Long Term Stability of DNA Stored in Norgen's Saliva DNA Preservative

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INTRODUCTION

In recent years, attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of saliva. The isolation of high quality DNA from saliva is not without its problems however. The number of DNA-containing cells found in saliva can vary significantly from individual to individual. Adequate amounts of saliva must therefore be collected to ensure that DNA can be extracted in an amount sufficient for testing. As enzymes that degrade DNA are found in saliva, methods must also be employed to protect the DNA in the sample. For currently available collection devices which do not employ preservative this requires that the saliva be collected into approved cryovials, held on ice and then frozen at -20°C as soon as possible. The addition of preservative to the collection vials eliminates the need to immediately process or freeze the saliva samples and allows the samples to be shipped at ambient temperature.

Norgen Biotek Corp. has developed a Saliva DNA Preservative which allows for the long-term preservation of saliva samples at ambient room temperature, making this buffer ideal for saliva storage and shipping. This buffer is available as a product on its own, and is also included with our different saliva DNA collection, preservation, storage and purification devices and kits. The Saliva DNA Preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh specimens. This preservative stabilizes the DNA for long-term storage at ambient temperature. Since the buffer prevents the growth of microorganisms and inactivates viruses it also allows the samples to be handled and shipped safely. The DNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR. In this application note, the long-term stability of saliva DNA in Norgen's Saliva DNA Preservative is analyzed.

Preserved saliva samples were stored at -20°C, 4°C, room temperature and 55°C for up to 12 months, to demonstrate the stability of saliva DNA in Norgen's Saliva DNA Preservative at the various temperatures that may be encountered during shipping and some instances of short-term storage. The preserved saliva sample at room temperature was further stored up to 32 months, to demonstrate the long-term storage of the preserved DNA. DNA was subsequently isolated from the various samples after different time points, and the DNA was analyzed through visual analysis on gels, as well as through the use of the DNA as a template in real-time PCR.

METHODS AND MATERIALS

DNA Isolation

Saliva samples were collected from numerous donors and mixed, and then an equal volume of Norgen's Saliva DNA Preservative was added to the saliva. Aliquots of the preserved DNA saliva samples were stored at -20°C, 4°C, room temperature and 55°C for up to 12 months. Saliva DNA was subsequently isolated at various time points from 0.5 mL of the saliva/preservative sample using Norgen's Saliva DNA Isolation Kit (Cat. RU45400).

The preserved samples stored at room temperature were further stored for up to 32 months, and again saliva DNA was isolated at various time points from 0.5 mL of the saliva/preservative sample using Norgen's Saliva DNA Isolation Kit (Cat. RU45400).

Gel electrophoresis

For visual analysis, aliquots of DNA from the final DNA elutions were loaded onto a 1% agarose TAE gel and run for 25 minutes at 150 V. Gel photos were taken using an AlphaImagerTM IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA was then used as the template in real-time PCR reactions. For the saliva samples stored at the 4 different temperatures for up to 1 year, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture (SYBR Green) containing 2.5 mM 5S primer pair. The PCR samples were amplified under the real-time program; 95°C for 5 minutes for an initial denaturation, 40 cycles of 95°C for 15 second for denaturation and 60°C for annealing and extension. The reaction was run on an iCycler iQ real-time system (Bio-Rad).

For the saliva samples that were stored at room temperature for up to 32 months, 20 ng of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 2.5 mM GAPDH primer pair and TaqMan probe (FAM). The PCR samples were amplified under the real-time program; 95°C for 5 minutes for an initial denaturation, 50 cycles of 95°C for 15 second for denaturation and 60°C for annealing and extension. Again, the reaction was run on an iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Saliva samples often need to be stored for a period of time prior to DNA isolation and analysis. Traditionally saliva samples are held on ice and then frozen at -20°C as soon as possible, however such storage leads to increased costs and is not always convenient, especially in resource-limited settings. The ability to safely store saliva samples at ambient temperatures without any degradation of the DNA is ideal. Here, saliva samples are mixed with Norgen's Saliva DNA Preservative and stored at -20°C, 4°C, room temperature and 55°C for up to 12 months, to demonstrate the stability of saliva DNA in Norgen's Saliva DNA Preservative at the various temperatures that may be encountered during shipping and some instances of short-term storage.

The preserved saliva samples at room temperature were further stored up to 32 months, to demonstrate the long-term storage of the preserved DNA. Saliva DNA was isolated from the saliva samples stored at the 4 different temperatures for up to 1 year in triplicate at time 0, 1 month, 2 months, 4 months, 6 months and 12 months. The isolated saliva DNA was subsequently run on a 1% agarose TAE gel for visual analysis. There was no evidence of DNA degradation after the saliva samples were stored for 1 year at all the temperatures in Norgen's Saliva DNA Preservative. Furthermore, the size of the DNA was maintained over 24 kb for the entire 12 month period (data not shown).

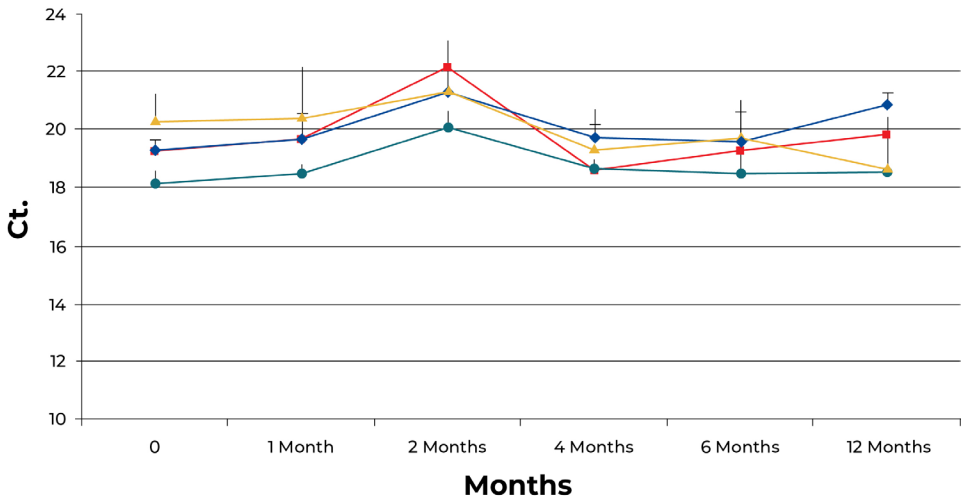

Figure 1. Real-Time PCR detecting the 5S gene from saliva DNA isolated from different temperatures (-20°C, 4°C room temperature and 55°C) at different time points (time 0, 1 month, 2 months, 4 months, 6 months and 12 months). The quality of the saliva DNA stored at all 4 temperatures did not change from 0 month to 12 months.

These isolated saliva DNA samples were then used as the template in a real-time PCR reaction to detect the 5s gene. DNA samples must be of a high quality in order to be used successfully in downstream amplification reactions. As it can be seen in Figure 1, the DNA isolated from the saliva samples stored at -20°C, 4°C, room temperature and 55°C for all the time points could all be successfully amplified using real time PCR.

Furthermore, the quality of the saliva DNA stored at all 4 temperatures did not change from 0 months to 12 months, as indicated by the fact that the Ct value remained consistent. Therefore, saliva DNA can be stored in Norgen's Saliva DNA Preservative at temperatures ranging from -20°C to 55°C for up to 1 year.

The preserved saliva samples at room temperature were further stored up to 32 months to demonstrate the long-term storage of the preserved DNA. Saliva DNA was subsequently isolated at 1 week, 1 month, 4 months, 20 months and 32 months from 0.5 mL of the saliva/preservative sample using Norgen's Saliva DNA Isolation Kit (Cat. RU45400). For visual analysis, 200 ng of the purified DNA was run on an agarose TAE gel **(Figure 2)**.

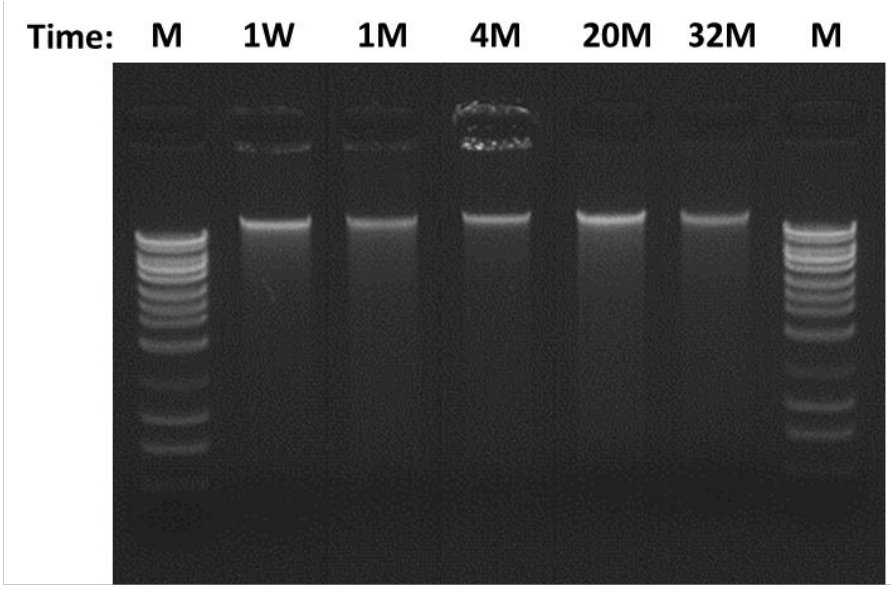

Figure 2. Stability of DNA preserved in Norgen's Saliva DNA Preservative at ambient temperature for up to 32 months. M: Norgen's UltraRanger 1 Kb DNA Ladder (Cat. 12100).

As it can be seen, there is no evidence of DNA degradation after the saliva samples are stored for 32 months at ambient temperatures in Norgen's Saliva DNA Preservative. Furthermore, the size of the DNA was maintained over 24 kb for the entire 32 month period.

Next, 20 ng aliquots of the purified DNA from the saliva samples stored at room temperature for up to 32 months were used as the template in a TaqMan real-time PCR reaction to detect the GAPDH gene. Each reaction was run in duplicate. The results of the amplification can be seen in Figure 3, and the average Ct values from the amplification are graphed in **Figure 4**.

As it can be seen the DNA isolated from the saliva samples stored at room temperature from 1 week up to 32 months could all be successfully amplified using real time PCR. Furthermore, the quality of the saliva DNA stored at room temperature did not change from 1 week to 32 months, as indicated by the fact that the Ct value remained consistent. Therefore, saliva DNA can be stored in Norgen's Saliva DNA Preservative at room temperature for up to 32 weeks with no signs of degradation.

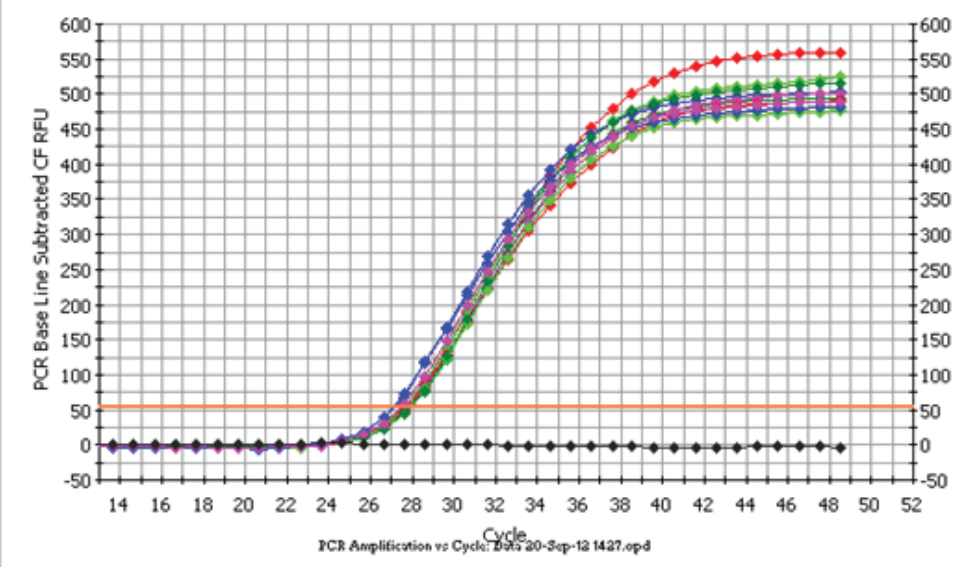

Figure 3. Real-time PCR detecting the GAPDH gene from saliva DNA stored at room temperature for up to 32 months. DNA was isolated at various time points (1 week, 1 month, 4 months, 20 months, 32 months) and was used as the template in the reaction. The reactions were run in duplicate. The DNA was of a high quality and could be amplified from all time points tested up to 32 months.

It should be noted that the saliva samples stored at 55°C for 1 year can allow us to extrapolate results based on accelerated-aging. According to the Arrhenius equation, the higher the temperature the faster a given chemical reaction will proceed, including DNA degradation.

Accordingly, the rate of a chemical reaction typically decreases by half for every 10°C decrease in temperature. Therefore the rate of chemical degradation of DNA at room temperature can be expected to be 6 -fold slower than the rate of degradation at 55°C. Based on this calculation the saliva preserved at 55°C for 12 months indicates equal stability to saliva preserved for 5 years at room temperature (24°C). Therefore, based on the successful PCR results for samples stored at 55°C shown in Figure 1 above, saliva samples stored in Norgen's Saliva DNA Preservative are stable at room temperature for up to 5 years.

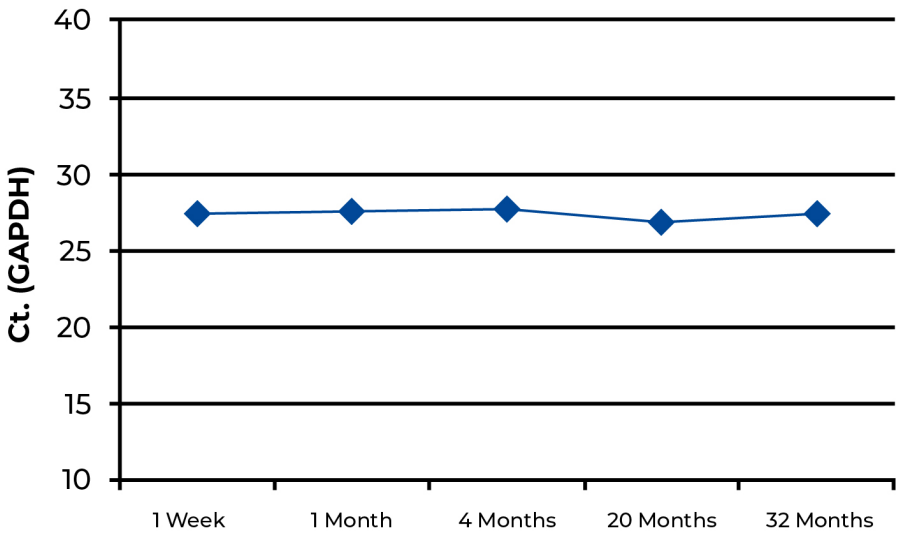

Figure 4. A real-time PCR reaction to detect the GAPDH gene from saliva DNA stored at room temperature for up to 32 months was performed. DNA was isolated at various time points (1 week, 1 month, 4 months, 20 months, 32 months) and was used as the template in the reaction, and the average Ct values for each time point were plotted. The quality of the saliva DNA did not change over the 32 months.

CONCLUSIONS

Saliva DNA can be stored in Norgen's Saliva DNA Preservative at temperatures ranging from -20°C to 55°C for up to 1 year with no signs of degradation. Based on real-time data, saliva DNA can be stored in Norgen's Saliva DNA Preservative at room temperature for up to 32 months with no signs of degradation. Based on accelerated-aging, saliva DNA can be stored in Norgen's Saliva DNA Preservative for up to 5 years at room temperature.

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Saliva DNA Isolation Kit	50 Preps	RU45400

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