The Effect of Elution Volume on DNA Quantity and Quality Using Norgen's Saliva DNA Isolation Kit

APPLICATION NOTE #55

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INTRODUCTION

Saliva is a useful bodily fluid for diagnostic and research purposes. Collection is non-invasive and practical, as DNA isolated from saliva can be used for the screening and detection of biomarkers of cancer and autoimmune disorders, as well as for genotyping and more[1,2] . Norgen Biotek Corp. has developed a simple method for the collection, preservation, and storage of DNA from saliva using Individual Saliva DNA Collection and Preservation Devices (Cat# 35710). Donors simply collect their saliva directly into the Collection Tube and add Norgen's Saliva DNA Preservative. The preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh specimens. This buffer stabilizes the DNA for long-term storage at ambient temperatures. Since the buffer prevents the growth of microorganisms and inactivates viruses, it also allows the samples to be handled and shipped safely. Congruently, Norgen has also developed a Saliva DNA Isolation Kit that is fast, reliable, and very customer-friendly, eluting saliva DNA of the highest quality and yield that can be used directly in sensitive downstream diagnostic assays such as PCR.

Investigators utilizing saliva in their research have unique needs, based on their downstream applications and sample volume. For some studies, the highest yield of DNA possible from individual samples is required, while other researchers have an array of downstream applications, thus requiring a large elution volume to accommodate the necessary sample volume. Therefore, investigators must ensure that their saliva DNA isolation method allows for flexible elution volumes, i.e. eluting in a small elution volume does not compromise DNA yield, and eluting in a larger volume does not render the sample too dilute for downstream applications.

The purpose of this study is to demonstrate the ability of Norgen's Saliva DNA Isolation Kit to elute saliva DNA using increasing elution volumes, and the impact on DNA quantity and quality.

METHODS AND MATERIALS

Sample collection

Four milliliters of saliva was collected from three different participants. All samples were preserved in Norgen's saliva preservative, and pooled together.

Saliva DNA extraction

DNA was extracted from all saliva samples using Norgen's Saliva DNA Isolation Kit (Cat# 45400), as per the manufacturer's instruction. Briefly, saliva samples were incubated at 55°C for 1 hour prior to DNA isolation. After inverting each saliva sample, 500 µL of preserved saliva was added to new microcentrifuge tube. Samples were then incubated at 55°C for 20 minutes with 20 µL of Proteinase K, Binding Solution was added along with ethanol, and samples were bound, washed and eluted as per manufacturer's instruction.

Spectrophotometry

Saliva DNA quantity and quality was measured using the UltraSpec 2100 Pro (Fisher Scientific). Briefly, 50 µL of each saliva DNA elution was diluted with 450 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

Real-Time PCR

The purified DNA was then used as the template in a realtime TaqMan PCR reaction. Briefly, 9 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 125 nM GAPDH primer pair, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (BioRad).

RESULTS AND DISCUSSION

The use of saliva as a diagnostic medium is steadily increasing, as collection is easy and non-invasive. Coinciding with the demand for a reliable, robust saliva DNA isolation method is the increasing number of downstream applications used for analyzing the isolated saliva DNA. With some studies requiring high DNA concentrations, and others requiring large elution volumes to be used in a number of downstream applications, researchers must be confident that their saliva DNA isolation method has been optimized for both ends of the spectrum.

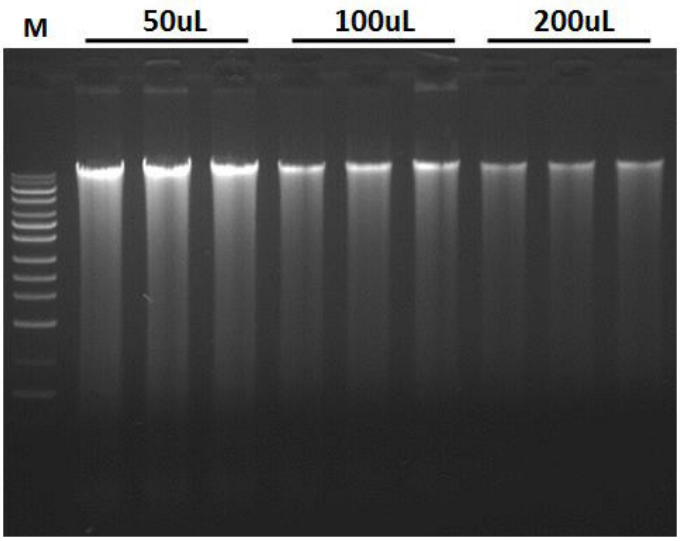

Figure 1. Resolution of DNA isolated from pooled saliva collected from 3 donors preserved using Norgen's Saliva Preservative, and isolated using Norgen's Saliva DNA Isolation Kit. Twenty microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. M= Norgen's UltraRanger DNA Ladder.

One issue with a low elution volume is that DNA may be left behind on the column, thus wasting a portion of the DNA available in the sample. The main concern with a large elution volume is that samples may be too dilute for some downstream applications. An ideal saliva DNA isolation kit will minimize both of these issues by demonstrating a linear relationship between elution volume and DNA concentration.

In this study, saliva was collected from three different participants, preserved in Norgen's saliva preservative, and pooled together. DNA was then isolated from 500 µL of preserved saliva, and run on a 1.0%, 1X TAE agarose gel (Figure 1). The increase in saliva DNA concentration from decreasing elution volumes is clearly demonstrated in Figure 1, with the relationship between elution volume and DNA yield being evidently linear.

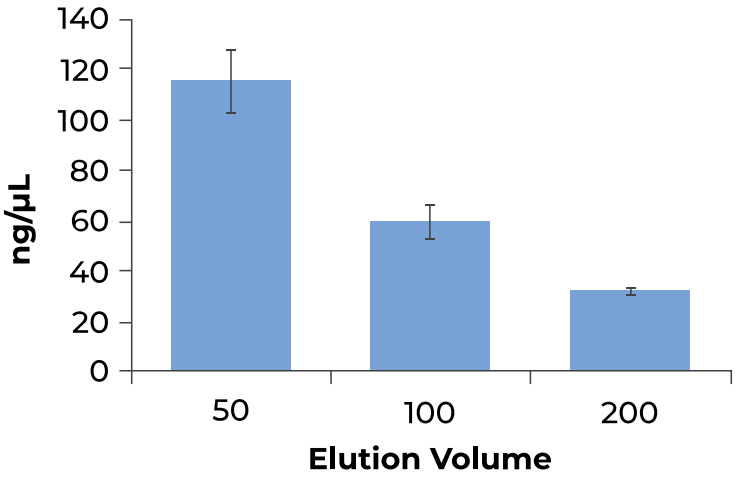

Figure 2. Linear decrease in DNA concentration corresponding to a linear increase in elution volume. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

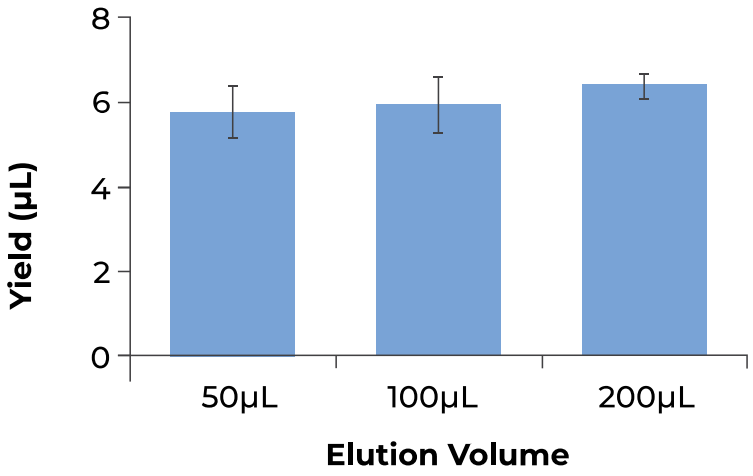

Figure 3. Slight increase in DNA yield corresponding to an increase in elution volume. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). When samples were eluted in 50 µL or 100 µL, DNA yields were on average 90% and 93% that of the samples eluted in 200 µL, respectfully.

DNA concentrations were then measured using the UltraSpec 2100 Pro (Fisher Scientific), with calculated DNA yields correlating with the gel photo in Figure 1 (Figure 2). Once again, the linear relationship between elution volume and DNA concentration can be seen.

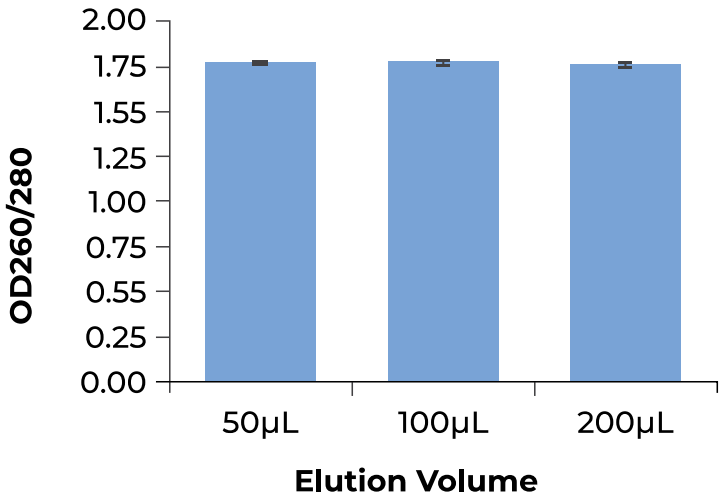

Figure 4. Virtually no change in A260:A280 when elution volume is adjusted. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and spectrophotometry measurements were taken using the UltraSpec 2100 Pro (Fisher Scientific).

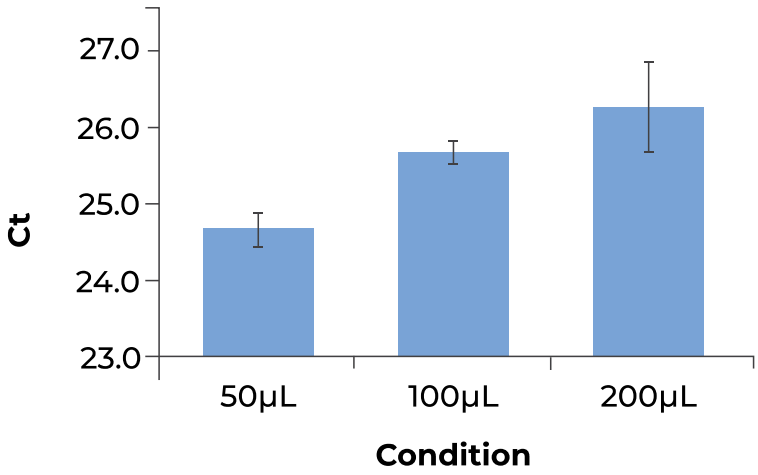

Figure 5. Linear decrease in Ct value corresponding to a linear increase in saliva volume processed. Nine microliters of saliva DNA template was used in a 20 µL TaqMan® qPCR reaction involving GAPDH primers, using the iCycler Thermal Cycler (BioRad Laboratories).

In order to determine the amount of DNA compromised by decreasing elution volumes, DNA yields were calculated based on the concentrations determined by spectrophotometry (Figure 3). As can be seen, DNA yields increase slightly when the elution volume increases. The percent recovery from 50 µL and 100 µL elutions was then determined to be 90% and 93%, respectfully, compared to the 200 µL control.

One concern with altering sample elution volume would be a change in DNA quality. High quality saliva DNA is required in most sensitive downstream applications, and is thus extremely important when evaluating a saliva DNA isolation method. Two methods were used in this study to demonstrate DNA quality: the A260:A280 ratio and the Ct values generated from a TaqMan qPCR reaction.

The A260:A280 ratio is a common measurement of nucleic acid quality. In this study, a cuvette-based spectrophotometry method was used to determine the A260:A280 of the saliva DNA samples (UltraSpec 2100 Pro; Fisher Scientific). The A260:A280 values were then graphed, and can be visualized in Figure 4. As can be seen, the A260:A280 values were not significantly affected by adjusting the elution volume, and with the average A260:A280 of all samples being ~1.75, this is indicative of minimal to no RNA contamination.

The final method of DNA quality determination was through the use of a TaqMan Real-Time PCR method. In order to assess sample inhibition, 9 µL of sample was used in the reaction. The Ct values were then graphed, and depicted in Figure 5. It was found that none of the saliva samples exhibited inhibition, and the decrease in Ct values was linear with the decrease in the elution volume (higher concentrated samples).

CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Elution Volumes can be Decreased to Allow for Higher DNA Concentrations using Norgen's Saliva DNA Isolation Kit. In this report, we demonstrate the ability to decrease the volume of elution in order to increase DNA concentration in a sample. This is important for many downstream applications requiring high DNA concentrations for optimal results.
2. Decreasing Elution Volumes Does not Significantly Compromise DNA Yields. When DNA elutions were decreased to a quarter of the recommended volume, DNA yields only deceased by ~10%. For customers wishing to recover 100% of the DNA bound to the column, they can simply elute in 200 µL.
3. Norgen's Saliva DNA Isolation Kit is Consistent and Reliable. We have demonstrated through the use of multiple sample replicates that Norgen's kit consistently isolates high quality DNA, despite the volume samples were eluted in.
4. Norgen's Saliva DNA Isolation Kit Elutes DNA Ready for Sensitive Downstream Applications. When 9 µL of sample was used in a sensitive TaqMan qPCR reaction, no inhibition was observed, and Ct values corresponded to the volume of elution used, in a linear fashion.

REFERENCES

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2. Streckfus CF, and Bigler LR. (2002). Saliva as a diagnostic fluid. Oral dis. 8: 69-76.

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