APPLICATION NOTE #56

Comparative Study of DNA Isolated from Saliva Preserved in Norgen's Preservative Using Norgen's Saliva DNA Isolation Kit Versus Qiagen's QIAamp DNA Blood Mini Kit

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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] .

DNA content in saliva is highly variable from sample to sample, and DNA yields are influenced by many factors, including the health status of the individual. Leukocytes and other blood cells are routinely found in the saliva of both healthy and diseased individuals[3] , and age, gender and immune status can highly impact the number of epithelial cells found in saliva. As a result, researchers can expect to find some saliva samples containing high concentrations of DNA, and others containing very little. Therefore, the most ideal saliva DNA isolation method must be consistent and reliable, capturing the highest yield of DNA from all samples, including those which contain very small amounts of DNA.

The purpose of this study is to compare two commercially available saliva DNA isolation kits: Norgen's Saliva DNA Isolation Kit (Cat# 45400) and Qiagen's QIAamp DNA Blood Mini Kit (Cat# 51104) for their ability to isolate high quality, and high quantities of saliva DNA from a variety of samples.

METHODS AND MATERIALS

Sample collection

Two milliliters of saliva was collected from 18 different participants. All samples were preserved in Norgen's saliva preservative, and were all processed at the same time.

Saliva DNA extraction

DNA was extracted from all saliva samples using Norgen's Saliva DNA Isolation Kit (Cat# 45400), or Qiagen's QIAamp DNA Blood Mini Kit (Cat# 51104), 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, 200 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 then added along with ethanol, and samples were bound, washed and eluted as per manufacturer's instruction. For the QIAamp DNA Blood

Mini Kit, samples were mixed with 20 µL of Protease (supplied with the kit), 200 µL Buffer AL, and incubated for 10 minutes at 55°C. After the additional of ethanol, samples were bound, washed and eluted as per manufacturer's protocol.

Spectrophotometry

Saliva DNA quantity and quality was measured using the UltraSpec 2100 Pro (Fisher Scientific). Fifty microliters 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), 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 extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

The use of saliva as a diagnostic medium is steadily increasing, as collection is easy and non-invasive. As a result, the need for a reliable, robust saliva DNA isolation method is increasingly becoming apparent. As the results of any study are solidified by sample numbers, the fewer samples that must be discarded due to low DNA concentrations, the more reliable the interpretation of results becomes.

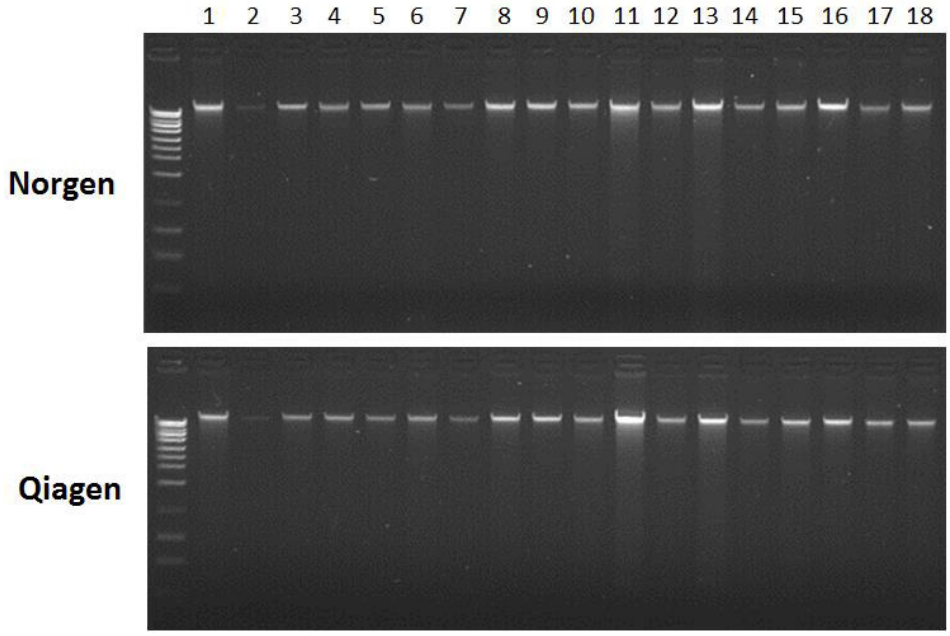

Figure 1. Purified saliva DNA isolated from swabs collected by two donors (A and B), run on a 1X TAE 1.0% agarose gel. High quality saliva DNA was isolated from both samples, with donor A showing a higher yield of DNA than donor B.

In this study, saliva was collected from 18 different participants and preserved in Norgen's saliva preservative. DNA was then isolated from all samples using either Norgen's or Qiagen's kit, and run on a 1.0% 1X TAE agarose gel **(Figure 1)**. Both Norgen's and Qiagen's kit successfully isolated DNA from all saliva samples. Norgen's kit was found to isolate higher yields of DNA on average from the same sample, compared to Qiagen's kit. To determine the DNA yields isolated from all samples, spectrophotometry readings were taken using the UltraSpec 2100 Pro (Fisher Scientific). Correlating with the gel photo, Norgen was found to isolate higher DNA yields compared to Qiagen, with an average difference of 1.7 µg more DNA isolated using Norgen's kit compared to Qiagen's **(Figure 2A)**. When individual samples yields were graphed **(Figure 2B)**, Norgen was found to isolate higher DNA yields from all 18 individual samples, when compared to Qiagen.

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.

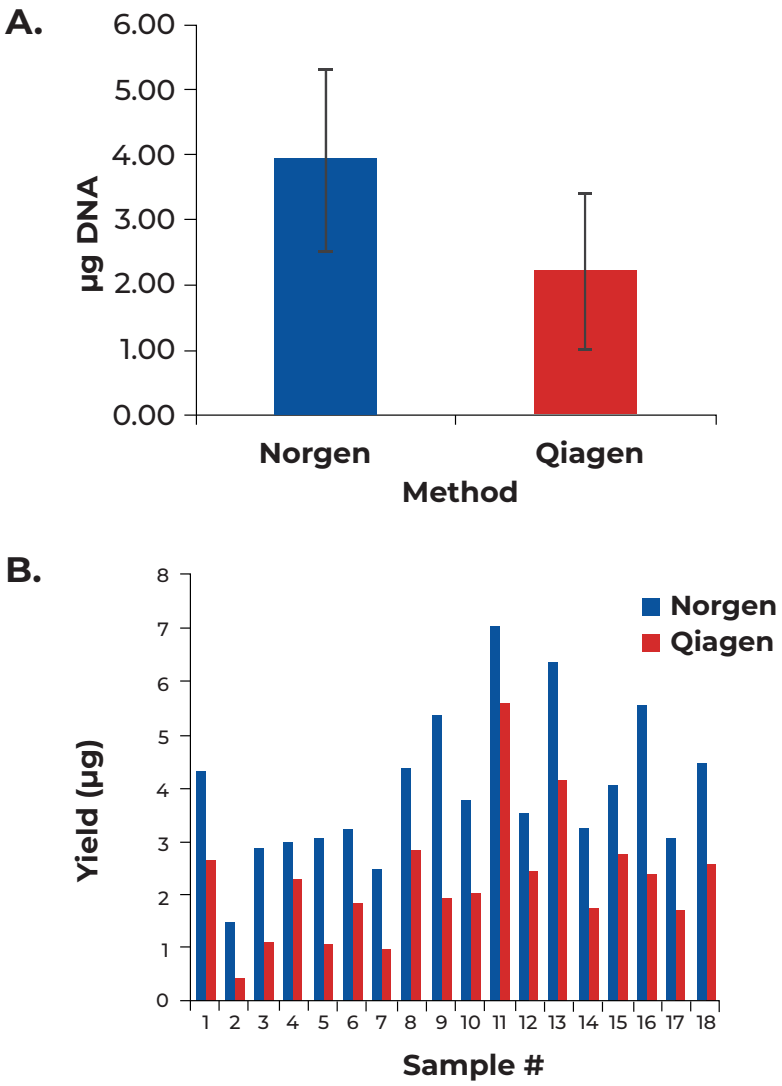

Figure 2. The difference in yield isolated from 18 different samples using Norgen's vs. Qiagen's kit. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). A) The average DNA yield from all samples. B) The DNA yield isolated from individual samples. Norgen consistently isolates a higher yield of saliva DNA compared to Qiagen, from all samples.

The first method of sample quality determination used in this study was the A260:A280 ratio **(Figure 3)**, which was determined by using a cuvette-based spectrophotometry method (UltraSpec 2100 Pro; Fisher Scientific). Norgen and Qiagen displayed very similar A260:A280 ratios, on average, indicating both methods isolate high quality DNA from saliva.

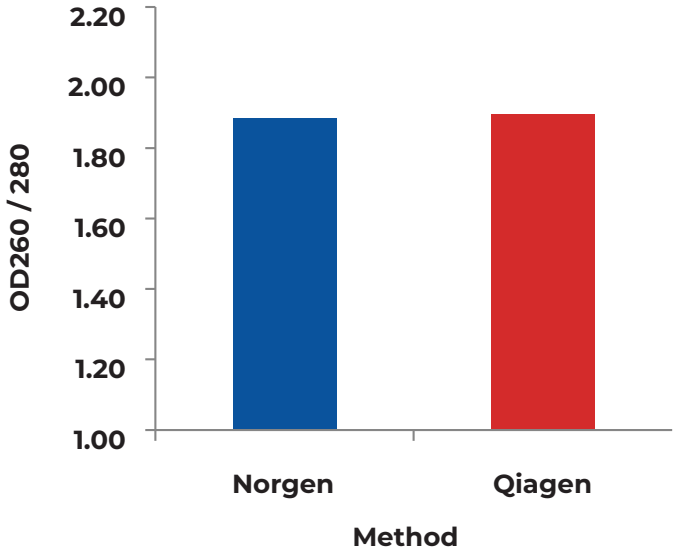

Figure 3. Similar A260:A280 ratios observed between Norgen and Qiagen saliva DNA samples. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and spectrophotometry measurements were taken using the UltraSpec 2100 Pro (Fisher Scientific).

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 4. As can be seen, Norgen and Qiagen performed similarly in the PCR, with Norgen showing overall lower Ct values compared to Qiagen **(Figure 4A)**. When individual sample Ct values were graphed **(Figure 4B)**, Norgen and Qiagen samples were fairly consistent between each other, with similar Ct values being generated from the same sample using both kits.

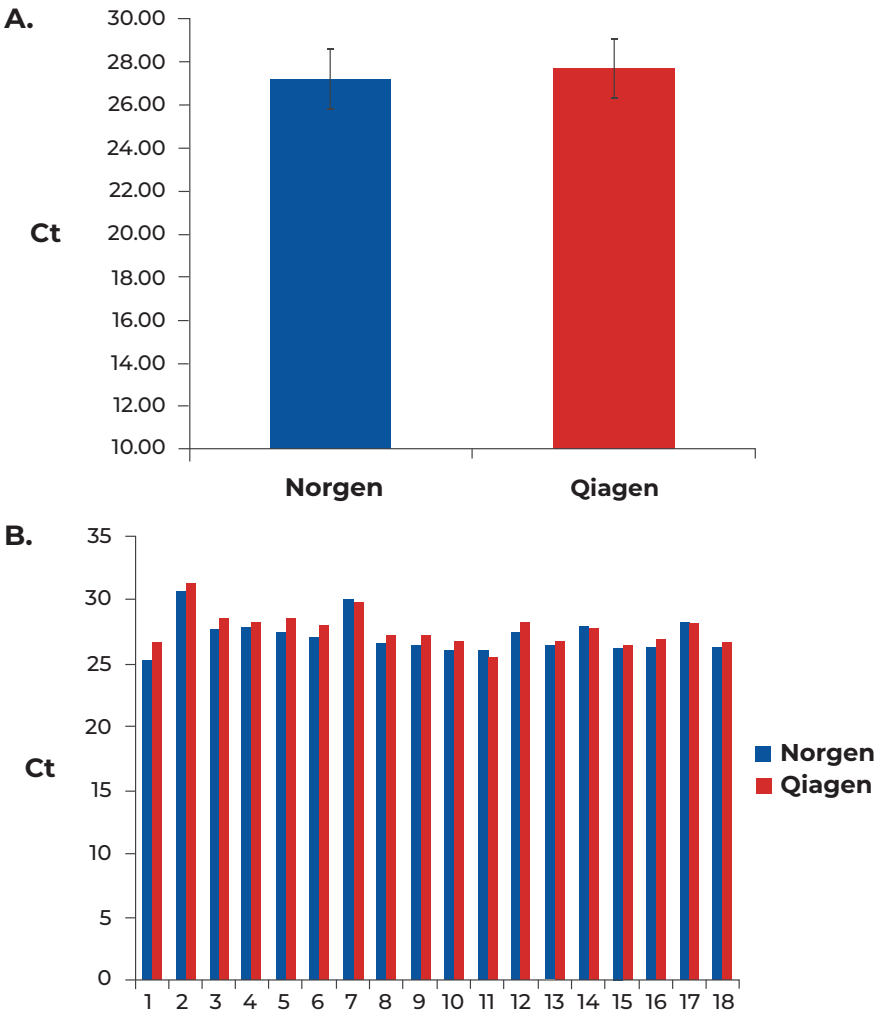

Figure 4. Ct values generated from 18 individual saliva samples isolated using Norgen's and Qiagen's kits. Nine microliters of saliva DNA template was used in a 20 µL TaqMan® qPCR reaction involving GAPDH primers, using the iCycler Thermal Cycler (BioRad Laboratories).

CONCLUSIONS

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

1. Norgen's Saliva DNA Isolation Kit can be Used to Isolate DNA from a Range of Different Saliva Samples. In this study, 18 different samples were used to demonstrate the robustness of both Norgen's and Qiagen's kit. Both methods were able to isolate DNA from all 18 samples, ranging from low to high amounts of saliva DNA.

2. Norgen's Saliva DNA Isolation Kit Consistently Isolates Higher DNA Yields from Saliva, Compared to Competitor Kits. Norgen's kit was found to isolate higher DNA yields from all saliva samples processed when compared to Qiagen's kit. On average, Norgen's kit isolated 1.7 µg more DNA from the same sample that was processed by Qiagen's kit.

3. Norgen's Saliva DNA Isolation Kit Isolates High Quality DNA Ready for Sensitive Downstream Applications. In this study, through the use of A260:A280 ratios and Ct values generated through qPCR, we have shown that Norgen's kit isolates the highest quality DNA, and performs similarly too, or slightly better than, the leading competitor kit in sensitive downstream applications such as qPCR.

4. Norgen's Saliva DNA Isolation Kit Elutes DNA Free from PCR Inhibitors. In this study we put Norgen's and Qiagen's saliva DNA samples to the true inhibitor test by using 9 µL in a 20 µL reaction; an amount that would completely inhibit a reaction had inhibitors been present in the sample.

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