Stabilization of cfDNA in urine using a preservative reagent during sample processing, transport, and storage for use with all non-assayed instrumentation

Jianbing Qin, Ph.D.; Sheila E. Norton; and Bradford A. Hunsley
Research and Development Division, Streck, Inc., La Vista, NE 68128, USA

Abstract

Background

Detection of circulating cell-free DNA (cfDNA) in blood plasma derived from tumor, fetus and transplanted organs has been well documented. These circulating cfDNAs can pass from the blood through the kidney barrier into urine. The obvious advantage of urine sampling makes urine a useful source of fetal and tumor DNA for the development of noninvasive prenatal and cancer diagnostic and prognostic tests. However, the inherent instability of cfDNA in urine hinders its clinical utility. Nucleated cells in urine can also release genomic DNA into urine leading to an increased DNA background during sample processing and storage. This study was designed to develop an innovative, customer friendly and cost-effective preservative reagent that can preserve the original proportion and integrity of cfDNA in urine post specimen collection.

Methods

The effect of different Streck Urine Preservative concentrations on stability of urinary cfDNA was examined and compared to untreated urine samples. The first-voided morning urine samples were diluted 10:1, 20:1 or 30:1 with the preservative reagent and stored at room temperature with untreated urine specimens in parallel. Aliquots were removed at specified time points. cfDNA was purified from urine and quantified by a Droplet Digital PCR (ddPCR) assay. To study the effect of storage temperature on cfDNA concentration, urine samples were stored at either 6 °C or 37 °C. Similarly, cfDNA was extracted at various time points and quantified by ddPCR.

Results

Untreated urine samples showed a significant decrease in cfDNA concentrations of both oncogene KRAS and housekeeping gene β-actin on day 4 and 7 post specimen collection at room temperature. In contrast, cfDNA concentrations remained stable for at least 7 days in urine samples treated 10:1 to 30:1 with Streck Urine Preservative. Moreover, the preservative reagent also stabilized cfDNA in urine at 6 °C or 37 °C for up to 7 days.

Conclusion

Our results show that Streck Urine Preservative is capable of preserving circulating cfDNA in urine over a wide range of dilution ratios within temperature fluctuations that can occur during urine sample handling, storage and transportation. This novel urine preservative reagent could provide a method for obtaining high quality stabilized urinary cfDNA for clinical diagnostics development and application.

Introduction

The discovery of cell-free nucleic acids in the circulation has opened up new opportunities for non-invasive diagnostic applications in cancer testing and prenatal diagnosis[1]. Since the confirmation of cfDNA presence in urine[2], there has been much interest in the potential utility of urinary DNA for clinical diagnostic development. The main advantage of urine over other body fluids (e.g.,, blood) is that urine sampling is truly non-invasive and it can be obtained safely and in large amounts with very limited training. When employing urinary cfDNA, however, it is important to minimize release of cellular DNA from nucleated cells and stabilize cfDNA following urine collection since cfDNA targets are present at low quantities and degrade rapidly[3,4]. Therefore, it is necessary to address pre-analytical issues that arise during the time between urine collection and cfDNA isolation. These include delays in urine processing and specimen storage temperature. Such conditions may cause cellular DNA contamination and subsequently alter cfDNA levels circulating in urine. Thus, in order to obtain reproducible results, it is essential to standardize the pre-analytical procedure for urine sample handling. cfDNA preservation and stabilization in urine should be an integral part of the non-invasive diagnostic test development using urine as the source of genetic material. This study was undertaken to develop a novel and easy preservative reagent that can maintain the cfDNA concentration in urine post specimen collection.

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Materials and Method

Urine Collection

The first-voided morning urine collected from healthy volunteers was treated with Streck Urine Preservative and stored at specified temperatures with untreated samples in parallel.

- **Sample Processing**

  Aliquots of urine (5 mL) were removed from each sample on day 0, 4 and 7, respectively. These aliquots were centrifuged at room temperature at 4000 rpm for 10 minutes. 4 mL of supernatant was carefully removed without disturbing pellets and transferred to a new tube using a pipette followed by cfDNA extraction.

- **Urine cfDNA Isolation**

  Urine cfDNA was purified using the commercially available QIAamp Circulating Nucleic Acid Kit (QIAGEN, Santa Clarita, CA). For optimal results, the manufacturer's recommended protocol was modified slightly by increasing the duration of the proteinase K treatment from 30 min to 1 hour at 60 °C.

- **Droplet Digital PCR (ddPCR)**

  The PCR was performed using the QX100 Droplet Digital PCR system (Bio-Rad, Hercules, CA). The KRAS copy number assay kit was purchased from Applied Biosystems (Foster City, CA). Primers and the probe for the ddPCR quantification of human β-actin were purchased from Integrated DNA Technologies (Coralville, IA).

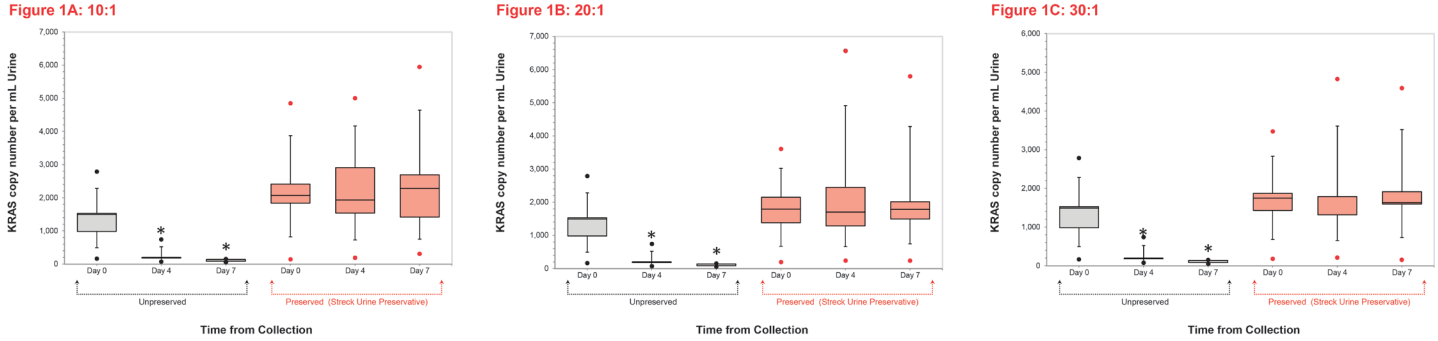

Figure 1. Urinary cfDNA stabilized by Streck Urine Preservative over a wide range of dilution ratio at room temperature. Urine was treated 10:1 (A), 20:1 (B) or 30:1 (C) with Streck Urine Preservative and stored at room temperature. On day 0, 4 and 7, cfDNA was isolated from urine samples and quantified by ddPCR. The concentration of KRAS gene decreased significantly in untreated urine on day 4 and 7, whereas it remained stable in all treated urine samples for at least 7 days post specimen collection. Similar results were observed for the house keeping gene β-actin (data not shown).

*Box plots show the median (line inside the box) and 75th and 25th percentiles (limits of the box). The upper and lower error bars indicate the 90th and 10th percentiles, respectively. The upper most and lower most dots indicate the maximum and minimum values. (n=5, *p <0.05)*

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Figure 2: Temperature Study

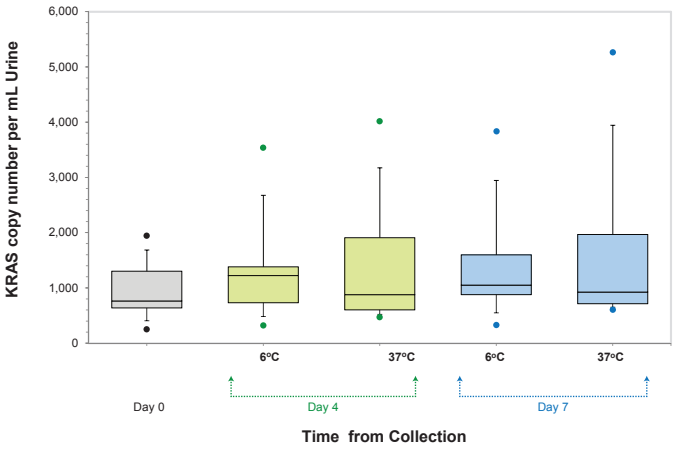

Figure 2. Urinary cfDNA stabilized by Streck Urine Preservative at various storage temperatures. Urine samples were treated 20:1 with Streck Urine Preservative and stored at 6 °C or 37 °C, respectively. cfDNA was isolated from urine samples at specified time points and quantified by ddPCR. The cfDNA (KRAS) was stabilized in all treated urine samples for at least 7 days at either 6 °C or 37 °C. No statistically significant difference in cfDNA concentration was found between stored samples and day 0 samples. Similar results were observed for the house keeping gene β-actin (data not shown). (n=5)

Conclusion

The novel urine preservative reagent can stabilize cfDNA in urine and minimize the post-sampling urinary DNA background for an extended period of time at various storage temperatures. This new methodology provides clinical laboratories with great flexibility and convenience in urine sample processing, handing and storage for urinary nucleic acid testing as it eliminates the necessity for immediate separation of supernatant after urine collection and refrigerating/freezing urine for transport.

Acknowledgments

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