DNA isolated from SalivaGard™ *HT* DNA is compatible with FDA-cleared eSensor® Warfarin Sensitivity Test

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Introduction

Diagnostic genetic tests are increasingly important as point-of-care and precision medicine become more common. Saliva represents a highly attractive source of genomic DNA for genetic testing because of the simple and noninvasive manner in which it can be obtained. However, since these samples are temperature-sensitive and prone to degradation, proper preservation is required to guarantee accuracy of test results. To this end, Biomatrica has developed the SalivaGard™ *HT* DNA device, which allows for both collection of saliva and preservation of salivary genomic DNA for up to 1 year at ambient temperature. GenMark Dx's eSensor® Warfarin Sensitivity Test (WST) is a genetic test that identifies patients at risk for warfarin-related adverse events. This test, recently cleared by the FDA for use with DNA isolated from saliva samples, allows for the detection and genotyping of three mutations correlating with warfarin sensitivity: CYP450 2C9 (*2 and *3) and VKORC1 (-1639G>A). Here, we show that genomic DNA purified from saliva collected in SalivaGard™ *HT* DNA devices is directly compatible with downstream genetic analysis, specifically GenMark Dx's FDA-cleared eSensor® Warfarin Sensitivity Test.

Results

Saliva samples from five donors were collected in SalivaGard™ *HT* DNA devices. Genomic DNA was extracted from stabilizer-saliva mixtures and quantitated by UV-Vis spectroscopy (Table 1). Purified DNA eluates were then analyzed by both eSensor® Warfarin Sensitivity Test and bi-directional sequencing (Figure 1). The genotypes and predicted phenotypes listed in Table 2 were obtained with the eSensor® Warfarin Sensitivity Test. Bi-directional sequencing of each sample at the loci of interest agreed fully (100% accurate test results) with the eSensor® Warfarin Sensitivity Test results for all donors tested, as shown in Table 3.

Figure 1 – Experimental Workflow

Sample collection and stabilization in SalivaGard *HT* DNA device

Automated DNA extraction

eSensor® genetic analysis

SalivaGard™ *HT* DNA is for Research Use Only. Not for use in diagnostic procedures.

Table 1 – Summary of DNA extraction results

Donor	DNA eluate concentration (ng/µL)	A260/A280
1	9.55	1.85
2	61.95	1.85
3	35.91	1.89
4	21.84	1.89
5	17.74	1.91

Table 2 – Summary of eSensor results

Donor	430C>T (*2)	1075A>C (*3)	-1639G>A	Expected warfarin metabolism	Expected warfarin sensitivity
1	C/C	A/A	G/G	Normal	Low
2	C/C	A/C	G/A	Slow	Intermediate
3	C/C	A/A	G/G	Normal	Low
4	C/C	A/A	G/A	Normal	Intermediate
5	C/C	A/A	G/A	Normal	Intermediate

Table 3 - Sequencing results

Donor	Genotype (*2, *3, -1639G>A)	Agreement with eSensor® Warfarin Sensitivity Test result
1	+/+, +/+, +/+	100 %
2	+/+, +/-, +/-	100 %
3	+/+, +/+, +/+	100 %
4	+/+, +/+, +/-	100 %
5	+/+, +/+, +/-	100 %

Conclusion

This study highlights the compatibility of genetic samples collected and stabilized in SalivaGard™ *HT* DNA with downstream *in vitro* diagnostic tests. For all donors tested in the FDA-cleared eSensor® Warfarin Sensitivity Test, diagnostic genotype results matched bi-directional sequencing results.

Materials and Methods

Saliva collection: Saliva was collected from 5 donors with SalivaGard™ *HT* DNA devices per product IFU.

DNA extraction: Genomic DNA was purified from a 200 µL aliquot of each stabilized saliva sample using a NucleoMag® 200 µL Blood kit (Macherey-Nagel) run on a KingFisher™ Flex instrument (Thermo Fisher).

DNA quantitation: DNA quantity and purity was obtained by UV-Vis spectrophotometry at 260nm and 280nm on an HTX Synergy instrument (Biotek).

eSensor® Warfarin Sensitivity Test: 20 µL of each purified genomic DNA sample was tested for warfarin sensitivity on an XT-8 instrument (GenMark).

DNA sequencing confirmation: PCR primers were designed against regions spanning 50bp upstream and downstream at each of 3 warfarin sensitivity polymorphism locations (Integrated DNA Technologies). In addition, the forward and reverse primers for each location were tailed with M13F or M13R sequences, respectively to increase ease of sequencing (primer sequences available upon request). These regions were then amplified by PCR from purified genomic DNA isolated from each donor, and the resulting PCR products were then sequenced in both directions to confirm the results generated by the eSensor WST.