Human Saliva Stabilization and Sample Collection Standardization Prevent Genomic DNA Degradation Enabling Reliable and Reproducible Test Results

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Introduction

Saliva is an easy-to-use specimen that offers the possibility to examine a wide range of analytes, including human genomic DNA (gDNA) as well as microbial or pathogenic nucleic acids. In contrast to blood sampling, saliva collection is non-invasive and may even be performed at home without the involvement of medical personnel. As long as nucleic acid stability is maintained, the sample could be shipped to the testing laboratory at ambient temperature.

In this study, we evaluated the stability of gDNA in samples collected and stored in PreAnalytiX PAXgene® Saliva Collector in comparison to unstabilized saliva samples. When working with saliva samples, standardized preanalytical workflows are of high importance in order to reduce errors and allow for reproducible and reliable analytical test results. Therefore, experiments presented here were performed according to ISO 4307:2021-10 Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for saliva – Isolated human DNA.

Data showed that human gDNA in unstabilized saliva samples underwent rapid degradation starting as early as at the time of collection and this degradation was continuously increasing during the first days following saliva collection. However, if the human saliva sample was collected using the PreAnalytiX PAXgene® Saliva Collector, the gDNA was stabilized and did not show any signs of degradation for at least 24 months of sample storage at room temperature.

Methods

Saliva was collected from consented, apparently healthy adult donors into PAXgene Saliva Collectors and 15 ml tubes without a stabilization reagent (n = 6–10). PAXgene Saliva stabilized samples, as well as unstabilized samples, were stored at room temperature (RT) or at 4 °C. gDNA was extracted from 200 µl of the stabilized and unstabilized saliva samples directly after collection and after 1 hour, 2 hours, 3 hours, 4 hours, 1 day, 2 days, 3 days, 4 days, 7 days, and 14 days storage using the QIAGEN QIAamp® DNA Mini Kit manually or automated on the QIAGEN QIAcube® Connect or the Gentra Puregene Cell Kit. The gDNA samples were analyzed for human DNA quantity and degradation status using the QIAGEN Investigator® Quantiplex® Pro RGQ Kit. The Investigator Quantiplex Pro Kit detects a small (91 bp) and a longer amplification product (353 bp) targeting the same locus. The longer target is more susceptible to DNA degradation, which allows for the assessment of the degradation status of the DNA in form of the degradation index. The degradation index is the quantification of the small fragment divided by the quantification of the long fragment. While an ideal degradation index of 1 indicates no degradation, values above 5 indicate DNA fragments of less than 300 bp. DNA profile was determined with the Agilent 4200 TapeStation® System using Genomic DNA ScreenTape® analysis.

Results:

The results of this study demonstrated that human saliva gDNA degrades quickly in unstabilized samples, whereas DNA integrity is maintained in PAXgene Saliva Collector stabilized samples.

1. **Onset of human gDNA degradation during first four hours after saliva collection in unstabilized samples.**
   While DNA yield (µg/ml human saliva) as determined by amplification of the 91 bp amplification product for gDNA quantification using Investigator Quantiplex Pro Kit and DNA integrity were not impaired in PAXgene Saliva samples during the course of the storage, an onset of degradation was detected in unstabilized saliva samples when assessing the degradation index (Figure 1). Also, TapeStation Analysis revealed that already after 4 hours, the DNA profile in unstabilized samples was affected whereas DNA profile was maintained in PAXgene Saliva samples (Figure 2).

Figure 1

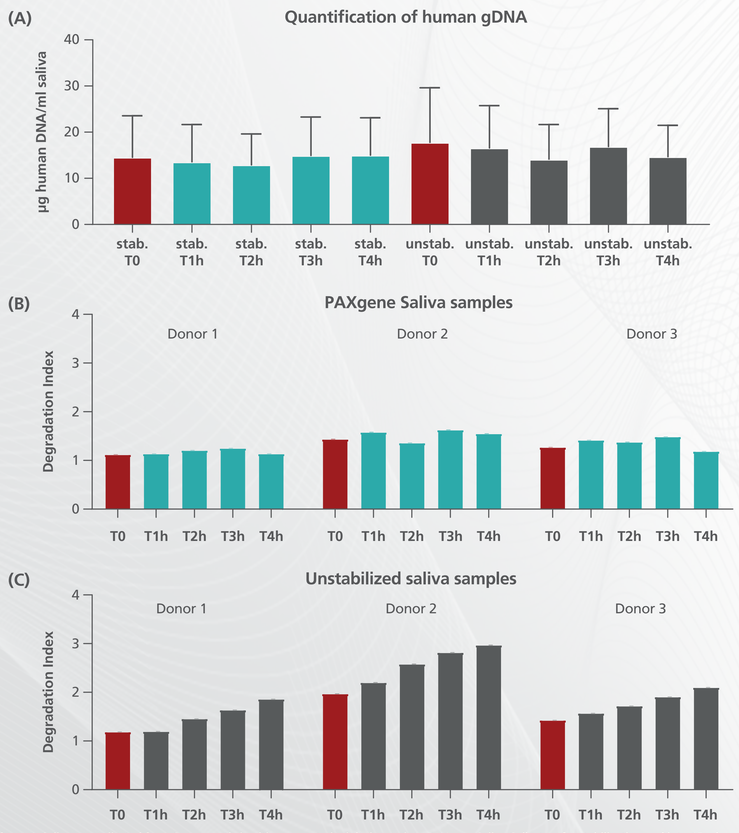

Figure 1: Saliva was collected from 6 apparently healthy consented adult donors into PAXgene Saliva Collectors for stabilization and into 15 ml tubes without stabilization reagent. Stabilized and unstabilized samples were kept at RT for 4 hours. Human gDNA extraction was performed automated directly after collection (T0) and every hour (T1h-T4h) using the QIAGEN QIAamp® DNA Mini Kit on the QIAcube Connect. Human gDNA was quantified in PAXgene Saliva Collector stabilized samples (green) and unstabilized samples (grey) (A). The degradation index was determined and is shown in three representative stabilized samples (B) and unstabilized samples (C) using Investigator Quantiplex Pro RGQ Kit.

Figure 2

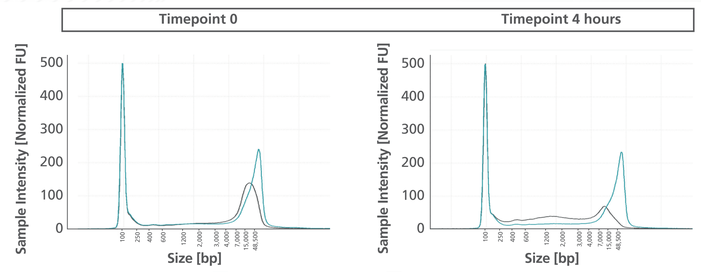

Figure 2: Representative profiles of human gDNA samples (n = 1). Human gDNA profile was determined with the Agilent 4200 TapeStation System using Genomic DNA ScreenTape analysis in PAXgene Saliva Collector stabilized samples (green) and unstabilized saliva samples (grey) at timepoint 0 directly after collection (left graph) and after the saliva sample was stored for 4 hours (right graph).

2. **Severe degradation of human gDNA within the first 4 days after saliva collection in unstabilized samples.**
   Results show that yield determined by quantification of a 91 bp product using the Investigator Quantiplex Pro assay decreased continuously in unstabilized samples stored at RT. This is in line with an increased degradation index in the same samples (Figure 3). While gDNA yield determined by qPCR remained more stable in unstabilized saliva samples stored at 4°C, these samples also showed an increase in degradation as detected by the degradation index (Figure 3). In addition, unstabilized samples stored at RT or 4°C showed a decline of the gDNA profile already after one day which was further deteriorating over storage time (Figure 4). In contrast, PAXgene Saliva samples remained stable during storage at RT, gDNA yield determined by qPCR and DNA profile were maintained at the same level in comparison to T0 (Figure 3 and 4).

Figure 3

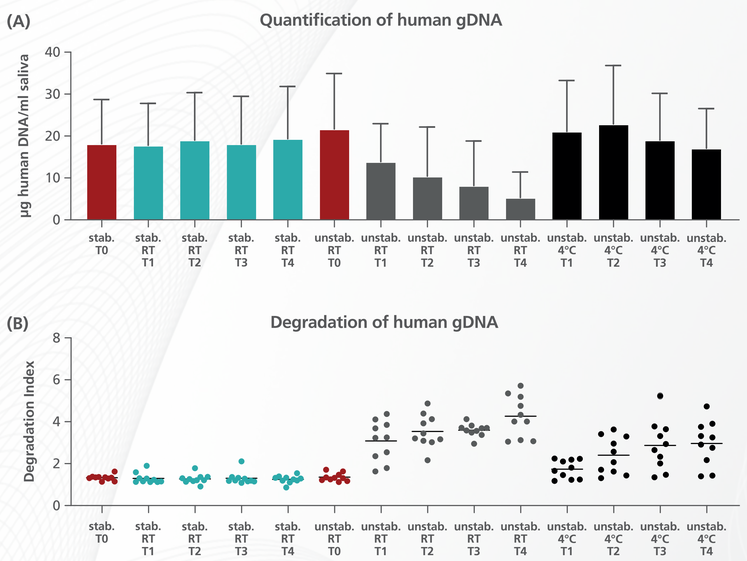

Figure 3: Saliva was collected from 10 apparently healthy consented adult donors into PAXgene Saliva Collector for stabilization and into 15 ml tubes without stabilization reagent. PAXgene saliva samples were kept at RT, unstabilized samples were kept both at RT and at 4°C for 4 days. Human gDNA extraction was performed directly after collection (T0) and on every following day (T1-T4) using the QIAGEN QIAamp® DNA Mini Kit. Human gDNA was quantified (A) and the degradation index was determined in stabilized samples (green) and unstabilized samples (grey – RT; black – 4°C) using Investigator Quantiplex Pro RGQ Kit.

Figure 4

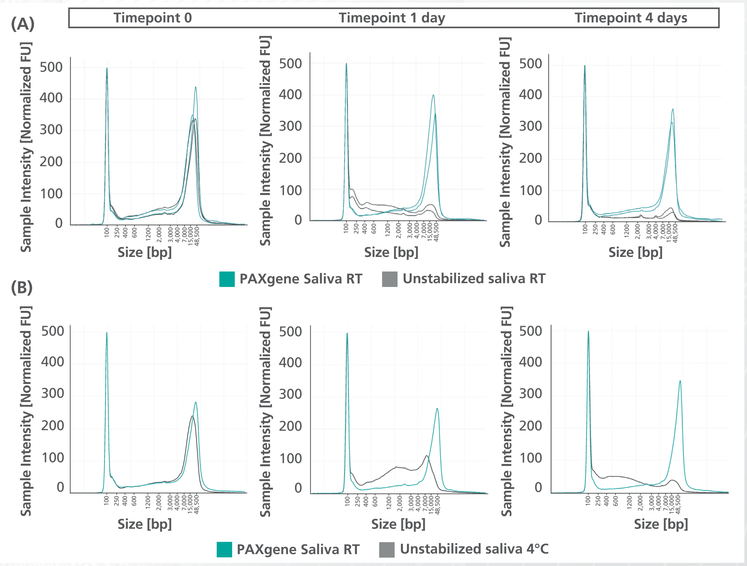

Figure 4: Representative profiles of human gDNA samples in A (n = 2) and B (n = 1). Human gDNA profile was determined with the Agilent 4200 TapeStation System using Genomic DNA ScreenTape analysis in PAXgene Saliva Collector stabilized samples (green) and unstabilized saliva samples (grey) at timepoint 0 directly after collection (left) and after the saliva sample was stored for 1 day (middle) and 4 days (right) at RT (upper panel) or 4°C (lower panel).

3. **Complete degradation of human gDNA in unstabilized saliva samples after 14 days storage at room temperature**
   Results show that gDNA yield determination by qPCR using the Investigator Quantiplex Pro Kit failed in unstabilized samples stored at RT for 7 and 14 days, indicating that DNA was degraded and could not be efficiently amplified, while gDNA yield determined by qPCR was maintained in PAXgene Saliva stabilized samples. The degradation index in unstabilized samples increased significantly in comparison to PAXgene stabilized samples which did not show any increase even after 14 days of storage at RT (Figure 5B). The DNA profile of 10 PAXgene Saliva and 10 unstabilized samples after 14 days of storage at RT is shown in Figure 5C. The DNA profile in PAXgene Saliva samples was maintained over the course of two week (14 days) but showed severe fragmentation in unstabilized samples (Figure 5C).

Figure 5

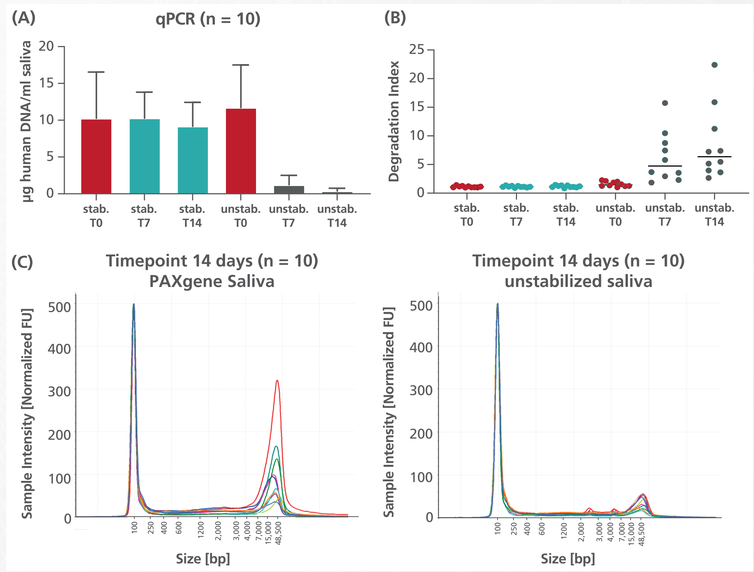

Figure 5: Saliva was collected from 10 apparently healthy consented adult donors into PAXgene Saliva Collector for stabilization and into 15 ml tubes without stabilization reagent. PAXgene Saliva samples and unstabilized saliva samples were kept at RT for up to 14 days. Human gDNA extraction was performed directly after collection (T0) and after 7 and 14 days (T7 and T14, respectively) using the QIAGEN QIAamp® DNA Mini Kit. Human gDNA was quantified (A) and the degradation index was determined in stabilized samples (green) and unstabilized samples (grey) using Investigator Quantiplex Pro RGQ Kit. Human gDNA profile was determined with the Agilent 4200 TapeStation System using Genomic DNA ScreenTape analysis in 10 PAXgene Saliva Collector stabilized samples (left) and 10 unstabilized saliva samples (right) after the samples were stored at RT for 14 days (C).

4. **Human gDNA levels are maintained for at least 24 months in saliva samples stabilized with the PAXgene Saliva Collector**
   A long-term stability study demonstrated that gDNA yield and degradation index, as determined by qPCR, were maintained for at least 24 months of storage at RT in PAXgene Saliva stabilized samples (this long-term stability study is still ongoing).

Figure 6

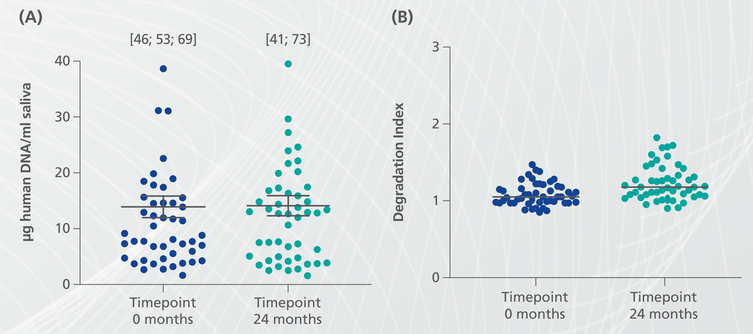

Figure 6: Saliva was collected from 49 apparently healthy consented adult donors into the PAXgene Saliva Collector. DNA was extracted from 200 µl stabilized saliva using Gentra Puregene Cell Kit immediately after stabilization (Timepoint 0 months, blue) and after 24 months of storage at room temperature (15–25°C) (Timepoint 24 months, green). Human DNA yield (A) and gDNA degradation index (B) were determined with Investigator Quantiplex Pro RGQ Kit.

Conclusion:

Data presented in this study show that human genomic DNA from human saliva is stabilized when collected with the PAXgene Saliva Collector. The pre-filled stabilization solution in the PAXgene Saliva Collector helped to preserve human gDNA levels by protecting DNA from degradation for at least 24 months of storage at room temperature. In unstabilized samples, however, gDNA degradation started within hours after collection, as detected by the Investigator Quantiplex Pro Kit and TapeStation Analysis, and increased rapidly. After one day only, qPCR results were no longer reliable in unstabilized samples as the severe DNA degradation affected the amplification of the qPCR target.