) and after 3 days (T3) of room temperature storage. For each condition, three technical replicates were extracted from 50 mL urine samples using the nRichDX Revolution Max 50 cfDNA Kit. cfDNA yield and fragment integrity were measured using the Agilent cfDNA High Sensitivity ScreenTape assay. Mutation detection was performed using a multiplex qPCR assay targeting the four hotspot mutations.

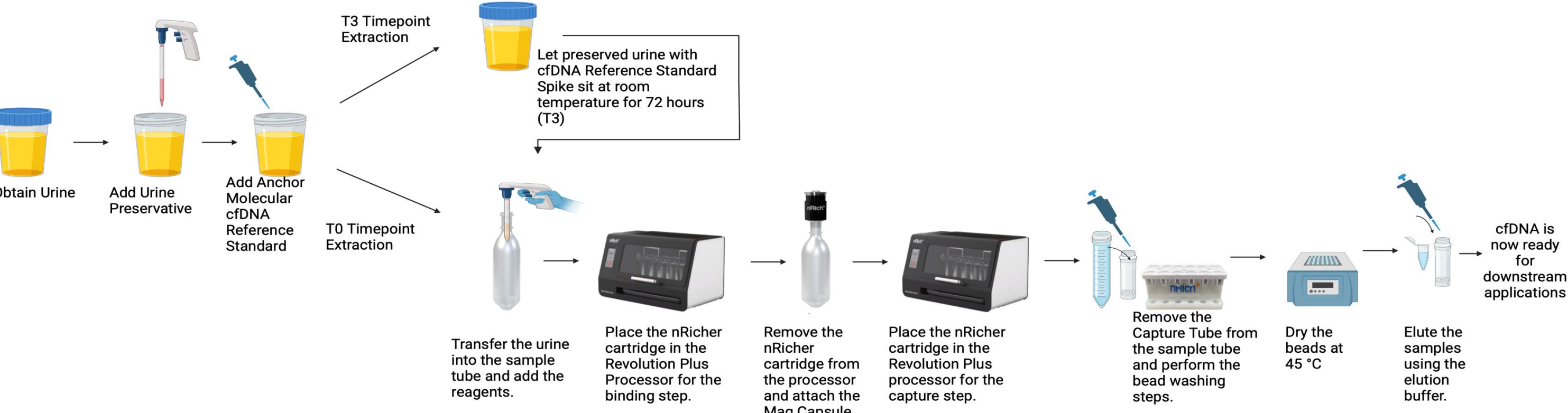


Figure 1. Urine cfDNA extraction workflow. Preserved urine spiked with a cfDNA reference standard was extracted at T0 and T3 using the nRichDX Revolution Max50 cfDNA Kit; cfDNA was bound, washed, dried, eluted, and collected for downstream analysis.

RESULTS



Figure 2. PCR Ct values for IDH1, PIK3CA, PTEN, and TP53 (wild-type) in urine samples stabilized with different preservatives and stored for 72 hours. Ct measurements at 0 and 72 hours were comparable, indicating no significant degradation over the storage period.

TapeStation cfDNA Concentration (pg/μL)		
Urine Preservative	cfDNA Concentration at T0	cfDNA Concentration at T3
Tris EDTA (pH 8.0)	2130	2280
Streck	1880	995
UAS	1420	1260

Table 1. cfDNA concentration (pg/μL) within the 50–700 bp window (mono/di/tri-nucleosomal cfDNA; excludes high-molecular-weight gDNA) quantified on the Agilent TapeStation cfDNA ScreenTape. Urine was collected into Tris-EDTA (pH 8.0), Streck, or UAS preservatives, processed with the nRichDX kit, and analyzed at t = 0 h (baseline) and after a 72 h hold at room temperature (≈20–25 °C). For Tris-EDTA, cfDNA concentrations were 2130 and 2280 pg/μL at T0 and T3, respectively, indicating stable to slightly increased recovery. Streck-preserved urine showed a decrease from 1880 to 995 pg/μL over 72 hours, whereas UAS demonstrated a more modest decline from 1420 to 1260 pg/μL.

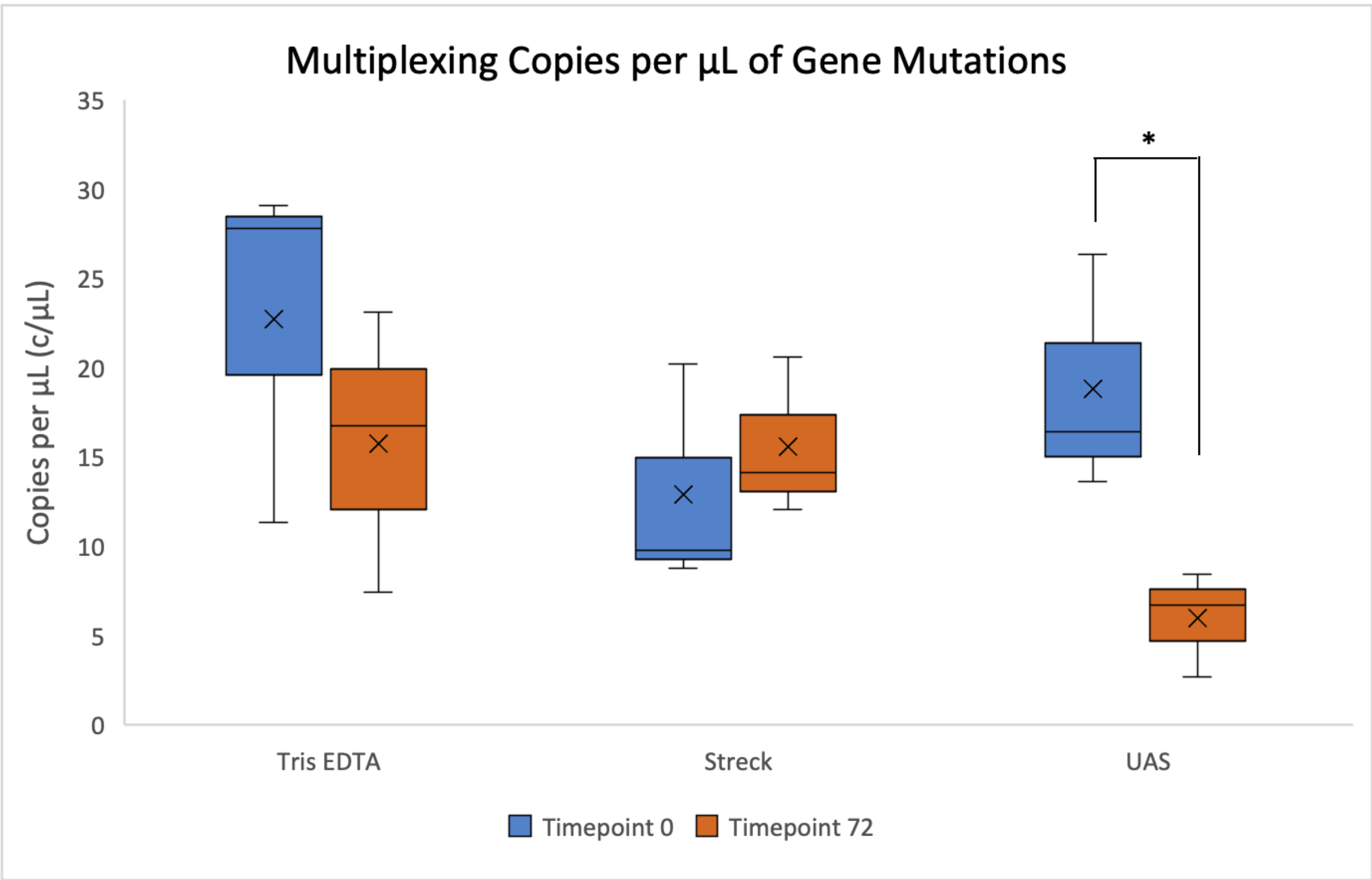


Figure 3. Mutation-signal stability by preservative. Multiplex PCR quantified IDH1, PIK3CA, PTEN, and TP53 from cfDNA at 0h and 72h at room temperature. Copy numbers (copies/μL) were calculated from an on-plate standard curve. UAS showed a significant T0→T72 change ($P < 0.05$); all other preservatives maintained stable copy numbers across loci.

CONCLUSION

Using the nRichDX Revolution Max 50 cfDNA Kit, mutation-specific qPCR robustly detected the Anchor Molecular cfDNA Reference Standard from 50 mL urine preserved with Tris-EDTA, Streck, or UAS at baseline and after 72 h at room temperature. Mean Ct drift from T0→T3 was +0.53 (Tris-EDTA; ~1.4× fewer copies), −0.32 (Streck; ~0.8× change), and +1.67 (UAS; ~3.2× fewer copies), indicating preservative-dependent stability. All conditions remained within the assay's quantitative window, confirming sufficient cfDNA recovery and effective inhibitor removal for downstream mutation detection. Practically, the data support 72-h ambient collection/transport with Tris-EDTA or Streck. Overall, the findings highlight the feasibility of urine as a stable and scalable liquid biopsy sample type for breast cancer genotyping, longitudinal monitoring, and remote collection workflows.

