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PreAnalytiX®

A QIAGEN / BD Company

# PAXgene®

## *Urine Liquid Biopsy Set*

**For Research Use Only**

Not for use in diagnostic procedures

REF **769143**

## I. Introduction

### PAXgene Urine Liquid Biopsy Set

The PAXgene Urine Liquid Biopsy Set consists of a urine collection cup (PAXgene Urine Collection Cup) and a nucleic acid stabilizing tube (PAXgene Urine Liquid Biopsy Tube). It is intended for the collection, storage and transport of human urine specimen and stabilization of cell-free DNA (cfDNA) profile. The set is part of a complete preanalytical workflow for molecular testing, including the QIAGEN® QIAamp® Circulating Nucleic Acid Kit or the QIAGEN QIAsymphony® Circulating DNA Kit.

The PAXgene Urine Liquid Biopsy Set is for research use only. Not for use in diagnostic procedures.

### PAXgene Urine Liquid Biopsy Tube

The PAXgene Urine Liquid Biopsy Tube is a plastic, closed, evacuated tube intended for direct sampling from the PAXgene Urine Collection Cup, stabilization of cell free DNA (cfDNA) profile, storage and transport of a human urine specimen. The tube is part of a complete preanalytical workflow for molecular testing, including cfDNA isolation using QIAGEN QIAamp Circulating Nucleic Acid Kit or the QIAGEN QIAsymphony Circulating DNA Kit.

The PAXgene Urine Liquid Biopsy Tube is for research use only. Not for use in diagnostic procedures.

### Components of the PAXgene Urine Liquid Biopsy Set

The PAXgene Urine Liquid Biopsy Set consists of the PAXgene Urine Collection Cup and the PAXgene Urine Liquid Biopsy Tube.

The PAXgene Urine Collection Cup is a plastic BD Vacutainer® Urine Collection Cup for collection of up to 120 mL of human urine. The cup enables transfer of urine into the evacuated PAXgene Urine Liquid Biopsy Tube without the need to open the tube or the cup. Directly after urine collection, the unstabilized urine is transferred into the PAXgene Urine Liquid Biopsy Tube for stabilization of urine. This technology allows for convenient and safe collection and stabilization of urine with consistent ratio of urine to additive.

The PAXgene Urine Collection Cup is for research use only. Not for use in diagnostic procedures.

The PAXgene Urine Liquid Biopsy Tube allows direct sampling of 10 mL human urine from the PAXgene Urine Collection Cup. The additive is non-crosslinking and does not modify biomolecules like cfDNA. Stabilization of cfDNA levels is accomplished by minimizing degradation of cfDNA, minimizing release of genomic DNA (gDNA), and minimizing microbial growth during sample storage.

Following centrifugation and separation from cellular components, stabilized cfDNA can be isolated from the urine supernatant by manual or automated procedures using the QIAGEN QIAamp Circulating Nucleic Acid Kit or the QIAGEN QIAsymphony Circulating DNA Kit, respectively.

The PAXgene Urine Liquid Biopsy Set was developed in accordance with the European Committee for Standardization Standard *CEN/TS 17811:2022 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for urine and other body fluids — Isolated cell free DNA during development and verification/validation.*

The PAXgene Urine Liquid Biopsy Set performance has been established in studies in which cfDNA was isolated from human urine derived from PAXgene Urine Liquid Biopsy Tube (see Section V. Performance Characteristics). Users must validate the performance of the product in their laboratory for specific research applications.

### Product Features

PAXgene Urine Liquid Biopsy Set REF 769143

20 tubes: PAXgene Urine Liquid Biopsy Tube (RUO, sterile, 10.0 mL draw volume, 1.5 mL additive)

20 cups: PAXgene Urine Collection Cup (RUO, sterile, up to 120 mL urine volume)

**Additional tubes**

| | |
|---|---|
| PAXgene Urine Liquid Biopsy Tube REF 769114 | RUO • Sterile • 50 tubes/case |
| 10.0 mL draw volume | 16 × 100 mm tube • 1.5 mL additive |
| Safety-engineered BD Hemogard™ closure | Red stopper • Yellow shield |

## II. Summary and Explanation

The PAXgene Urine Liquid Biopsy Set, which is comprised of the PAXgene Urine Collection Cup and the PAXgene Urine Liquid Biopsy Tube, enables standardized collection, stabilization, transport, and storage of urine specimens. Following centrifugation and separation from cellular components, stabilized cfDNA can be isolated from the urine supernatant by manual or automated procedures using the QIAGEN QIAamp Circulating Nucleic Acid Kit or the QIAGEN QIAsymphony Circulating DNA Kit, respectively.

## III. Principle of Procedure

The PAXgene Urine Collection Cup is used to collect up to 120 mL of human urine. The evacuated PAXgene Urine Liquid Biopsy Tube contains 1.5 mL of additive and is used to sample urine directly from the PAXgene Urine Collection Cup via a needle integrated in the blue cap of the cup. The stopper of the PAXgene Urine Liquid Biopsy Tube is pierced by the needle and the vacuum in the tube enables a flow of 10 mL urine from the PAXgene Urine Collection Cup, through a straw (part of collection cup cap) into the tube. Using the PAXgene Urine Liquid Biopsy Set for urine stabilization according to the handbook assures a consistent ratio of urine to additive. The additive in the tube is non-crosslinking. Stabilization of cfDNA levels is accomplished by minimizing degradation of cfDNA, minimizing release of gDNA, and minimizing microbial growth during sample storage. Cellular components are separated by centrifugation and cfDNA is isolated from the urine supernatant. Isolation of cfDNA from this supernatant can be performed by manual or automated methods, using the QIAGEN QIAamp Circulating Nucleic Acid Kit or the QIAGEN QIAsymphony Circulating DNA Kit, respectively.

## IV. Specimen Collection and Processing

**A. Required urine handling and processing accessories (not included with PAXgene Urine Liquid Biopsy Set).**

1. Labels for positive specimen identification.
2. 15 mL conical bottom centrifugation tubes for urine centrifugation.
3. Disposal containers for biohazard waste and biohazard sharps for used empty collection cups and used blue cup caps.

**B. Recommendation on steps to do before specimen collection**

1. For detailed instructions on the urine collection procedure, please refer to "Urine Collection Procedure with PAXgene® Urine Collection Cup." These instructions are accessible on the product webpage for the PAXgene Urine Liquid Biopsy Set, under resources, on **www.preanalytix.com**. The instructions for use for the urine collection procedure with PAXgene Urine Liquid Biopsy Set should be printed and provided to donating individuals prior to specimen collection.

2. Store cup at 4–25°C prior to use. The sterile interior is maintained until the blue cap is removed for urine collection.
3. Properly label the cup with the donating individual's name, the urine collection date and any additional information required by your facility's policy for sample identification.
4. Check that the collection cup is provided to the donating individual with the intact warning label on the blue cup cap.

   **Caution:** SHARP NEEDLE IS LOCATED UNDER THE CAP WARNING LABEL. DO NOT REMOVE THE WARNING LABEL ON THE TOP OF THE CAP. IF THE LABEL IS REMOVED, REPLACE WITH A NEW CUP.
5. Follow your facility's guidelines for best practices for urine collection.
6. Instruct the urine donating individual to collect a minimum of 15 mL and up to 120 mL of urine. A minimum of 15 mL is recommended to be present in the cup to fill one tube.

**C. Procedure for specimen collection**

1. Verify you have a PAXgene Urine Collection Cup and instructions on how to collect urine.
2. Place the collection cup aside. Wash hands thoroughly with soap and water before starting collection.
3. Remove the blue screw cap of the PAXgene Urine Collection Cup. Place the cap on a flat surface with the "straw" facing upwards. Be careful not to touch the inside of the cap or straw. Do not remove the warning label on top of the blue cup cap.

   **Caution:** SHARP NEEDLE IS LOCATED UNDER THE CAP WARNING LABEL. DO NOT REMOVE THE WARNING LABEL ON THE TOP OF THE CAP. IF THE LABEL IS REMOVED, CALL LABORATORY PROFESSIONAL TO RECEIVE A NEW CUP.
4. Collect up to 120 mL of urine. A minimum of 15 mL is recommended to be present in the cup to fill one tube.
5. Male instructions for collection: Bring the collection cup closer to the end of the penis and void urine into the cup. DO NOT touch the inside or lip of the cup with the hands or any other part of the body. Once cup is filled, void remainder of urine into the toilet.

   Female instructions for collection: Bring the collection cup closer to the urinary opening and void urine into the cup. DO NOT touch the inside or lip of the cup with the hands or any other part of the body. Once cup is filled, void remainder of urine into the toilet.

   **Important:** Please void urine only after opening the blue cap of the collection cup. Do not void into the cup through the hole in the blue cup cap.
6. Close the cup firmly with the blue cap, touching ONLY the outside surfaces of the cap and cup.

**D. Procedure for storage and transport of unstabilized urine in the PAXgene Urine Collection Cup**

1. Store cup prior to use at 4–25°C. The sterile interior is maintained until the blue cup cap is removed for urine collection.
2. Properly label the cup with individual name, collection date and time and any additional information required by your facility's policy for sample identification.
3. For transport of cup to the laboratory, provide adequate warning using labeling and packaging to protect against inadvertent needlesticks caused by sharp located under label on top of the blue cup cap. Treat the screw cap of the PAXgene Urine Collection Cup as a contaminated sharp.
4. After specimen collection, storage in the PAXgene Urine Collection Cup at the specimen collection site should be minimized. The cup does not contain any preservative.
5. The urine specimen should arrive directly without intermediate storage, upright and vibration-free at 15–25°C (including room temperature) to the lab professional in order to ensure rapid further processing.
6. Ensure the time of specimen collection is recorded and urine is stabilized by transferring into PAXgene Urine Liquid biopsy Tube within 15 minutes of collection.

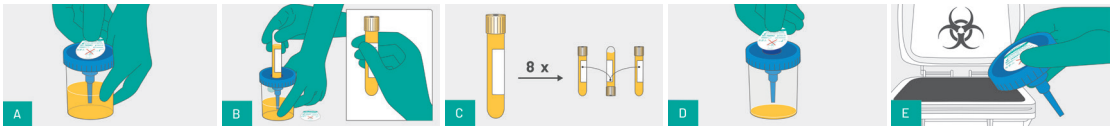
   **Note:** The time between urine donation and stabilization of the specimen in the PAXgene Urine Liquid Biopsy Tube is crucial for the successful stabilization of cfDNA. Degradation of cfDNA progresses more rapidly in unstabilized urine compared to other body fluids like blood. Therefore, the time between collection and stabilization should be reduced as much as possible.

**E. Procedure for stabilization of urine specimen in the PAXgene Urine Liquid Biopsy Tube**

1. Ensure that the PAXgene Urine Liquid Biopsy Tube is at 15–25°C (including room temperature) prior to use.
2. Place the PAXgene Urine Collection Cup on a clean, flat surface. If the sample volume is limited, the cup can be tilted.
3. Pull back the label on the blue cup cap to reveal the integrated transfer device as indicated in **Image A**.
4. Insert the PAXgene Urine Liquid Biopsy Tube with the yellow tube closure facing down into the cavity of the blue cup cap. Push quickly and firmly to puncture the stopper and hold the tube down to begin filling the tube as indicated in **Image B**.

   **Note:** Pushing the tube into the cavity of the blue cup cap slowly may result in tube underfilling.
5. Hold the PAXgene Urine Liquid Biopsy Tube in place until the tube stops filling.
6. Remove the PAXgene Urine Liquid Biopsy Tube from the integrated transfer device.
7. Mix the urine and additive by inverting the tube 8 times. A complete inversion is defined as turning the filled tube upside down once and then returning it to an upright position as indicated in **Image C**.

   **Note:** If more than one PAXgene Urine Liquid Biopsy Tube will be filled from the same urine sample, the blue cap of the PAXgene Urine Collection Cup should be unscrewed, slightly lifted and screwed back on without lifting it off completely. Repeat steps 4 to 7 for each additional tube (see Section XII. Ordering Information PAXgene Products for ordering additional PAXgene Urine Liquid Biopsy Tubes).
8. Place the label back on the blue cup cap to cover the exposed needle as indicated in **Image D**.
9. Dispose the blue cap from the PAXgene Urine Collection Cup into a biohazard sharps container as indicated in **Image E**.
10. Discard leftover urine according to your facility's policy.
11. Discard the empty PAXgene Urine Collection Cup into the biohazard waste container.



**F. Stabilized urine storage**

1. Prior to centrifugation, urine can be stored at 15–25°C (including room temperature) for up to 10 days or at 30°C for up to 7 days or at 37°C for up to 3 days or at 2–8°C for up to 10 days (see Section V. Performance Characteristics).

   **Note:** Do not freeze or store unprocessed urine-filled tubes below 2°C. For sample freezing, see Section H.

**G. Urine processing for isolation of cfDNA (or freezing of supernatant)**

1. Centrifuge the PAXgene Urine Liquid Biopsy Tube at 15–25°C (including room temperature) for 15 minutes at 1,900 × g using a balanced swing-out bucket centrifuge. If braking is preferred, it is recommended to use medium level braking, but should be validated for your specific workflow.
2. Decant the supernatant into a 15 mL conical bottom centrifugation tube, making sure to not disturb the pellet (cellular fraction).
3. Centrifuge the 15 mL conical bottom centrifugation tube at 15–25°C (including room temperature) for 10 minutes at 1,900 × g using a balanced swing-out bucket centrifuge. Transfer the urine supernatant into a new tube for further processing or storage.
4. Isolate cfDNA from the urine supernatant in accordance with the instructions provided with the cfDNA sample preparation kit according to Section I (Kit examples are provided in Section XII. Ordering Information PAXgene Products) or freeze the urine (see Section H. Stabilized urine supernatant storage [freezing]).

**H. Stabilized urine supernatant storage (freezing)**

For storage beyond 10 days, process urine samples as described in Section G and freeze the urine supernatant in a freezer compatible secondary container at –20°C or –80°C. cfDNA is stable in urine supernatant stored in a suitable container for up to 6 months at –20°C (long term study ongoing) or for up to 6 months at –80°C (long term study ongoing). Furthermore, cfDNA is stable for up to 3 freeze-thaw cycles at –20°C or –80°C (tested during storage of at least 7 days). Thaw the urine supernatant for 2 hours at 15–25°C (including room temperature) before starting the isolation of cfDNA.

**I. Isolation of cfDNA from urine supernatant**

cfDNA isolation should be performed in accordance with the instructions provided with

1. QIAamp Circulating Nucleic Acid Kit (10 mL supplementary protocol, visit **www.preanalytix.com** PAXgene Urine Liquid Biopsy Set)

   **Note:** Additional reagents. See Section XII. Ordering Information.
2. QIAGEN QIAsymphony Circulating DNA Kit* (10 mL customized protocol, visit **support.qiagen.com**)

   **Note:** Other sample input volumes are also possible, please visit **support.qiagen.com**

   *QIAGEN QIAsymphony Circulating DNA Kit is not available in all countries. For further details please visit **support.qiagen.com**.

**J. Freezing and thawing of cfDNA eluates**

It is recommended to store cfDNA eluates at 2–8°C for up to 1 month or at –20°C or –80°C for up to 3 months (long-term study ongoing), or for up to 3 freeze-thaw cycles at –20°C or –80°C (tested during storage for 1 month).

## V. Performance Characteristics

**A. Introduction**

The cfDNA profile in urine is impacted by storage after urine collection. Enzymatic degradation reduces the concentration of cfDNA, and gDNA released from human cells and bacteria dilute the cfDNA. The proper use of the PAXgene Urine Liquid Biopsy Set minimizes these processes and enables consistent research outcomes.

To assess the effect of cfDNA degradation separately from the effect of gDNA release, a spike-in approach was used. Cell-free male urine supernatant, containing male specific cfDNA, was spiked into female urine. Detection of male specific cfDNA allowed evaluation of cfDNA degradation without the effects of released gDNA from cells. Detection of an autosomal target allowed evaluation of cfDNA profile changes induced by gDNA release and degradation occurring in parallel. The results of these studies are described in the following sections. In all studies, urine sample collection, stabilization and processing were performed following the PAXgene Urine Liquid Biopsy Set instructions for use in this handbook. Sample centrifugation was performed according to instructions in Section G, unless specified otherwise.

**Note:** The concentration of cfDNA in urine may be low and varies considerably due to many factors including among different individuals, sex and health status of the donating individual, time of collection and hydration status.

Users must validate the performance of the product in their laboratory for specific research applications.

**B. Comparison of PAXgene Urine Liquid Biopsy stabilized and unstabilized urine**

Second urine of the day was collected from consented, apparently healthy adult females into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate urine pools (4 pools total) that were tested for each of the below described conditions.

Male unstabilized second urine of the day, used as a spike-in, was centrifuged within 4 hours of urine collection. The cell-free supernatant, containing male cfDNA, was added to the female urine pools in a consistent ratio.

One part of the spiked urine pool was stabilized using the PAXgene Urine Liquid Biopsy Tube and the second part was left unstabilized and cooled on ice until processing.

Unstabilized samples were centrifuged according to the instructions provided in Section G of this handbook and cfDNA was isolated within 4 hours of urine collection ($T_0$ unstabilized).

Stabilized urine samples were centrifuged following the same instructions and cfDNA was isolated within 4 hours of urine collection ($T_0$ stabilized) or the stabilized whole urine samples were stored for 10 days prior to centrifugation ($T_{10}$ stabilized). The 10-day storage of whole urine included initial storage for 4 hours at room temperature on the lab bench (RT), followed by 3 days at 5°C, followed by 6 days at 12°C and 8 hours at RT.

After centrifugation, cfDNA isolation from 10 mL of the urine supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit for all samples.

cfDNA was quantified in triplicate (4 pools with 3 replicates, n = 12) with the QIAGEN Investigator® Quantiplex® Pro RGQ assay on the QIAGEN Rotor-Gene® Q real-time PCR cycler. The relative cfDNA yield was calculated as the ratio of the male specific DNA in stabilized urine samples compared to the yield of male qPCR (quantitative polymerase chain reaction) target in unstabilized samples of the same urine pool.

cfDNA levels in PAXgene Urine Liquid Biopsy Tube stabilized samples ($T_0$ stabilized) were preserved during 10-day storage condition tested in this study and were higher than in unstabilized samples at initial timepoint ($T_0$ unstabilized, dashed red line) indicating that a significant loss of cfDNA target has already occurred in unstabilized urine within 4 hours of collection (Figure 1, Table 1).

**Figure 1. Relative cfDNA yield comparison of unstabilized and PAXgene Urine Liquid Biopsy Tube stabilized urine**

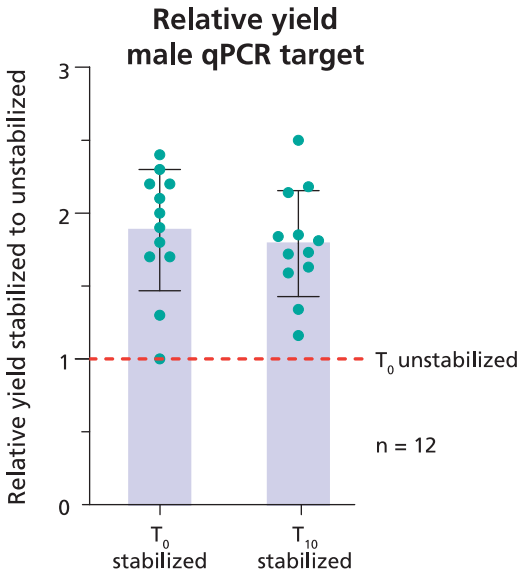



Figure 1 depicts the yield of spiked male urine cfDNA in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube at the day of urine collection ($T_0$ stabilized) or after urine storage for 10 days ($T_{10}$ stabilized) relative to unstabilized urine cooled until processing on the day of urine collection ($T_0$ unstabilized, set to 1). cfDNA was isolated with the QIAGEN QIAsymphony DSP Circulating DNA Kit. Data include 4 urine pools with 3 qPCR replicates (n = 12). Mean and SD are denoted.

**Table 1. Data summary: Relative cfDNA yield comparison of unstabilized and PAXgene Urine Liquid Biopsy Tube stabilized urine**

| | Relative yields stabilized to unstabilized urine: Male qPCR target | |
|---|---|---|
| | $T_0$ stabilized | $T_{10}$ stabilized |
| **n** | 12 | 12 |
| **Mean** | 1.9 | 1.8 |
| **Standard deviation** | 0.4 | 0.4 |

## C. Storage of whole human urine in the PAXgene Urine Liquid Biopsy Tube

### C.1 cfDNA stabilization by minimization of degradation and minimization of gDNA release with the PAXgene Urine Liquid Biopsy Set

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate urine pools (4 female pools and 4 male pools, 8 pools in total). Different urine pools were tested with different conditions.

Male unstabilized second urine of the day, used as a spike-in for female urine pools, was centrifuged within 4 hours of urine collection. The cell-free supernatant, containing male cfDNA, was added to the female urine pools in a consistent ratio.

After stabilization of the spiked urine using the PAXgene Urine Liquid Biopsy Tube, the urine samples were either centrifuged according to the instructions in Section G of this handbook and cfDNA was isolated within 4 hours of urine collection ($T_0$) or urine samples were stored under various conditions prior to processing ($T_X$).

cfDNA isolation from 8 mL of the supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit.

cfDNA was quantified in triplicate (male qPCR target: 4 female urine pools with 3 replicates, n = 12; autosomal qPCR target: 8 urine pools with 3 replicates, n = 24) with the QIAGEN Investigator Quantiplex Pro RGQ assay on the QIAGEN Rotor-Gene Q real-time PCR cycler.

The relative cfDNA yield was calculated as the ratio of either the male specific DNA yield (Figure 2) or the autosomal target DNA yield (Figure 3) after urine sample storage ($T_X$) compared to the yield in the same stabilized urine pool before storage ($T_0$).

cfDNA levels remained stable due to minimized cfDNA degradation in unprocessed whole urine stabilized in the PAXgene Urine Liquid Biopsy Tube when stored for up to 3 days at 37°C, for up to 7 days at 30°C, for up to 10 days refrigerated (2–8°C), for up to 10 days at 15°C, for up to 10 days at room temperature (lab bench storage) or for up to 10 days at 25°C (Figure 2, Table 2).

**Figure 2. cfDNA stabilization by minimization of degradation during whole urine storage**

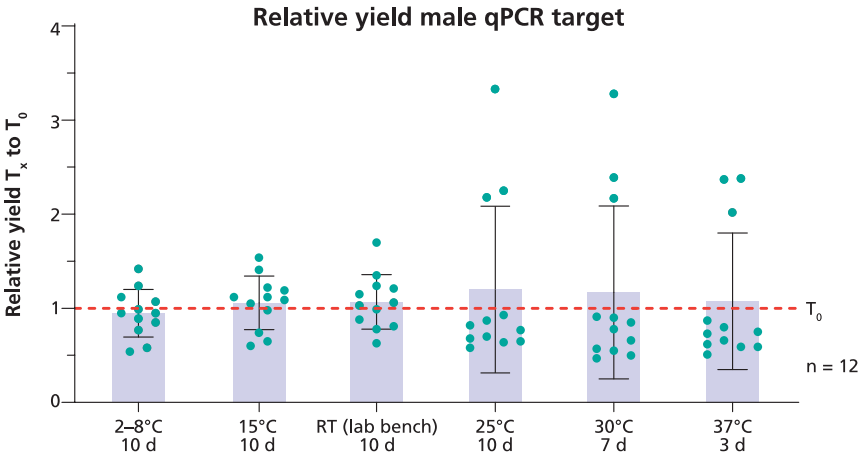



Figure 2 depicts the yield of spiked male urine cfDNA in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage ($T_X$; where x equals 3, 7, or 10 days storage) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Circulating DNA Kit and analyzed with the Investigator Quantiplex Pro RGQ assay. Data include 4 urine pools with 3 qPCR replicates. Mean and SD are denoted.

**Table 2. Data summary: cfDNA stabilization by minimization of degradation during whole urine storage**

| | Relative yields $T_X$ to $T_0$: Male qPCR target | | | | | |
|---|---|---|---|---|---|---|
| | 2–8°C 10 days | 15°C 10 days | RT (lab bench) 10 days | 25°C 10 days | 30°C 7 days | 37°C 3 days |
| **n** | 12 | 12 | 12 | 12 | 12 | 12 |
| **Mean** | 0.9 | 1.1 | 1.1 | 1.2 | 1.2 | 1.1 |
| **Standard deviation** | 0.3 | 0.3 | 0.3 | 0.9 | 0.9 | 0.7 |

cfDNA profile was stabilized by minimization of gDNA release in unprocessed whole urine stabilized in the PAXgene Urine Liquid Biopsy Tube when stored for up to 3 days at 37°C, for up to 7 days at 30°C, for up to 10 days refrigerated (2–8°C), for up to 10 days at 15°C, for up to 10 days at room temperature (lab bench storage) or for up to 10 days at 25°C (Figure 3, Table 3).

**Figure 3. cfDNA stabilization by minimization of gDNA release during whole urine storage**

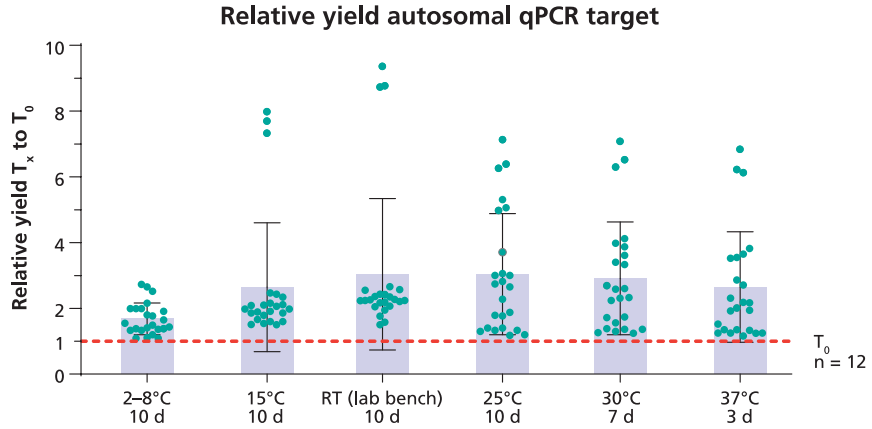



Figure 3 depicts the yield of an autosomal qPCR target in urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage ($T_X$, where x equals 3, 7, or 10 days storage) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Circulating DNA Kit and analyzed with the Investigator Quantiplex Pro RGQ assay. Data include 8 urine pools with 3 qPCR replicates. n = 24. Mean and SD are denoted.

**Table 3. Data summary: cfDNA stabilization by minimization of gDNA release during whole urine storage**

| | Relative yields $T_X$ to $T_0$: Male qPCR target | | | | | |
|---|---|---|---|---|---|---|
| | 2–8°C 10 days | 15°C 10 days | RT (lab bench) 10 days | 25°C 10 days | 30°C 7 days | 37°C 3 days |
| **n** | 24 | 24 | 24 | 24 | 24 | 24 |
| **Mean** | 1.7 | 2.6 | 3.0 | 3.0 | 2.9 | 2.6 |
| **Standard deviation** | 0.5 | 2.0 | 2.3 | 1.8 | 1.7 | 1.7 |

### C.2 cfDNA stabilization by minimization of bacterial growth during urine storage in the PAXgene Urine Liquid Biopsy Tube

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate urine pools (4 female pools and 4 male pools, 8 pools in total). Different urine pools were tested with different conditions.

After stabilization of the urine using the PAXgene Urine Liquid Biopsy Tube, urine samples were either centrifuged according to the QIAsymphony DSP Virus/Pathogen Midi Kit Handbook and microbial DNA was isolated from the pellet within 4 hours of urine collection ($T_0$) or the urine samples were stored under various conditions prior to processing ($T_X$).

Microbial DNA isolation from the urine pellet of 800 µL stabilized urine was performed using the QIAGEN QIAsymphony DSP Virus/Pathogen Midi Kit.

Microbial DNA was quantified in triplicate (8 pools with 3 replicates, n = 18–24) with a 16S qPCR assay on the QIAGEN Rotor-Gene Q real-time PCR cycler. The relative yield was calculated as the ratio of 16S DNA (16S qPCR target) after urine sample storage ($T_X$) compared to the yield in the same stabilized urine pool before storage ($T_0$).

For all tested storage conditions, no increase in microbial DNA was detected in stabilized urine samples after storage in comparison to urine samples not stored ($T_0$). Hence, minimized bacterial growth during urine storage was observed in unprocessed whole urine stabilized in the PAXgene Urine Liquid Biopsy Tube when stored for up to 3 days at 37°C, for up to 7 days at 30°C, for up to 10 days refrigerated (2–8°C), for up to 10 days at 15°C, for up to 10 days at room temperature (lab bench storage) or for up to 10 days at 25°C (Figure 4, Table 4).

**Figure 4. cfDNA stabilization by minimization of bacterial growth during whole urine storage**

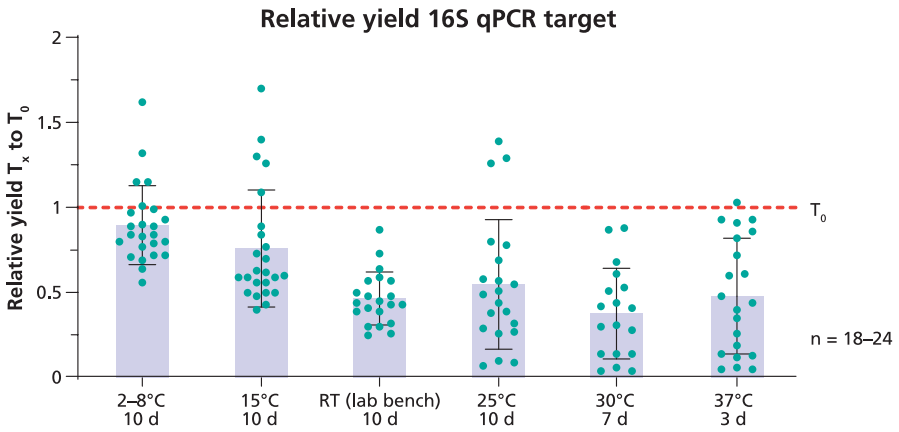



Figure 4 depicts the yield of microbial DNA (16 qPCR target) in urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage ($T_X$, where x equals 3, 7, or 10 days storage) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Virus/ Pathogen Kit and analyzed on the Rotor-Gene Q real-time PCR cycler. Data include 6 to 8 urine pools with 3 qPCR replicates. n = 18–24. Mean and SD are denoted.

**Table 4. Data summary: cfDNA stabilization by minimization of bacterial growth during whole urine storage**

| | Relative yields $T_X$ to $T_0$: 16S qPCR target | | | | | |
|---|---|---|---|---|---|---|
| | 2–8°C 10 days | 15°C 10 days | RT (lab bench) 10 days | 25°C 10 days | 30°C 7 days | 37°C 3 days |
| n | 24 | 24 | 21 | 21 | 18 | 21 |
| Mean | 0.9 | 0.8 | 0.5 | 0.5 | 0.4 | 0.5 |
| Standard deviation | 0.2 | 0.3 | 0.2 | 0.4 | 0.3 | 0.3 |

## D. Transport of whole human urine in the PAXgene Urine Liquid Biopsy Tube

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate urine pools (4 female pools and 4 male pools, 8 pools in total) that were tested for each of the below described conditions.

Male unstabilized second urine of the day, used as a spike-in for female urine pools, was centrifuged within 4 hours of urine collection. The cell-free supernatant, containing male cfDNA, was added to the female urine pools in a consistent ratio.

After stabilization of the spiked urine using the PAXgene Urine Liquid Biopsy Tube, urine samples were either centrifuged according to the instructions in Section G of this handbook and cfDNA was isolated within 4 hours of urine collection ($T_0$) or the urine samples were stored at room temperature (lab bench storage) for 10 days with no transport (RT storage 10 days) or subjected to 10 days of real-world transportation including air shipment in insulated packaging at ambient temperature without (Transport 10 days) or with further vibration and dropping (Transport and transport simulation 10 days) prior to processing. During transportation, the temperatures fluctuated but did not exceed the extremes and durations specified in Section F, Stabilized urine storage.

cfDNA isolation from 10 mL of the supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit.

cfDNA was quantified in triplicate (male qPCR target: 4 male urine pools with 3 replicates, n = 12; autosomal qPCR target: 8 urine pools with 3 replicates, n = 24) with the QIAGEN Investigator Quantiplex Pro RGQ assay on the QIAGEN Rotor-Gene Q real-time PCR cycler. The relative cfDNA yield was calculated as the ratio of either the male specific DNA yield (Figure 5) or an autosomal DNA target yield (Figure 6) after urine sample storage or transport compared to the yield in the same stabilized urine pools before storage ($T_0$).

cfDNA levels remained stable due to minimized cfDNA degradation in unprocessed whole urine stabilized in the PAXgene Urine Liquid Biopsy Tube when stored, transported or transported with additional transport simulation (Figure 5, Table 5).

**Figure 5. cfDNA stabilization by minimization of degradation during whole urine storage and transport**

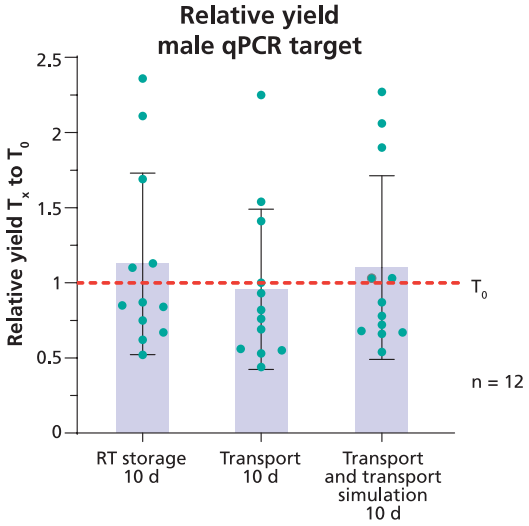



Figure 5 depicts the yield of spiked male urine cfDNA in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage, transport or transport and transport simulation ($T_X$, where x equals 10 days) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Circulating DNA Kit and analyzed with the Investigator Quantiplex Pro RGQ assay. Data include 4 urine pools with 3 qPCR replicates. n = 12. Mean and SD are denoted.

**Table 5. Data summary: cfDNA stabilization by minimization of degradation during whole urine storage and transport**

| | Relative yields $T_X$ to $T_0$: Male qPCR target | | |
|---|---|---|---|
| | RT storage 10 days | Transport 10 days | Transport and transport simulation 10 days |
| n | 12 | 12 | 12 |
| Mean | 1.1 | 1.0 | 1.1 |
| Standard deviation | 0.6 | 0.5 | 0.6 |

cfDNA profile was stabilized by minimization of gDNA release in unprocessed whole urine stabilized in the PAXgene Urine Liquid Biopsy Tube when stored, transported or transported with additional transport simulation (Figure 6, Table 6).

**Figure 6. cfDNA stabilization by minimization of gDNA release during whole urine storage and transport**

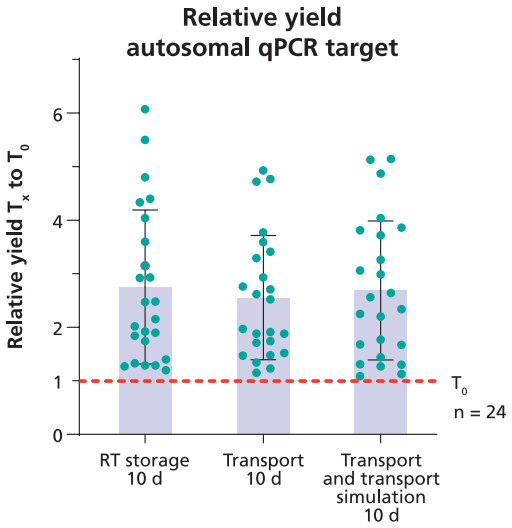



Figure 6 depicts the yield of an autosomal qPCR target in urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage, transport or transport and transport simulation ($T_X$, where x equals 10 days) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Circulating DNA Kit and analyzed with the Investigator Quantiplex Pro RGQ assay. Data include 4 urine pools with 3 qPCR replicates. n = 12. Mean and SD are denoted.

**Table 6. Data summary: cfDNA stabilization by minimization of gDNA release during whole urine storage and transport**

| | Relative yields $T_X$ to $T_0$: Autosomal qPCR target | | |
|---|---|---|---|
| | RT storage 10 days | Transport 10 days | Transport and transport simulation 10 days |
| n | 24 | 24 | 24 |
| Mean | 2.8 | 2.6 | 2.7 |
| Standard deviation | 1.4 | 1.2 | 1.3 |

**E. Freezing of urine supernatant after whole urine stabilization in the PAXgene Urine Liquid Biopsy Tube**

Second urine of the day was collected from consented, apparently healthy adult females into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate urine pools (4 pools total) that were tested for each of the below described conditions.

Male unstabilized second urine of the day, used as a spike-in, was centrifuged within 4 hours of urine collection. The cell-free supernatant, containing male cfDNA, was added to the female urine pools in a consistent ratio.

After stabilization of the spiked urine using the PAXgene Urine Liquid Biopsy Tube, urine samples were centrifuged to generate urine supernatant. Processing by centrifugation for cell removal was performed according to the instructions in Section G of this handbook.

cfDNA was isolated from 8 mL of the urine supernatant using the QIAGEN QIAsymphony DSP Circulating DNA Kit within 4 hours of urine collection ($T_0$) or after freezing of the stabilized urine supernatant in a secondary container at various conditions ($T_X$). As cells were removed before storage, cfDNA degradation could be directly assessed and results are shown below.

cfDNA was quantified in triplicate (4 pools with 3 replicates, n = 12) with the QIAGEN Investigator Quantiplex Pro RGQ assay on the QIAGEN Rotor-Gene Q real-time PCR cycler. The relative cfDNA yield was calculated as the ratio of the male specific DNA after urine supernatant storage ($T_X$) compared to the yield in the same stabilized urine supernatant before storage ($T_0$).

cfDNA levels remained stable due to minimized cfDNA degradation in frozen stabilized urine supernatant when stored for at least up to 6 months at –20°C or for at least up to 6 months at –80°C. Up to 3 freeze-thaw cycles were tested during 10 days storage at –20°C or –80°C. (Figure 7, Table 7).

**Figure 7. cfDNA stabilization by minimization of degradation during frozen urine supernatant storage**

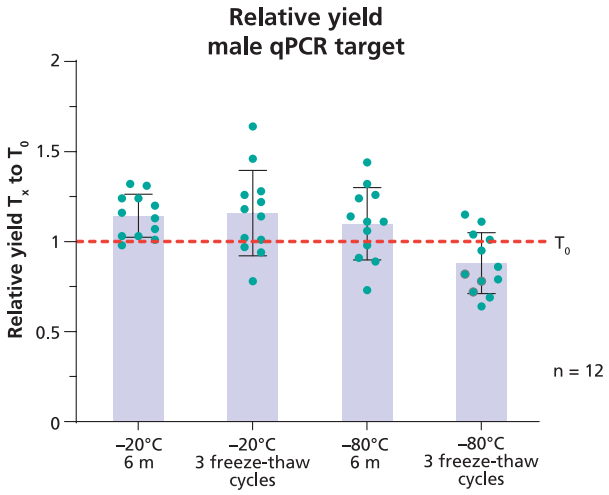



Figure 7 depicts the yield of spiked male urine cfDNA in female urine stabilized with the PAXgene Urine Liquid Biopsy Tube after frozen urine supernatant storage ($T_X$, where x equals 1 month or 6 months) relative to stabilized urine processed within 4 hours of urine collection ($T_0$, set to 1). cfDNA was isolated with the QIAsymphony DSP Circulating DNA Kit and analyzed with the Investigator Quantiplex Pro RGQ assay. Data include 4 urine pools with 3 qPCR replicates, n = 12. Mean and SD are denoted.

**Table 7. Data summary: cfDNA stabilization by minimization of degradation during frozen urine supernatant storage**

| | Relative yields $T_X$ to $T_0$: Male qPCR target | | | |
|---|---|---|---|---|
| | –20°C 6 months | –20°C 3 freeze-thaw cycles | –80°C 6 months | –80°C 3 freeze-thaw cycles |
| **n** | 12 | 12 | 12 | 12 |
| **Mean** | 1.1 | 1.2 | 1.1 | 0.9 |
| **Standard deviation** | 0.1 | 0.2 | 0.2 | 0.2 |

## F. Stability and compatibility with downstream analysis of cfDNA eluates isolated from the PAXgene Urine Liquid Biopsy Tube

cfDNA can be isolated from stabilized urine supernatant from the PAXgene Urine Liquid Biopsy Tube after centrifugation for separation of cellular components. cfDNA isolation can be performed manually using the QIAGEN QIAamp Circulating Nucleic Acid Kit or automated using the QIAGEN QIAsymphony DSP Circulating DNA Kit. The isolated cfDNA eluates are compatible with various downstream technologies such as qPCR and dPCR (digital polymerase chain reaction).

### F.1 Compatibility with qPCR

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate a urine pool (4 female pools and 4 male pools, 8 pools in total).

After stabilization of the urine using the PAXgene Urine Liquid Biopsy Tube, urine samples were either centrifuged according to the instructions in Section G of this handbook and cfDNA was isolated within 4 hours of urine collection ($T_0$) or the urine samples were stored prior to processing ($T_{10}$). The 10 days storage of whole urine included initial storage for 4 hours at 15–25°C (including room temperature), followed by 3 days at 5°C, followed by 6 days at 12°C and 8 hours at RT.

cfDNA isolation from 10 mL of the supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit or the QIAGEN QIAamp Circulating Nucleic Acid Kit.

qPCR was performed in triplicate (8 pools with 3 replicates, n = 24) with the QIAGEN Investigator Quantiplex Pro RGQ assay on the QIAGEN Rotor-Gene Q real-time PCR cycler assessing the amplification of an internal DNA control. The $\Delta C_T$ between cfDNA eluates and qPCR controls (qPCR standards) was calculated.

All samples, QIAsymphony as well as QIAamp cfDNA eluates, showed a $\Delta C_T$ below 1, indicating no negative effects of the cfDNA eluates on amplification of the internal control (Figure 8). Hence, cfDNA of high quality compatible with qPCR can be isolated from urine stabilized with the PAXgene Urine Liquid Biopsy Tube manually as well as automated.

**Figure 8. cfDNA eluate compatibility with qPCR**

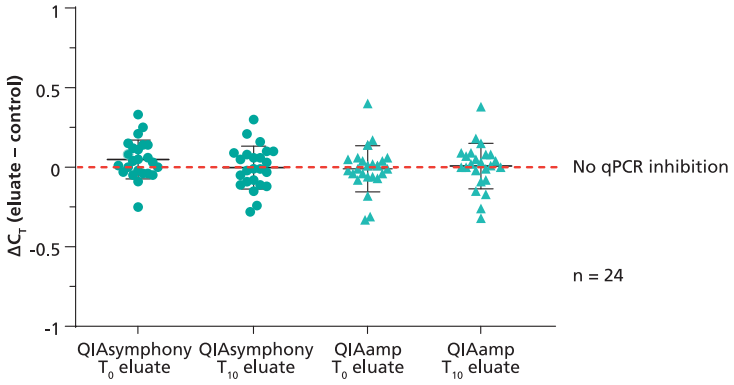



Figure 8 depicts the $\Delta C_T$ calculated between cfDNA eluates and qPCR control samples for the amplification of a qPCR internal control. cfDNA was isolated from urine stabilized with the PAXgene Urine Liquid Biopsy Tube after urine storage for 10 days ($T_{10}$) or urine processed within 4 hours of urine collection ($T_0$). The isolation was performed using the QIAGEN QIAsymphony DSP circulating DNA Kit or the QIAGEN QIAamp Circulating Nucleic Acid Kit. qPCR was performed with the Investigator Quantiplex Pro RGQ assay. Data include 8 urine pools with 3 qPCR replicates, n = 24. Mean and SD are denoted.

**Table 8. Data summary: cfDNA eluate compatibility with qPCR**

| | $\Delta C_T$ (eluate – control) | | | |
|---|---|---|---|---|
| | QIAsymphony $T_0$ eluate | QIAamp $T_0$ eluate | QIAsymphony $T_{10}$ eluate | QIAamp $T_{10}$ eluate |
| **n** | 24 | 24 | 24 | 24 |
| **Mean** | 0.05 | –0.01 | 0.03 | 0.03 |
| **Standard deviation** | 0.12 | 0.14 | 0.15 | 0.16 |

### F.2 Compatibility with dPCR

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate a urine pool (4 female pools and 4 male pools, 8 pools in total).

After stabilization of the urine using the PAXgene Urine Liquid Biopsy Tube, urine samples were centrifuged according to the instructions in Section G of this handbook and cfDNA was isolated within 4 hours of urine collection.

cfDNA isolation from 10 mL of the supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit or the QIAGEN QIAamp Circulating Nucleic Acid Kit.

cfDNA was quantified in triplicate (8 pools with 3 replicates, n = 24) with a QIAGEN dPCR LNA Assay assessing the copy number of target gene PIK3CA.

Detection of cfDNA by dPCR was possible for all samples demonstrating compatibility with dPCR. Variations in copy numbers reflect donor to donor variations.

**Figure 9. cfDNA eluate compatibility with dPCR**

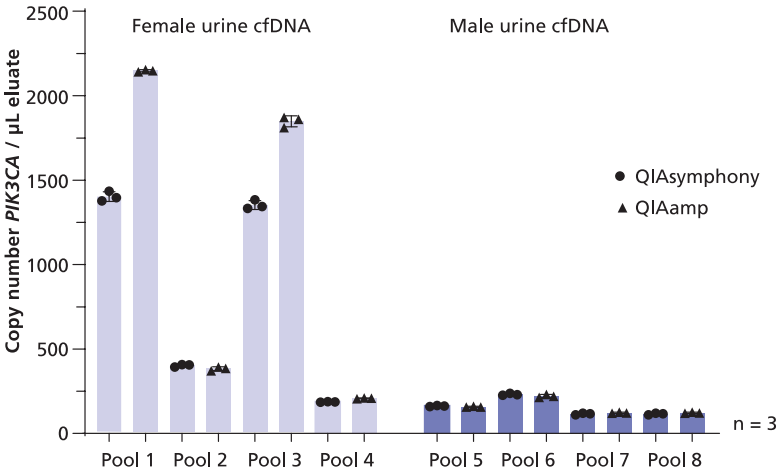



Figure 9 depicts copy numbers of PIK3CA / µL of cfDNA eluates isolated either with the QIAsymphony DSP Circulating DNA Kit or the QIAamp Circulating Nucleic Acid Kit from urine stabilized with the PAXgene Urine Liquid Biopsy Tube. dPCR was performed using the QIAGEN dPCR LNA PIK3CA Assay. Data include 8 urine pools with 3 qPCR replicates, n = 24. Mean and SD are denoted.

**Table 9. Data summary: cfDNA eluate compatibility with dPCR**

| | PIK3CA copy numbers: Female urine pools | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pool 1 | | Pool 2 | | Pool 3 | | Pool 4 | |
| | QIAsymphony | QIAamp | QIAsymphony | QIAamp | QIAsymphony | QIAamp | QIAsymphony | QIAamp |
| **n** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Mean** | 1,391 | 2,137 | 391 | 372 | 1,342 | 1,837 | 176 | 208 |
| **Standard deviation** | 29 | 7 | 8 | 11 | 26 | 32 | 2 | 2 |

| | PIK3CA copy numbers: Male urine pools | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pool 5 | | Pool 6 | | Pool 7 | | Pool 8 | |
| | QIAsymphony | QIAamp | QIAsymphony | QIAamp | QIAsymphony | QIAamp | QIAsymphony | QIAamp |
| **n** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Mean** | 153 | 159 | 231 | 222 | 116 | 122 | 116 | 122 |
| **Standard deviation** | 3 | 3 | 6 | 9 | 4 | 4 | 4 | 4 |

### F.3 cfDNA eluate stability

Second urine of the day was collected from consented, apparently healthy adults into PAXgene Urine Collection Cups. Urine from at least 3 individuals was mixed to generate a urine pool (1 female pool and 1 male pool, 2 pools in total).

Of each pool, 12 samples were stabilized (24 samples in total) using the PAXgene Urine Liquid Biopsy Tube. The stabilized whole urine samples were stored 10 days at 25°C ($T_{10}$) prior to processing.

Processing by centrifugation for cell removal was performed according to the instruction in Section G of this handbook. cfDNA isolation from the supernatant was performed using the QIAGEN QIAsymphony DSP Circulating DNA Kit or the QIAGEN QIAamp Circulating Nucleic Acid Kit.

cfDNA eluates were pooled by gender and cfDNA isolation protocol:

- Female $T_{10}$ QIAsymphony cfDNA pool
- Female $T_{10}$ QIAamp cfDNA pool
- Male $T_{10}$ QIAsymphony cfDNA pool
- Male $T_{10}$ QIAamp cfDNA pool

The cfDNA pools were aliquoted and aliquots were stored under various conditions. cfDNA quantification was performed with 9 replicates per each cfDNA eluate pool (9 replicates * 2 eluate pools = 18 replicates per condition) with the QIAGEN Investigator Quantiplex Pro RGQ assay on the QIAGEN Rotor-Gene Q real-time PCR cycler before and after cfDNA eluate storage at various conditions.

The mean $C_T$ values showed less variation than 3× assay standard deviation (1.2 $C_T$) and therefore cfDNA eluates were considered to be stable over at least 1 month at 2–8°C, 3 month at –80°C or 3 month at –80°C. Up to 3 freeze-thaw cycles were tested during 1 month storage at –20°C or –80°C (Figure 10, Table 10, Table 11).

**Figure 10. cfDNA eluate stability**

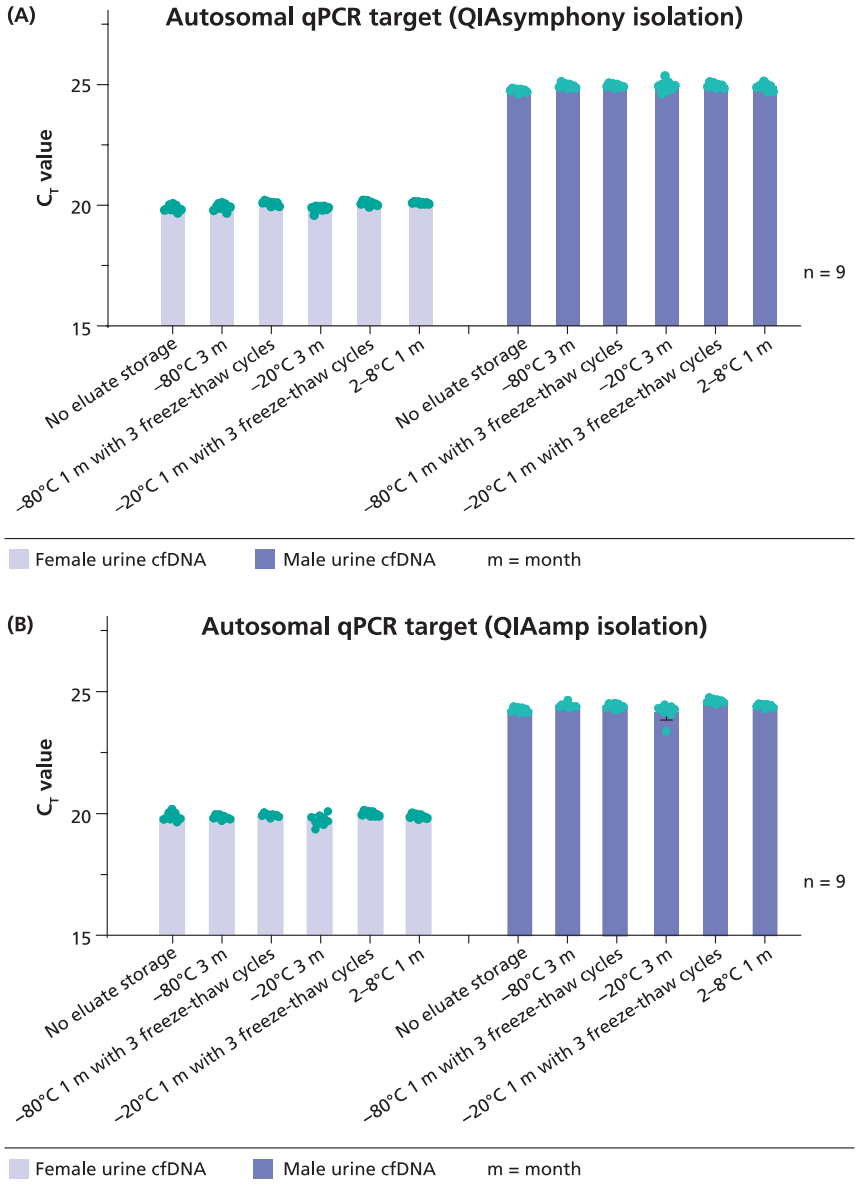



Figure 10 depicts $C_T$ values of cfDNA eluate pools before storage and after eluate storage at various conditions as assessed with the Investigator Quantiplex Pro RGQ assay. cfDNA was isolated either with the QIAGEN QIAsymphony DSP Circulating DNA Kit (A.) or the QIAGEN QIAamp circulating Nucleic Acid Kit (B.) from PAXgene Urine Liquid Biopsy stabilized urine. Data include 9 qPCR replicates, n = 9. Mean and SD are denoted.

**Table 10. Data summary: Quantification of QIAsymphony eluates during eluate storage**

| | $C_T$ values: Autosomal qPCR target female QIAsymphony cfDNA eluates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No eluate storage | –80°C 1 month | –80°C 3 months | –80°C 3 freeze-thaw cycles | –20°C 1 month | –20°C 3 months | –20°C 3 freeze-thaw cycles | 2–8°C 1 month |
| **n** | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| **Mean** | 19.88 | 20.06 | 19.94 | 20.07 | 20.06 | 19.86 | 20.07 | 20.09 |
| **Standard deviation** | 0.12 | 0.10 | 0.14 | 0.09 | 0.06 | 0.12 | 0.09 | 0.04 |

| | $C_T$ values: Autosomal qPCR target male QIAsymphony cfDNA eluates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No eluate storage | –80°C 1 month | –80°C 3 months | –80°C 3 freeze-thaw cycles | –20°C 1 month | –20°C 3 months | –20°C 3 freeze-thaw cycles | 2–8°C 1 month |
| n | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Mean | 24.75 | 25.00 | 24.95 | 24.95 | 24.98 | 24.93 | 24.96 | 24.91 |
| Standard deviation | 0.07 | 0.08 | 0.09 | 0.07 | 0.09 | 0.21 | 0.09 | 0.14 |

**Table 11. Data summary: Quantification of QIAamp eluates during eluate storage**

| | $C_T$ values: Autosomal qPCR target female QIAamp cfDNA eluates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No eluate storage | –80°C 1 month | –80°C 3 months | –80°C 3 freeze-thaw cycles | –20°C 1 month | –20°C 3 months | –20°C 3 freeze-thaw cycles | 2–8°C 1 month |
| n | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Mean | 19.79 | 19.79 | 19.75 | 19.84 | 19.85 | 19.64 | 19.90 | 19.80 |
| Standard deviation | 0.17 | 0.06 | 0.09 | 0.06 | 0.12 | 0.22 | 0.09 | 0.08 |

| | $C_T$ values: Autosomal qPCR target male QIAamp cfDNA eluates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No eluate storage | –80°C 1 month | –80°C 3 months | –80°C 3 freeze-thaw cycles | –20°C 1 month | –20°C 3 months | –20°C 3 freeze-thaw cycles | 2–8°C 1 month |
| n | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Mean | 24.25 | 24.45 | 24.43 | 24.41 | 24.68 | 24.16 | 24.62 | 24.42 |
| Standard deviation | 0.10 | 0.12 | 0.09 | 0.11 | 0.12 | 0.32 | 0.09 | 0.07 |

## VI. Limitations

1. This tube and cup are for research use only. Not for use in diagnostic procedures. The clinical utility and the performance characteristics of the PAXgene Urine Liquid Biopsy Tube and Set as part of an in vitro diagnostic procedure have not been established.
2. The quantity of urine drawn should be approximately 10 mL for the PAXgene Urine Liquid Biopsy Tube, but this volume may vary depending on various factors such as altitude, ambient temperature, barometric pressure, tube age, and filling technique.
3. Due to the dead space in the cup, approximately 2.25 mL of the urine specimen will be unavailable in the cup resting on a flat surface. The cup may be tilted to fill the tube, and then approximately 0.6 mL of the urine specimen will be unavailable.

## VII. Warnings and Precautions

### A. Warnings:

1. The additive in this tube is a mild irritant and may cause irritation to the eyes and skin upon direct contact:
   - If inhaled, move to fresh air. If symptoms persist, call a physician.
   - In case of skin contact, wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.
   - In case of eye contact, remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
   - If accidentally swallowed, obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person.

   **Note:** For more information, please consult the appropriate safety data sheets (SDSs). The PAXgene Urine Liquid Biopsy Set SDS is available online in convenient and compact PDF format at **www.qiagen.com/safety**. The PAXgene Urine Liquid Biopsy Tube SDS is available online in convenient and compact PDF format at **regdocs.bd.com/regdocs/sds**.
2. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from splatter, leakage, and potential exposure to pathogens that could be present in urine. Handle all urine samples and urine collection devices according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any urine exposure since there is a risk of transmission of infectious diseases.
3. A sharp needle is located under the PreAnalytiX label on top of the blue cup cap. Do not remove the PreAnalytiX label on top of blue cap until ready to transfer urine into the tubes.
4. Excessive centrifugation speed (over 10,000 × g) may cause PAXgene Urine Liquid Biopsy Tube breakage, exposure to urine and possible injury.
5. The PAXgene Urine Liquid Biopsy Tube is not designed for use with open urine collection cups requiring manual sample transfer into the tube. It is not recommended to manually fill the PAXgene Urine Liquid Biopsy Tube from a syringe or other methods due to increased risk of biohazard exposure, needlestick injury and incorrect urine to additive ratio that may affect product performance. Additionally, removal of the stopper may compromise the sterility of the tube and increase risk of sample contamination.
6. After use, discard all urine collection cups and tubes and accessories in dedicated containers approved for their disposal.
7. Do not reuse the PAXgene Urine Liquid Biopsy Set.
8. Do not use the PAXgene Urine Liquid Biopsy Set after the expiration date printed on the label.

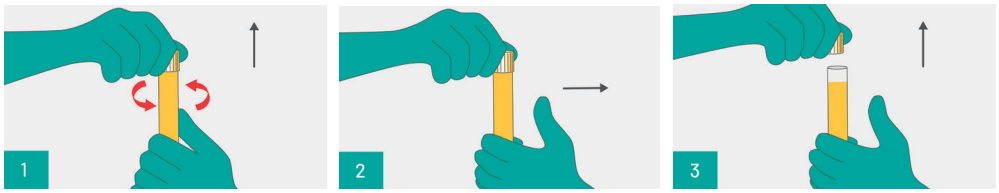
**B. Precautions:**

1. Examine cups and tubes prior to use. Do not use cups and tubes if foreign matter is present inside the cup or tube.
2. The PAXgene Urine Liquid Biopsy Tube additive may have a slightly yellow appearance; this does not affect the performance of the additive.
3. The PAXgene Urine Liquid Biopsy Tube may have a slight blue-gray tint; this does not affect the performance of the tube.
4. Do not shake the filled urine cup.
5. Consistently inverting the tube 8 times immediately after collection is recommended for optimal stabilization.
6. Filled tubes may be at risk for breakage if dropped from above benchtop height. Exercise caution when handling filled tubes to reduce the risk of possible exposure.
7. Underfilling of PAXgene Urine Liquid Biopsy Tubes will result in an incorrect urine-to-additive ratio and may lead to incorrect analytical results or poor product performance.
8. Remove the BD Hemogard closure (yellow tube cap) with a twist and pull motion. Removal by rolling with the thumb is not recommended (for detailed instructions see Section IX. Removal of BD Hemogard Closure).
9. The concentration of cfDNA in urine may be low and varies considerably by many factors including among different individuals, sex and health status of the donating individual, time of collection and hydration status.

## VIII. Storage

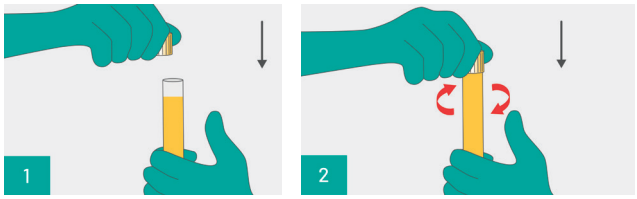
Store the unused PAXgene Urine Liquid Biopsy Set at 4–25°C. Brief temperature excursions from –10°C to 40°C are permitted. The PAXgene Urine Liquid Biopsy Tube additive may have a slightly yellow appearance; this does not affect the performance of the additive. The PAXgene Urine Liquid Biopsy Tube may have a slight blue-gray tint; this does not affect the performance of the tube. Do not use any tube or cup after the expiration date printed on the label.

## IX. Removal of BD Hemogard Closure




1. Grasp the PAXgene Urine Liquid Biopsy Tube with one hand, placing the thumb under the BD Hemogard closure (for added stability, place arm on solid surface). With the other hand, twist the BD Hemogard Tube closure while simultaneously pushing up with the thumb of the other hand UNTIL THE TUBE STOPPER IS slightly LOOSENED.
2. Move thumb away before lifting closure. DO NOT use thumb to push closure off tube. If the tube contains urine, an exposure hazard exists.
3. Lift closure off tube. In the unlikely event of the plastic shield separating from the rubber stopper, DO NOT REASSEMBLE CLOSURE. Carefully remove rubber stopper from tube.

## X. Reinsertion of BD Hemogard Closure




1. Replace closure over tube.
2. Twist and push down firmly until stopper is fully reseated. Complete reinsertion of the stopper is necessary for the closure to remain securely on the tube during handling.

## XI. Technical Assistance

For technical assistance and more information regarding PAXgene Urine Liquid Biopsy Set, please see our Technical Support Center at **support.qiagen.com**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (more information at **www.qiagen.com**).

## XII. Ordering Information PAXgene Products

- **PAXgene Urine Liquid Biopsy Set (20) / REF 769143**

Content: 20 urine collection cups

| | |
|---|---|
| Up to 120 mL Urine | Sterile |
| 20 cups/case | RUO |

20 urine stabilization, transport and storage tubes

| | | |
|---|---|---|
| 10.0 mL • 16 × 100 mm | 1.5 mL additive | Sterile |
| BD Hemogard closure | 20 tubes/case | RUO |

- **PAXgene Urine Liquid Biopsy Tube (50) / REF 769114**

Content: 50 urine stabilization, transport and storage tubes

| | | |
|---|---|---|
| 10.0 mL • 16 × 100 mm | 1.5 mL additive | Sterile |
| BD Hemogard closure | 50 tubes/case | RUO |

### To order PAXgene Urine Liquid Biopsy Set and Tubes:

Go to: **www.preanalytix.com**

### QIAGEN Products*

**For cfDNA Isolation:**

**QIAamp Circulating Nucleic Acid Kit / REF 55114**

For 50 preps: QIAamp Mini Columns, Tube Extenders (20 mL), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors, and Collection Tubes (1.5 mL and 2 mL)

For 25 preps: The content of the kit is sufficient for 25 preparations when using Supplementary Protocol (Section I. Isolation of cfDNA from urine supernatant) rather than the 50 preparations specified in the QIAamp Circulating Nucleic Acid kit handbook. For 50 preparations starting from 10 mL stabilized urine, 220 mL of Buffer ACL (1x, catalog number 939017), 300 mL of Buffer ACB (1x, catalog number 1069275), and 30 mL of QIAGEN Proteinase K (3×, containing 10 mL each, catalog number 19133) must be purchased separately.

**QIAGEN QIAsymphony Circulating DNA Kit**

For 192 preps: Reagent cartridges, accessories and proteinase K vials for 192 preps of 2,000 µL or 4,000 µL each.

(QIAGEN QIAsymphony Circulating DNA Kit is not available in all countries. For further details please contact **support.qiagen.com**)

**To order QIAGEN circulating DNA preparation kits:**

Go to: **www.qiagen.com/shop**

** These catalog numbers represent typical sample preparation kits that can be used with the PAXgene Urine Liquid Biopsy Set.*

**QIAGEN – Customer Service**

Ordering **www.qiagen.com/shop** | Technical Support **support.qiagen.com** | **www.qiagen.com**

**BD – Customer Service**

Ordering **www.bdbiosciences.com/en-us/products/blood-collection** | Technical Support **www.bd.com/en-us/support** | Website **www.bd.com**

### Change History

| Revision | Date | Change Summary |
|---|---|---|
| 01 | 2024-09 | First Issue |

## SYMBOLS GLOSSARY

Please refer to product labeling for applicable symbols.

| Symbol | Meaning |
|---|---|
| | Manufacturer |
| EC REP | Authorized representative in the European Community |
| CH REP | Authorised representative in Switzerland |
| | Date of manufacture |
| | Use-by date |
| LOT | Batch code |
| REF | Catalogue number |
| SN | Serial number |
| STERILE | Sterile |
| STERILE A | Sterilized using aseptic processing techniques |
| STERILE EO | Sterilized using ethylene oxide |
| STERILE R | Sterilized using irradiation |
| STERILE | Sterilized using steam or dry heat |
| | Do not resterilize |
| NON STERILE | Non-sterile |
| | Do not use if package is damaged and consult ***instructions for use*** |
| STERILE | Sterile fluid path |
| STERILE EO | Sterile fluid path (ethylene oxide) |
| STERILE R | Sterile fluid path (irradiation) |
| | Fragile, handle with care |
| | Keep away from sunlight |
| | Keep dry |
| | Lower limit of temperature |
| | Upper limit of temperature |
| | Temperature limit |
| % | Humidity limitation |
| | Biological risks |
| | Do not re-use |
| | Consult *instructions for use* or consult electronic *instructions for use* |
| | Caution |
| LATEX | Contains or presence of natural rubber latex |
| IVD | In vitro diagnostic medical device |
| CONTROL – | Negative control |
| CONTROL + | Positive control |
| Σ | Contains sufficient for <*n*> tests |
| | For IVD performance evaluation only |
| | Non-pyrogenic |
| # | Patient number |
| | This way up |

| Symbol | Meaning |
|---|---|
| | Do not stack |
| | Single sterile barrier system |
| PHT DEHP BBP | Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP) |
| | Collect separately<br>Indicates separate collection for waste of electrical and electronic equipment required. |
| CE | CE marking; Signifies European technical conformity |
| | Device for near-patient testing |
| | Device for self-testing |
| Rx Only | This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner." |
| CC | Country of manufacture<br>"CC" shall be replaced by either the two letter or the three letter country code. |
| | Collection time |
| | Cut |
| | Peel here |
| | Collection date |
| | Keep away from light |
| $H_2$ | Hydrogen gas is generated |
| | Perforation |
| | Start panel sequence number |
| | End panel sequence number |
| | Internal sequence number |
| / | <Box #> / <Total Boxes> |
| MD | Medical device |
| | Contains hazardous substances |
| | Ukrainian conformity mark |
| FC | Meets FCC requirements per 21 CFR Part 15 |
| c UL us | UL product certification for US and Canada |
| UDI | Unique device identifier |
| | Importer |
| | Place patient label in framed area only |
| MR | Magnetic resonance (MR) safe |
| MR | Magnetic resonance (MR) conditional |
| MR | Magnetic resonance (MR) unsafe |
| This Product Contains Dry Natural Rubber | This Product Contains Dry Natural Rubber |
| For Export Only | For Export Only |

Note: Text layout in symbols is determined by label design.

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The PAXgene Urine Liquid Biopsy Set and Tube are made in the UK by BD for PreAnalytiX GmbH.



Patent **www.preanalytix.com/patents**

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