DNA from saliva: Oragene®•Dx performance as demonstrated through 510(k) validation studies

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Abstract

Molecular diagnostic tests allow for the rapid and accurate detection of biomarkers. Achieving high sensitivity and specificity of a diagnostic test requires a reliable and high quality sample. While traditionally, blood was considered the preferred sample type for diagnostic applications, DNA from saliva is non-invasive and is now proven to be a viable and beneficial alternative. DNA from saliva improves patient care and convenience with point-of-care sample collection while enabling efficient storage and transportation of stable samples at ambient temperature.

DNA from saliva is reliably and easily collected using Oragene®•Dx. Oragene•Dx is a device for the collection, stabilization and transportation of DNA from saliva and offers a non-invasive, reliable method for self-collection or assisted collection of samples. A 510(k) validation study was performed to demonstrate the safety, effectiveness and robustness of the product.

The Oragene•Dx validation studies demonstrated that prior to collection, the kits can be transported at temperatures ranging from -20°C to 50°C and can be stored at room temperature for up to 24 months. Once saliva is collected, Oragene•Dx/saliva can be transported at temperatures ranging from -20°C to 50°C without compromising the quality of the DNA present in the sample.

DNA from 450 samples (245 unique donors) extracted using the *Manual purification protocol for 0.5 mL* Oragene•Dx/saliva sample (DNA Genotek) was used to demonstrate performance characteristics for OGD-500 (DNA yield, DNA concentration and A_{260}/A_{280} ratio). A subset of 43 donors was used to demonstrate performance characteristics for OGD-575. Performance of DNA extracted from Oragene•Dx/saliva was analyzed on GenMark's eSensor® Warfarin Sensitivity Saliva Test.

Reproducibility was demonstrated in a study including triplicate samples from 10 donors (collected using three lots of OGD-500) tested on the eSensor® Warfarin Sensitivity Saliva Test by four different operators at three different sites. In this study, 100% concordance was observed for all samples.

Sample stability validation studies were conducted. Donors were asked to self-collect four saliva samples using Oragene•Dx (OGD-500). These studies support the claims that Oragene•Dx/saliva samples can be stored at room temperature (RT), 6°C ± 4°C or -20°C ± 5°C for 12 months, or at 50°C ± 5°C for 3 months without deterioration of DNA quality.

Interfering substances were assessed through additional experiments, there was no effect of endogenous and exogenous potentially interfering substances (i.e. amylase, hemoglobin, eating, drinking, chewing gum) on performance.

In conclusion, the Oragene•Dx validation studies demonstrated that Oragene•Dx allows for a viable alternative to blood for use in molecular diagnostic applications.

Device stability

Shelf-life conditions were evaluated by storing unused devices at room temperature (RT), 6°C ± 4°C or -20°C ± 5°C for up to 24 months. Other devices were exposed to multiple freeze/thaw cycles of -20°C ± 5°C/50°C ± 5°C. At all study time points a subset of devices were evaluated for physical and chemical properties to ensure the product specifications remained within acceptable tolerances. Another subset of devices was used to collect saliva from which DNA was extracted and analyzed for yield and A_{260}/A_{280} ratio.

Summary of pre-collection (device) stability study results

	Pre-collection storage temperature	RT	Freeze (-20°C)/ Thaw (50°C)	-20°C ± 5°C	6°C ± 4°C
	Time (months)	24		12	12
OGD-500	Chemical properties	✓	✓	✓	✓
	DNA yield†	✓	✓	✓	✓
	A_{260}/A_{280} ratio†	✓	✓	✓	✓

✓ Samples meet acceptance criteria for chemical properties, DNA yield and A_{260}/A_{280} ratio.
† Following pre-collection storage at indicated temperatures, devices were used to collect saliva samples from donors.

Sample stability

Thirty (30) donors each self-collected four saliva samples using Oragene•Dx format OGD-500 for a total of 120 samples. Samples were stored at room temperature (RT), 6°C ± 4°C or -20°C ± 5°C for 12 months, or at 50°C ± 5°C for 3 months. At the study time-point, DNA was extracted and analyzed for yield and A_{260}/A_{280} ratio. Samples stored at room temperature were analyzed for microbial content using a real-time PCR-based assay.

Summary of post-collection (sample) stability study results

	Post-collection storage temperature	-20°C ± 5°C	6°C ± 4°C	RT	50°C ± 5°C	Freeze (-20°C)/ Thaw (50°C)
	Time (months)	12	12	12	3	
OGD-500	DNA yield	✓	✓	✓	✓	✓
	A_{260}/A_{280} ratio	✓	✓	✓	✓	✓

✓ Samples meet acceptance criteria (yield ≥ 10 ng, A_{260}/A_{280} ratio 1.2 – 2.3).

Microbial content of samples stored at room temperature for 12 months

OGD-500		Baseline	12 months
Samples tested		29†	29†
% Microbial content	Mean ± SD	7.3% ± 5.5%	7.9% ± 5.5%
	Median	6.0%	5.9%
	Min, Max	0.6%, 22.1%	1.6%, 24.8%
	p-value	0.72	

† Insufficient sample obtained from 1 donor.

Volume tolerance

A total of 240 samples were collected using OGD-500 with modified fill lines in order to simulate both under and over spitting based on user adherence to the recommended Instructions for Use included with the kit that indicate a 2 mL volume. Collected samples ranged from as low as 0.58 mL of saliva to as much as 3.64 mL of saliva with a median collection volume of 2.00 mL.

Summary of eSensor® Warfarin Sensitivity Saliva Test results after re-testing for sample volume tolerance study

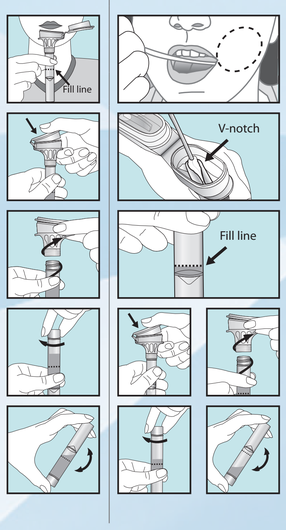
Range of collected saliva volume (mL)	Samples tested	Correct calls	Incorrect calls	No-calls†	% Correct calls
0.58 – 3.64	240	240	0	0	100%

† One first-pass no-call which was resolved upon re-testing.

Oragene•Dx format comparison

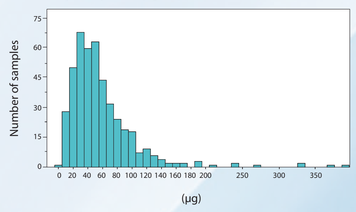
Format	Stabilizing liquid volume	Tube, funnel lid, small cap, instructions for use	Collection Sponge
OGD-500	2 mL	✓	–
OGD-510	1 mL	✓	–
OGD-575	0.75 mL	✓	✓

ORAgene®•Dx

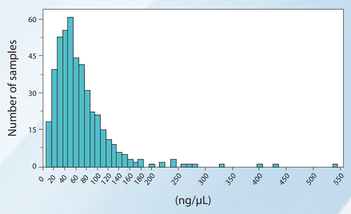


OGD-500
(n = 450 samples from 245 donors)

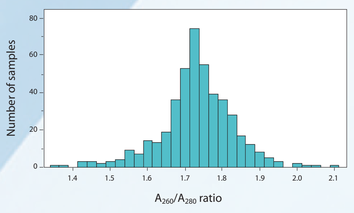
Summary for yield (µg)



Summary for DNA concentration



Summary for A_{260}/A_{280} ratio

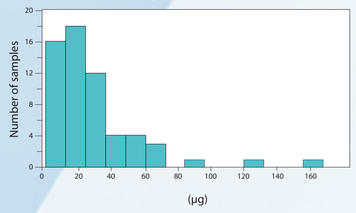


OGD-500 summary

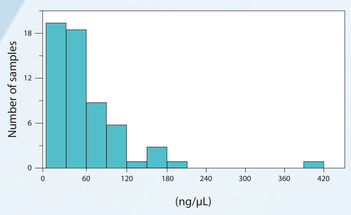
	DNA yield (µg)	DNA concentration (ng/µL)	A_{260}/A_{280} ratio
Mean ± SD	58.5 ± 47.0	68.1 ± 55.3	1.7 ± 0.1
Median	48.4	55.3	1.7
95% of samples	≥ 13.1	≥ 16.0	1.5 – 1.9

OGD-510
(n=60 samples from 60 donors)

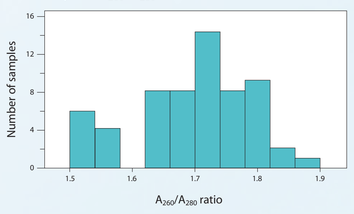
Summary for yield (µg)



Summary for DNA concentration



Summary for A_{260}/A_{280} ratio

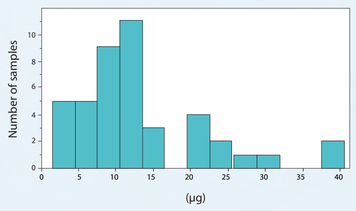


OGD-510 summary

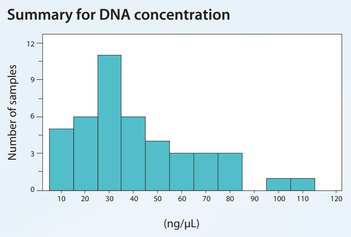
	DNA yield (µg)	DNA concentration (ng/µL)	A_{260}/A_{280} ratio
Mean ± SD	28.8 ± 28.1	62.2 ± 64.1	1.7 ± 0.1
Median	20.7	45.6	1.7
95% of samples	≥ 4.3	≥ 9.0	1.5 – 1.9

OGD-575
(n = 43 samples from 43 donors)

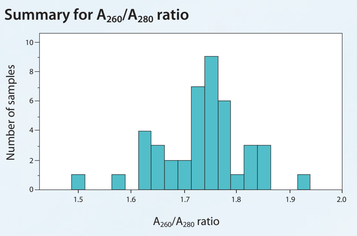
Summary for DNA yield



Summary for DNA concentration



Summary for A_{260}/A_{280} ratio



OGD-575 summary

	DNA yield (µg)	DNA concentration (ng/µL)	A_{260}/A_{280} ratio
Mean ± SD	13.5 ± 8.8	41.1 ± 24.6	1.7 ± 0.1
Median	11.0	33.2	1.7
95% of samples	≥ 3.8	≥ 11.2	1.6 – 1.9

Summary of eSensor® Warfarin Sensitivity Saliva Test results after re-testing for Oragene•Dx format comparison

Format	SNP	Samples tested	Correct calls	Incorrect calls	No-calls†	% Correct calls
OGD-500	2C9*2	45	45	0	0	100%
	2C9*3	45	45	0	0	100%
	VKORC1	45	45	0	0	100%
OGD-575	2C9*2	43	43	0	0	100%
	2C9*3	43	43	0	0	100%
	VKORC1	43	43	0	0	100%

† Three first-pass no-call results in OGD-575 were resolved upon re-testing.

Interfering substances

Both endogenous and exogenous potentially interfering substances were added separately to OGD-500/saliva samples from donors with known genotypes. Addition of tested substances had no effect as demonstrated through testing on the eSensor® Warfarin Sensitivity Saliva Test. All samples gave a correct call on the first-pass.

Summary of eSensor® Warfarin Sensitivity Saliva Test results for interfering substances

Endogenous substance	Samples tested	Correct calls	Incorrect calls	No-calls	% Correct calls
Control	30	30	0	0	100%
Amylase	30	30	0	0	100%
Hemoglobin	30	30	0	0	100%
IgA	30	30	0	0	100%
Total protein	30	30	0	0	100%

Exogenous substances	Collection time-point post activity	Samples tested	Correct calls	Incorrect calls	No-calls	% Correct calls
Eating	30 minutes	15	15	0	0	100%
Drinking	30 minutes	15	15	0	0	100%
Chewing gum	30 minutes	15	15	0	0	100%
Mouthwash	30 minutes	15	15	0	0	100%
Smoking	30 minutes	15	15	0	0	100%

Reproducibility

The device reproducibility study was conducted at three sites. Three samples (collected using three lots of OGD-500) from each of ten donors, covering all possible genotypes for three alleles for the eSensor® Warfarin Sensitivity Saliva Test, were tested in triplicate by four different operators at three different sites. Each operator extracted DNA from each sample using the same alcohol precipitation method, followed by determination of DNA concentration and A_{260}/A_{280} ratio for all samples by an independent operator at one of the sites. Four operators at three sites tested the extracted DNA samples on the eSensor® Warfarin Sensitivity Saliva Test.

Summary of device reproducibility DNA concentration, yield and A_{260}/A_{280} results

		Operator 1	Operator 2	Operator 3	Operator 4	Combined
Samples tested		87	87	90	90	354
DNA yield (µg)	Mean ± SD	74.89 ± 68.00	76.68 ± 61.76	69.59 ± 57.24	77.40 ± 68.36	74.62 ± 63.79
	Median	57.31	66.32	60.95	57.62	60.39
	95% of samples	≥ 23.23	≥ 26.56	≥ 18.13	≥ 26.02	≥ 23.47
DNA concentration (ng/µL)	Mean ± SD	86.76 ± 84.43	87.84 ± 70.76	80.21 ± 67.78	90.20 ± 86.69	86.24 ± 77.61
	Median	63.83	74.43	68.82	66.20	68.58
	95% of samples	≥ 25.87	≥ 29.58	≥ 20.42	≥ 28.98	≥ 26.74
A_{260}/A_{280}	Mean ± SD	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
	Median	1.9	1.8	1.9	1.8	1.9
	95% of samples	1.6 – 2.3	1.6 – 2.1	1.7 – 2.0	1.5 – 2.0	1.6 – 2.2

Summary of eSensor® Warfarin Sensitivity Saliva Test results after re-testing and investigation for device reproducibility study stratified by site and operator

Site	Operator	SNP	Samples tested	Correct calls	Incorrect calls†	No-calls‡	% Correct calls
Site 1	Operator 1	2C9*2	87	87	0	0	100%
		2C9*3	87	87	0	0	100%
		VKOR	87	87	0	0	100%
	Operator 2	2C9*2	87	87	0	0	100%
		2C9*3	87	87	0	0	100%
		VKOR	87	87	0	0	100%
Site 2	Operator 3	2C9*2	90	90	0	0	100%
		2C9*3	90	90	0	0	100%
		VKOR	90	90	0	0	100%
Site 3	Operator 4	2C9*2	90	90	0	0	100%
		2C9*3	90	90	0	0	100%
		VKOR	90	90	0	0	100%

† One first-pass incorrect call due to operator error resolved upon investigation.
‡ 46 first-pass no-calls were due to two runs (23 samples per run) invalidated due to DNA Contamination Monitor (DCM) failures. The other five first-pass no calls were low signal for the 2C9*2 allele (three), positive control failure (one) and contradictory score at the 2C9*3 allele (one). All were resolved upon re-testing.

Summary

Oragene•Dx is intended for use in the non-invasive collection of saliva samples. DNA from the saliva sample is isolated, stabilized, and suitable for use in FDA cleared molecular diagnostic applications. Saliva may be collected by spitting directly into the Oragene•Dx container or may be transferred into the Oragene•Dx container using a sponge. Saliva samples collected using Oragene•Dx are stabilized and can be transported and/or stored long-term at ambient conditions.

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