DNA from saliva: ORAcollect®•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 ORAcollect®•Dx. ORAcollect•Dx (OCD-100) is a device for the collection, stabilization and transportation of DNA from saliva and offers a non-invasive, reliable method for collection of samples. A 510(k) validation study was performed to demonstrate the safety, effectiveness and robustness of the product.

Validation studies demonstrated that prior to collection the ORAcollect•Dx 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, ORAcollect•Dx/saliva can be transported at temperatures ranging from -20°C to 50°C and can be stored at room temperature for up to 60 days without compromising the quality of the DNA present in the sample. DNA from 156 unique donors was extracted using the QIAamp® DNA Mini Kit (QIAGEN®) and used to demonstrate performance characteristics for ORAcollect•Dx (DNA yield, DNA concentration and A_{260}/A_{280} ratio). Performance of DNA extracted from ORAcollect•Dx/saliva was also demonstrated in a molecular diagnostic assay, the eSensor® Warfarin Sensitivity Saliva Test (GenMark Diagnostics).

Reproducibility studies were conducted to evaluate sample, device lot, operator and processing site variability. Replicate samples were tested on the eSensor® Warfarin Sensitivity Saliva Test and 100% concordance with bidirectional sequencing was observed for all samples. Interfering substances were assessed through additional experiments; there was no effect of endogenous and exogenous potentially interfering substances (e.g., amylase, hemoglobin, eating, drinking, chewing gum) on performance. Product robustness was demonstrated through user studies which showed samples collected under variable conditions still met performance characteristics for the product. In conclusion, these studies have validated the safety and effectiveness of ORAcollect•Dx and demonstrated it is a viable alternative to blood for use in molecular diagnostic applications.

Device stability

Shelf-life conditions were evaluated by testing devices that have been stored at room temperature (RT) for more than 24 months pre-collection. Additionally, separate devices were used to test simulated transport conditions where the devices were subjected to multiple freeze/thaw cycles pre-collection. At study time-points, a subset of devices was 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/concentration and A_{260}/A_{280} ratio.

	Pre-collection condition	Long-term storage (RT, 24 months)	Simulated transport (Freeze (-20°C)/ Thaw (50°C))
OCD-100	Chemical properties	✓	✓
	DNA concentration/yield	✓	✓
	A_{260}/A_{280} ratio	✓	✓

✓ Samples meet acceptance criteria (concentration ≥ 2 ng/µL, yield ≥ 0.01 µg, A_{260}/A_{280} ratio 1.2–2.3)

Sample stability

Two OCD-100 samples were collected from each of 30 donors for a total of 60 samples. Samples were stored at room temperature (RT) for 60 days or subjected to 3 freeze/thaw cycles. At the study time-point, DNA was extracted and analyzed for DNA concentration, yield, A_{260}/A_{280} ratio and microbial content using a real-time PCR-based assay. Downstream performance was tested using the eSensor® Warfarin Sensitivity Saliva Test (WST).

Summary of post-collection (sample) stability study results

	Post-collection condition	Long-term storage (RT, 60 days)	Simulated transport (Freeze (-20°C) / Thaw (50°C))
OCD-100	DNA concentration/yield	✓	✓
	A_{260}/A_{280} ratio	✓	✓
	WST*	100% agreement†	100% agreement†

✓ Samples meet acceptance criteria (concentration ≥ 2 ng/µL, yield ≥ 0.01 µg, A_{260}/A_{280} ratio 1.2–2.3)
* A subset of 10 donors was tested to assess downstream performance
† Agreement between WST and bi-directional sequencing

Microbial content of samples stored at room temperature for 60 days

OCD-100		Baseline	60 days
Samples tested		30	30
% microbial content	Mean ± SD	5.35% ± 3.79%	5.19 % ± 3.70%
	Median	4.38%	4.07%
	Min, Max	0.70%, 14.43%	0.86%, 14.45%
	p-value	0.8	

ORAcollect•Dx device performance data

Data from samples collected using the OCD-100 device was used in support of the performance characteristics for ORAcollect•Dx. In this study, 80% of the donors were naive to the collection device.

Overall data

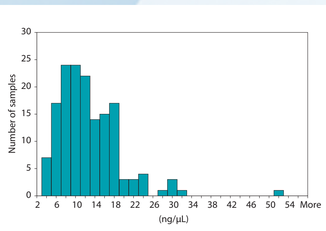
	Number of samples	DNA concentration (ng/µL)	DNA yield (µg)	A_{260}/A_{280} ratio
Mean ± SD	156	11.77 ± 6.59	2.94 ± 1.65	1.75 ± 0.15
Median	156	10.50	2.63	1.74
% samples meeting criteria	156	100%	100%	100%

Summary of method comparison genotyping results

	Samples tested	Correct calls	Incorrect calls	No calls	% agreement*
Final-pass	156	155	0	1†	99.4%

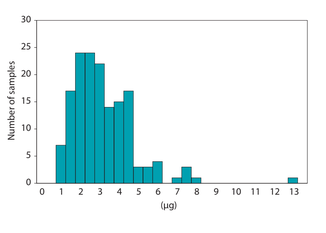
† Investigation of original and re-extracted DNA samples found no-call was likely due to extraction procedure
* Agreement between WST and bi-directional sequencing

Summary for DNA concentration (ng/µL)

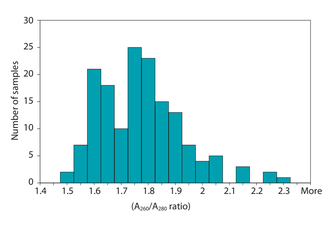


OCD-100
(n= 156 unique donors)

Summary for yield (µg)



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



Reproducibility

Three samples (collected using three lots of OCD-100) from each of ten donors, were processed by three different operators on multiple days. Each operator extracted DNA from each sample using the QIAGEN QIAamp DNA Mini Kit, followed by determination of DNA concentration and A_{260}/A_{280} ratio for all samples. Three operators tested the extracted DNA samples on the eSensor® Warfarin Saliva Sensitivity Test. Genotyping data was evaluated after first-pass results and all samples were concordant to bi-directional sequencing.

Summary of results stratified by operator

		Operator 1	Operator 2	Operator 3	Combined
Number of samples		20	20	20	60
DNA concentration (ng/µL)	Mean ± SD	11.80 ± 4.72	10.63 ± 4.12	10.54 ± 3.95	10.99 ± 4.24
	Median	10.61	9.86	9.95	10.33
Total DNA yield (µg)	Mean ± SD	3.08 ± 1.27	2.66 ± 1.03	2.64 ± 0.99	2.79 ± 1.10
	Median	2.70	2.46	2.49	2.61
A_{260}/A_{280} ratio	Mean ± SD	1.64 ± 0.07	1.66 ± 0.03	1.68 ± 0.06	1.66 ± 0.06
	Median	1.64	1.66	1.67	1.66

Summary of results stratified by operator

	SNP	Samples tested	Correct calls	Incorrect calls	No-calls	% agreement*
Operator 1	2C9*2	20	20	0	0	100%
	2C9*3	20	20	0	0	100%
	VKOR	20	20	0	0	100%
Operator 2	2C9*2	20	20	0	0	100%
	2C9*3	20	20	0	0	100%
	VKOR	20	20	0	0	100%
Operator 3	2C9*2	20	20	0	0	100%
	2C9*3	20	20	0	0	100%
	VKOR	20	20	0	0	100%
Combined	2C9*2	60	60	0	0	100%
	2C9*3	60	60	0	0	100%
	VKOR	60	60	0	0	100%

* Agreement between WST and bi-directional sequencing

For multi-site device reproducibility a total of 90 ORAcollect•Dx samples were extracted by three operators at three sites for a total of 270 sample aliquots extracted (90 aliquots per operator). Each operator analyzed the 90 extracted DNA samples for DNA concentration and A_{260}/A_{280} ratio.

		Site 1	Site 2	Site 3	Combined
Number of samples		90	90	90	270
DNA concentration (ng/µL)	Mean ± SD	11.01 ± 6.43	18.00 ± 8.71	13.87 ± 10.57	14.29 ± 9.17
	Median	9.70	16.04	11.58	12.50
Total DNA yield (µg)	Mean ± SD	2.75 ± 1.61	4.50 ± 2.18	3.47 ± 2.64	3.57 ± 2.29
	Median	2.42	4.01	2.89	3.12
A_{260}/A_{280} ratio	Mean ± SD	1.65 ± 0.06	1.79 ± 0.13	1.79 ± 0.07	1.74 ± 0.11
	Median	1.66	1.80	1.79	1.75

Interfering substances

Both endogenous and exogenous potentially interfering substances were tested with ORAcollect•Dx/saliva samples from donors with known genotypes. Endogenous substances were added separately to the ORAcollect•Dx samples post-collection. ORAcollect•Dx/saliva samples containing potentially interfering exogenous substances were collected 30 minutes after completion of the assigned activity. Addition of tested substances had no effect as demonstrated through testing on the eSensor® Warfarin Sensitivity Saliva Test. All samples gave a correct call after the final pass.

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

Endogenous substance	Samples tested	Correct call	Incorrect call	No-call	% agreement*
Amylase	14	14	0	0	100%
IgA	14	14	0	0	100%
Hemoglobin	14	14	0	0	100%
Total protein	14	14	0	0	100%

Exogenous substance	Collection time-point post activity	Samples tested	Correct call	Incorrect call	No-call	% agreement*
Eating	30 minutes	9	9	0	0	100%
Drinking	30 minutes	9	9	0	0	100%
Chewing gum	30 minutes	7	7	0	0	100%
Smoking	30 minutes	5	5	0	0	100%
Mouthwash	30 minutes	5	5	0	0	100%
Brushing teeth	30 minutes	9	9	0	0	100%

* Agreement between WST and bi-directional sequencing

Product robustness

Human factor studies demonstrated greater than 93% compliance to ORAcollect•Dx user instructions. Additionally, it was demonstrated through various studies that following the instructions for use, the ORAcollect•Dx product characteristics for DNA concentration, yield and A_{260}/A_{280} ratio were met and the samples collected with the device were able to successfully perform on eSensor® Warfarin Saliva Sensitivity Test (WST).

Summary

ORAcollect•Dx is intended for use in the non-invasive collection of saliva samples. Human DNA from the saliva sample is isolated, stabilized, and suitable for use in FDA cleared molecular diagnostic applications. Saliva samples collected using ORAcollect•Dx are stabilized and can be transported and/or stored long-term at ambient conditions.

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