A Comparative Study Between Two Saliva DNA Preservation Systems, and Two Column-Based Saliva DNA Purification Methods

APPLICATION NOTE #50

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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# 49000). 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. Competitor O's DNA saliva collection device works similarly to Norgen's saliva collection system, however the preservatives contained within the two collection devices are different. As these two preservation systems vary in composition, choosing a specific preservation system can impact the quantity and quality of the saliva DNA isolated.

Currently, there are two main categories of saliva DNA isolation methods: a spin column format and alcohol precipitation. In this study, we looked at two popular spin column methods for saliva DNA isolation, as well as the alcohol precipitation method. This report will focus on the spin column methods. The two spin column methods used include: Norgen's Saliva DNA Isolation Kit (Cat# 45400) and Competitor Q's DNA Blood Mini Kit (Cat# 51104).

The purpose of this study is to assess the differences between Norgen's and Competitor O's saliva DNA preservation systems with regards to DNA quantity and quality when used on the two aforementioned purification systems.

METHODS & MATERIALS

Sample Collection and Preservation

Two milliliters of saliva was collected from six different participants. All samples were preserved in 2 ml of either Norgen's saliva preservative or Competitor O's saliva preservative.

Saliva DNA Purification

Saliva DNA was purified using two different methods, with all methods being conducted by two different experimenters to assess experimenter variability and data reproducibility. DNA was extracted from all saliva samples using either Norgen's Saliva DNA Isolation Kit or the Competitor Q's DNA Blood Mini Kit, as per the manufacturer's instructions. Briefly, saliva samples were incubated at 55°C for 1 hour, prior to DNA isolation. After inverting each saliva sample, 400 µL of preserved saliva was added to new microcentrifuge tubes. Samples being isolated using the Norgen Saliva DNA Isolation Kit were 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. For the Competitor Q's DNA Blood Mini Kit, samples were mixed with 20 µL of protease (supplied with the kit), 400 µL Buffer AL, and incubated for 10 minutes at 55°C. After the addition of ethanol, samples were bound, washed and eluted as per manufacturer's protocol.

Agarose Gel Electrophoresis and Quantification

Ten percent of each elution was loaded on a 1X TAE 1.2% agarose gel. This equates to 20 µl of spin-column elutions, and 10 µl of alcohol precipitation elutions. Two microliters of all elutions were also measured using the NanoVue Plus™ nanospectrophotometer to assess DNA concentration, A260:A280 and A260:A230 ratios.

Real-Time PCR

The purified DNA was then used as the template in a realtime PCR (qPCR) reaction. Briefly, 2 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, 2.5 mM 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 72°C for 45 seconds for extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

Statistical Analyses

A two-tailed student t-test was performed on all averages calculated for yield, A260:A280 and A260:A230 ratios, as well as Ct values. A p-value of <0.05 was required for a finding to be considered significant.

RESULTS AND DISCUSSION

A) DNA Quantity

Saliva DNA was isolated from 6 different saliva samples using the Norgen Saliva DNA Isolation Kit and Competitor Q's DNA Blood Mini Kit. Twenty microliters from 200 µL of 5 out of 6 elutions were run on 1X TAE 1.2% agarose gel **(Figure 1).** It was found that the DNA isolated using the Competitor Q's DNA Blood Mini Kit was similar to the Norgen-isolated samples (which the preservative was optimized for). The purified DNA from each participant looked similar despite different methods and preservatives used, with the only obvious variability being found between the individual saliva samples themselves. Next, each purified sample was measured using the NanoVue Plus™ spectrophotometer, to assess saliva DNA concentration. The average DNA yields from both experimenters, using both preservatives and isolation methods, were calculated and are depicted in **Figure 2.**

While DNA yields look similar for both isolation methods on the gel (Figure 1), when yields were calculated, it was found that the Norgen Saliva DNA Isolation Kit produced slightly higher average saliva DNA yields compared to the

Competitor Q's DNA Blood Mini Kit. However, based on a two-tailed student t-test, the differences found between the Norgen isolation kit and the Competitor Q's isolation kit were not significant. The p-value for Competitor O's preservative for both isolations was 0.06762, and for Norgen's preservative, using both isolations, was 0.06403. Therefore, while Norgen's kit appears to isolate on average a higher yield of DNA, the differences found between Norgen's and Competitor Q's kit are not significant, and the kits can therefore be considered equal.

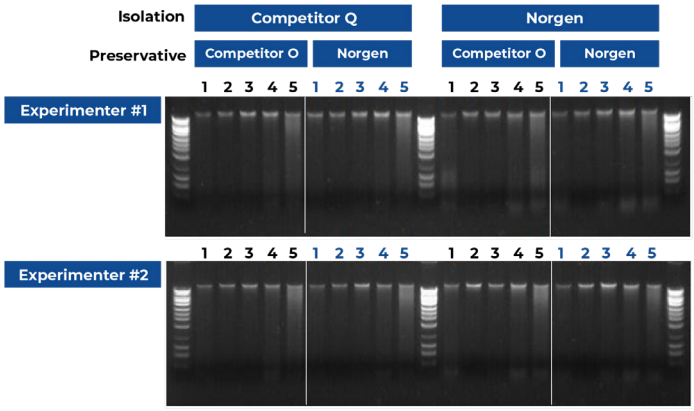

Figure 1. Saliva DNA from 5 donors, preserved using either Norgen's or Competitor O's Saliva Preservative, and isolated using both the Norgen Saliva DNA Isolation Kit and the Competitor Q's DNA Blood Mini Kit. The experiment was conducted using two different experimenters. Twenty microliters of 200 µL elutions were run on 1X TAE 1.2% agarose gel.

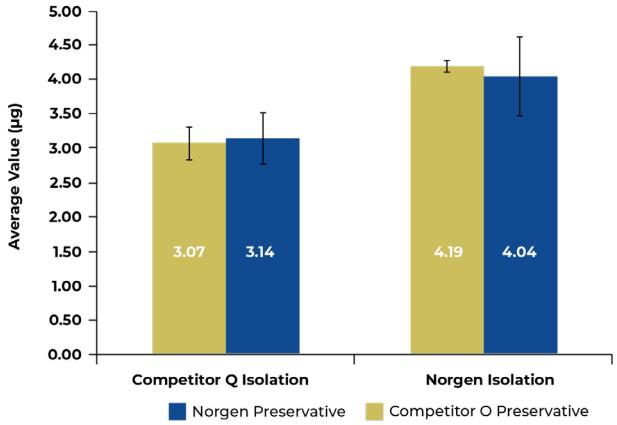

Figure 2. Average saliva DNA yields generated from six donors, using Norgen's preservative compared to Competitor O's preservative, isolated using Competitor Q's isolation kit compared to Norgen's isolation kit, from two experimenters. The averages between both experimenters, from each preservative and isolation method, were generated and depicted in the above graph.

B) DNA Quality

There are many common methods used to measure DNA quality. In this study, quality was assessed using the A260:A280 and A260:A230 ratios, as well as Ct values generated from a qPCR reaction. Similar to DNA yields, saliva DNA quality will vary from sample to sample, thus averages between two experimenters and six individual saliva samples were taken.

A260:A280 Ratios

A260:A280 ratios have classically been used to give a general idea of the amount of protein contamination in a DNA sample. In this study, the elutions from six different saliva samples, preserved in either Norgen's or Competitor O's preservative, and isolated using both the Norgen and Competitor Q kit, using two experimenters, were measured using nanospectrophotometry. The averages for both experimenters using all four conditions were taken, and depicted in **Figure 3.**

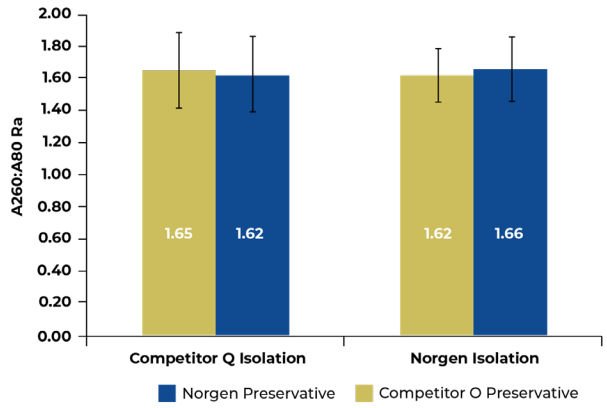

Figure 3. Average A260:A280 ratios generated from six purified saliva DNA samples, using Norgen's preservative compared to Competitor O's preservative, isolated using Competitor Q's isolation kit compared to Norgen's isolation kit, from two experimenters. The average A260:A280 generated between both experimenters, from each preservative and isolation method, were generated and depicted in the above graph.

For this quality measurement, Norgen and Competitor Q's appear to isolate similar quality DNA, based on the total average values generated from both kits. It was also found that the Norgen and Competitor O preservatives are both compatible with the Competitor Q kit, as well as the Norgen kit, as they both isolated similar quality DNA, using both methods.

A260:A230 Ratios

The A260:A230 ratio is useful in determining the relative amounts of contaminants in a purified DNA sample. Phenolate ions, thiocyanates or other organic compounds absorb at 230nm3. Therefore the presence of these contaminants in a sample will lead to a low A260:A230. Similar to the A260:A280 measurements, the A260:A230 average readings were also taken, based on the data generated from two different experimenters, using the four different conditions previously described. These averages have been depicted in **Figure 4.**

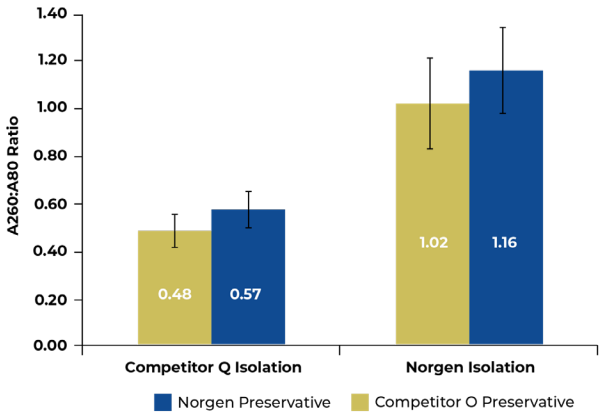

Figure 4. Average A260:A230 ratios generated from six purified saliva DNA samples, using Norgen's preservative compared to Competitor O's preservative, isolated using Competitor Q's isolation kit compared to Norgen's isolation kit, from two experimenters. The average A260:A230 generated between both experimenters, from each preservative and isolation method, were generated and depicted in the above graph.

For this quality measurement, based on a two-tailed student t-test, the difference found between the Norgen and Competitor Q isolation methods was found to be significant, with a p-value of 0.00026 for Competitor O's preservative, and 0.00031 for Norgen's preservative. The difference between the Norgen and Competitor O preservative performance for both isolation methods was found to be insignificant (p-value= 0.35391).

qPCR Results

Finally, all saliva samples were used in a real-time PCR reaction as a downstream quality measurement. The average Ct values across all six saliva samples, generated between two experimenters, using the aforementioned four conditions, are depicted in **Figure 5**. It was found that on average, both the Norgen and Competitor O preservatives perform similarly using both isolation methods, generating similar Ct values. It was also found that the Norgen and Competitor Q isolation methods were also nearly identical in their qPCR performance.

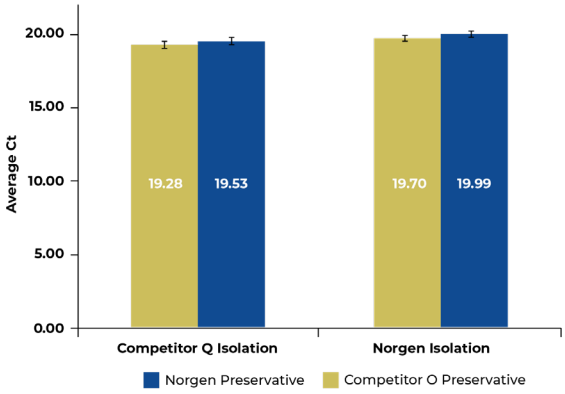

Figure 5. Average Ct values generated from six purified saliva DNA samples, using Norgen's preservative compared to Competitor O's preservative, isolated using Competitor Q's isolation kit compared to Norgen's isolation kit, from two experimenters.

CONCLUSIONS

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

1. **No significant differences were found between Norgen's saliva DNA preservative compared to Competitor O's preservative.** Both preservatives were found to generate comparable DNA yield and quality from all saliva samples, using two different isolation methods.
2. **No significant differences found in DNA yields** isolated using Norgen's vs. Competitor Q's isolation kits.
3. **No significant differences found in A260:A280 ratios or Ct values** generated from Norgen's vs. Competitor Q's isolation kits.
4. **Significant differences were found in A260:A230 ratios** generated between Norgen's and Competitor Q's kits. Norgen was found to have statistically significantly higher average A260:A230 ratios, compared to Competitor Q's kit. However, as this was the only significant finding, it can be concluded that both kits are nearly identical in their generation of the highest quality and quantity of DNA from saliva.

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