SalivaGard® *HT* DNA – A superior solution for DNA preservation in saliva samples

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INTRODUCTION

Saliva is a convenient alternative to blood as a biological sample in research and diagnostic applications as it can be collected non-invasively with limited training. SalivaGard *HT* DNA is a new device designed for high efficiency collection and automated processing of genomic DNA (gDNA) from saliva for genetic analyses. The device features an integrated stabilizer which contains optimized chemistry for the preservation of DNA upon saliva collection. The device has a spill-proof design that minimizes donor exposure to chemicals and contamination. Moreover, a pierceable cap eliminates the need to decap the device prior to laboratory processing of samples. Lastly, triple redundant identification markers further support automated processing and sample analysis. Here we show that SalivaGard *HT* DNA reproducibly generates higher DNA yields than the leading competitor over a variety of purification methods. gDNA isolated from this device can be immediately utilized for qPCR and other downstream genetic analyses.

METHODS

Saliva Samples. Saliva was collected in SalivaGard *HT* DNA or Oragene® Dx devices. Saliva samples were incubated as indicated. At each time-point, gDNA was isolated and stored at -20°C.

DNA Extraction Methods. gDNA was isolated from saliva samples using the following methods: NucleoMag® Blood (200 µL or 3 mL) (Macherey-Nagel) via KingFisher™ Flex (ThermoFisher), QIAsymphony® DSP, QIAamp® DNA Mini, and Gentra® Puregene® (Qiagen).

DNA Quantification. Total DNA concentration and purity were evaluated by UV-VIS for both DNA quantity and purity. Double-stranded DNA (dsDNA) concentration was quantified by Quant-iT™ PicoGreen® (Molecular Probes).

DNA Quality. DNA quality was examined by 1% agarose gel.

qPCR. Reactions were performed using iQ SYBR Green Supermix on a CFX96 Real-Time PCR Instrument (Bio-Rad).

eSensor® Warfarin Sensitivity Test. gDNA isolated from stabilized saliva was characterized using an XT-8 instrument (GenMark).

High-throughput Liquid Handling. Aspiration, mixing, and scanning tests were conducted with SalivaGard *HT* DNA devices on a Microlab Star instrument (Hamilton).

RESULTS

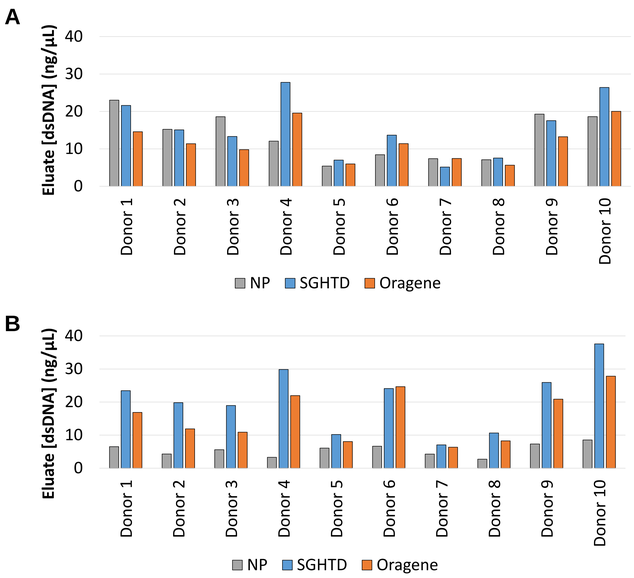


Figure 1. Samples extracted from SalivaGard *HT* DNA provide reproducibly higher DNA yields than samples from Oragene® Dx. Saliva was collected from 10 donors and 2 mL was dispensed into SalivaGard *HT* DNA (SGHTD) or Oragene® Dx devices or left non-protected (NP). At each timepoint, a 200 µL sample was removed and processed by Macherey-Nagel NucleoMag® Blood 200 µL kit. dsDNA yield was then quantified by PicoGreen®. **A)** dsDNA yields at time 0. **B)** dsDNA yields after 1 month at 25°C.

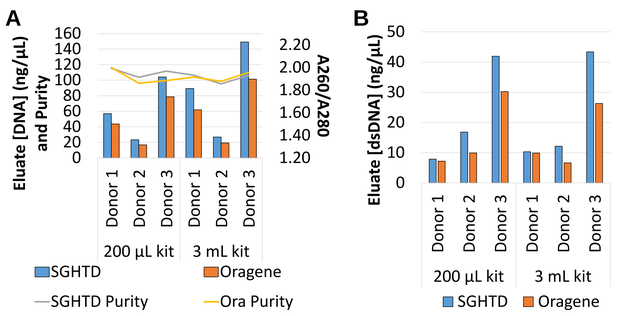


Figure 2. Scalability of DNA extraction sample volume. Saliva was collected from 3 donors in either SalivaGard *HT* DNA (SGHTD) or Oragene® Dx devices. Samples were then immediately extracted either by using the NucleoMag® Blood 200 µL or 3 mL kit. Elution volume of the small volume kit is 1/10th of the large volume kit. **A)** Comparative DNA quantitation (columns) and purity (lines) based on UV-VIS. **B)** Comparative dsDNA quantitation by PicoGreen®. **C)** gDNA quality by 1% agarose gel electrophoresis.

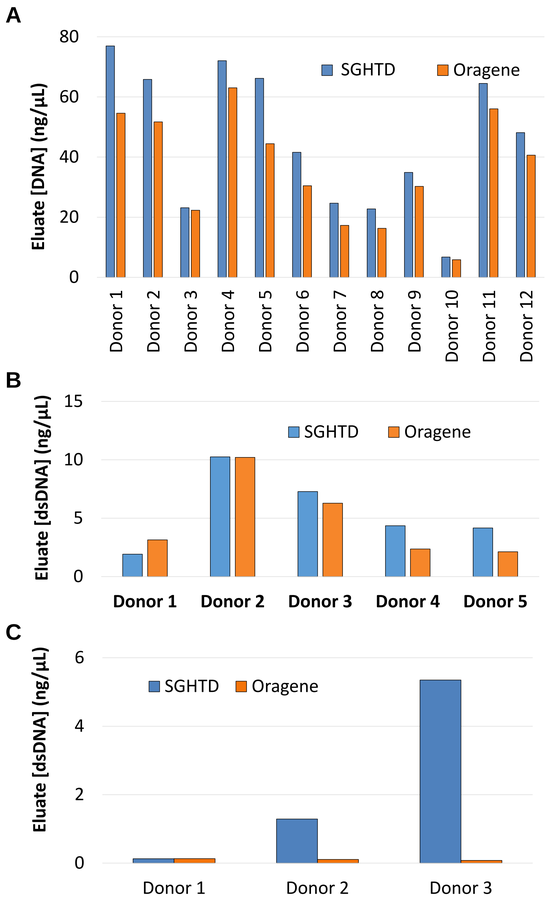


Figure 3. Comparison of DNA yields in additional DNA extraction kits between SalivaGard *HT* DNA and Oragene® Dx. gDNA yields of saliva stored and stabilized in SalivaGard *HT* DNA (SGHTD) or Oragene® Dx devices was also comparatively tested by the following methods: **A) QIAsymphony® DSP** (magnetic beads), UV-VIS DNA quantitation performed via QIAexpert; **B) QIAamp® DNA Mini** (spin columns), dsDNA quantified by PicoGreen®; and **C) Puregene®** (salt precipitation), dsDNA quantified by PicoGreen®.

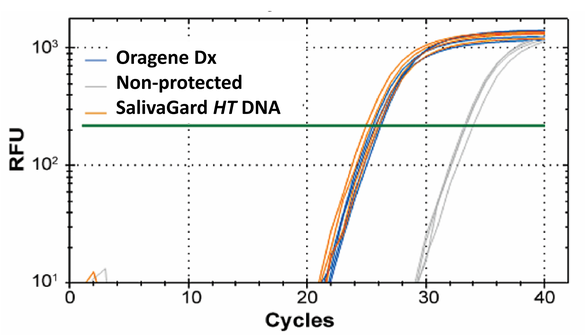
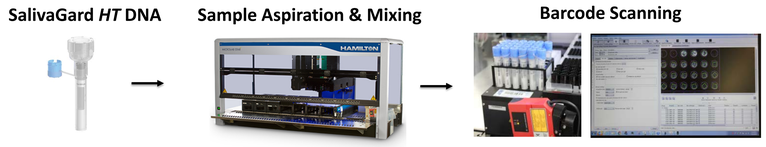


Figure 4. Detection of targets by qPCR. qPCR using gDNA isolated from saliva stored in Oragene® Dx, SalivaGard *HT* DNA, or non-protected saliva. Samples were amplified in duplicate using primers specific to the human RNase P gene.



Tip size	Transfer volume	Success
300 µL	200 µL	√
1 mL	1 mL	√
5 mL	2 mL	√

Figure 5. SalivaGard *HT* DNA compatibility with high-throughput liquid handling systems. Performance of SalivaGard *HT* DNA devices with a Hamilton Microlab Star liquid handler was evaluated. The pierceable cap of the device was fully accessible to all tip sizes tested. The devices could also be readily mixed and scanned by onboard features of the instrument.

Automated DNA extraction Using KingFisher™ Flex

eSensor® genetic analysis on XT-8

Gene	Warfarin Sensitivity Polymorphism	Result Accuracy
CYP450 2C9	430C>T (*2)	100%
CYP450 2C9	1075A>C (*3)	100%
VKORC1	-1639G>A	100%

Figure 6. Performance of gDNA isolated from saliva stored in SalivaGard *HT* DNA in genetic analyses. Saliva was collected from 5 donors in SalivaGard *HT* DNA devices. gDNA isolated from these samples was then submitted to GenMark for evaluation by the FDA-cleared eSensor® Warfarin Sensitivity Test. The results of this testing were found to be 100% accurate by subsequent DNA sequencing.

CONCLUSION

Our results show that the choice of saliva collection device is critical for gDNA yield and ease-of-use with automated liquid handlers.

gDNA can be purified from SalivaGard *HT* DNA samples by a variety of methodologies including magnetic beads, spin columns and salt precipitation with yields superior to the leading competitor.

Overall, SalivaGard *HT* DNA provides robust ambient stabilization of gDNA. The device is also directly compatible with high throughput liquid handling systems. gDNA purified from these devices can be readily used for downstream applications such as qPCR and other genetic analyses.