

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k110701

**B. Purpose for Submission:**

New device

**C. Measurand:**

Not applicable.

**D. Type of Test:**

Collection and stabilization of genomic DNA from saliva for use in molecular diagnostic testing

**E. Applicant:**

DNA Genotek Inc.

**F. Proprietary and Established Names:**

Oragene®•Dx Collection Device, models OGD-500, OGD-575, OXD-525 and OYD-500

**G. Regulatory Information:**

1. Regulation section:

21 CFR §862.1675 - Blood specimen collection device

2. Classification:

II

3. Product code:

OYJ - DNA specimen collection, saliva

4. Panel:

Chemistry (75)

**H. Intended Use:**

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

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.

3. Special conditions for use statement(s):

For professional use only.

For use in people 18 years of age or older.

The Oragene•Dx collection devices are only cleared for use with genotyping tests that have obtained FDA clearance for use with saliva samples obtained with these collection devices.

4. Special instrument requirements:

None.

**I. Device Description:**

The OGD-500, OGD-575, OXD-525 and OYD-500 formats of the Oragene•Dx collection device consist of a collection tube containing stabilizing liquid. The OGD-500, OGD-575, OXD-525 and OYD-500 devices all consist of a tube and funnel with lid attached. A small cap is provided to close the tube for transport and storage (funnel with lid is detached and discarded). The OXD-525 includes sponges to aid in saliva collection. The difference in the four models is the amount and/or concentrations of the reagents in the tube which vary because of the difference in the amount of saliva collected or distinct final sample + reagent volume.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Vacutainer PPT Plasma Preparation Tube

2. Predicate K number(s):

k972075

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Proposed Device</b>	<b>Predicate Device (k972075)</b>
Intended use	Intended for the collecting, processing, and transporting of a clinical specimen	Same
Indications for use	For use with molecular diagnostic applications	Same

<b>Differences</b>		
<b>Item</b>	<b>Proposed Device</b>	<b>Predicate Device (k972075)</b>
Collection device contents	Nucleic acid stabilizing solution	EDTA and barrier gel
Sample source	Human saliva	Human whole blood

**K. Standard/Guidance Document Referenced (if applicable):**

- ISO 13485: Medical Device – Quality Management Systems
- ISO 14971: Medical Device – Application of Risk Management in Medical Devices

**L. Test Principle:**

The Oragene•Dx collection device collects and stabilizes human DNA from saliva; it can also be used for the transportation and long-term room temperature storage of a sample. Oragene•Dx is a non-invasive alternative for collecting high quality and quantity DNA for use in molecular diagnostic applications that are cleared for use with the Oragene•Dx collection device.

All formats consist of a collection tube, stabilizing liquid and optional sponges for assisted collection. After saliva is collected, the stabilizing liquid is mixed with the sample. Saliva can be delivered directly by spitting or using provided sponges to transfer saliva into the device.

Upon contacting saliva cells, the stabilizing liquid lyses cellular and nuclear membranes to release and stabilize nucleic acids. Samples can be immediately processed, transported or stored for future use. Device and sample integrity are preserved during typical ambient transport and storage conditions for up to 12 months. DNA extraction from Oragene•Dx can be performed using alcohol precipitation or other methods for the purpose of molecular diagnostic applications.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

**Two reproducibility studies were performed.**

1. Device reproducibility using prepared sample panel: a study was performed to evaluate the reproducibility of the performance of the Oragene•Dx device (OGD-500 format) with the GenMark Diagnostics eSensor Warfarin Sensitivity Saliva Test (k110786) across multiple sites and operators.

Ten donors self-collected six saliva samples each (two samples per lot x three lots of OGD-500). Two samples collected using the same OGD-500 lot were pooled to generate three samples per donor. Triplicate aliquots of each sample from each donor were provided to four operators at three sites for DNA

extraction (purification)<sup>1</sup>. Site 2 and 3 each had one operator and Site 1 (internal) had two operators for a study total of four operators.

All purified genomic DNA samples were tested for concentration and  $A_{260}/A_{280}$  ratio at a single external DNA Testing Site, Site 3. The eSensor Warfarin Sensitivity Testing was performed by the same four operators at the three sites where DNA extraction was also performed.

All specimens yielded a DNA concentration of 2 ng/ $\mu$ L or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010  $\mu$ g).

Each patient specimen was tested nine times on the eSensor Warfarin Sensitivity Saliva Test by each operator, for a total of 36 tests per sample. Each no-call was re-run. After the first round of testing, total percent agreement between the results of the eSensor Warfarin Sensitivity Saliva Test and genotype (by sequencing) was 85.30%. Two first-pass runs were invalidated at Site 3 due to contamination in the PCR blank (DCM failures) which resulted in 47 no-calls. After re-testing of the genomic DNA samples, total percent agreement was 100%.

## 2. Reproducibility of Sample Collection, Processing and Testing Procedure:

A study was performed to evaluate the reproducibility of the entire Oragene Dx sample collection, processing and genotyping procedure. Samples were directly shipped from donors to investigational sites for analysis of DNA yield, concentration,  $A_{260}/A_{280}$  ratio and eSensor® Warfarin Sensitivity Test genotyping. Donors were selected based on their naivety to the OGD-500 device; all donors had used the product at most once previously.

The study was conducted at three sites: one internal site and two external clinical laboratories. Donors (n=15) were each shipped four OGD-500 devices. Each donor was asked to provide four samples: one sample was sent directly by the donors to each of the three sites. (One sample was sent to DNA Genotek for remediation purposes if needed.) Each operator extracted one aliquot of DNA from each saliva sample they received and determined the DNA concentration and  $A_{260}/A_{280}$  ratio using their laboratory's standard procedures, prior to testing the sample on the eSensor® Warfarin Sensitivity Test. One donor did not return any of their samples and thus was excluded from the study. 14 samples were tested once at each site for a study total of 42 tests.

All specimens yielded a DNA concentration of 2 ng/ $\mu$ L or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010  $\mu$ g).

First pass genotyping test results yielded a total percent agreement of 90.50% with four wrong calls. Three wrong calls were due to one patient specimen (one wrong call at each testing site). Upon sequencing the reference sample for this patient, it was determined that this donor had an interfering mutation

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<sup>1</sup> One of the six samples provided had to be excluded since it failed incoming study screening criteria.

at the site adjacent to the 2C9\*2 mutation: 429C>T. This mutation is described in the literature and is known to impact genotyping results at the \*2 loci. (This limitation is stated in the GenMark Diagnostics eSensor Warfarin Sensitivity Saliva Test package insert.) The other wrong call was for a patient specimen, the result was “low signal for all polymorphisms”. The sample was cloudy suggesting that it was compromised. Re-test of the extracted genomic DNA yielded the identical incorrect call. Total percent agreement after re-testing (donor with the 429C>T genotype was excluded from the analysis and one wrong call was obtained again using the genomic DNA extracted from the cloudy saliva specimen) was 97.40%. (A new sample was obtained from the latter donor, tested, and a correct result was obtained.)

b. *Linearity/assay reportable range:*

Not applicable.

c. *Tracability, Stability, Expected values (controls, calibrators, or methods):*

Pre-collection shelf-life stability: Shelf-life stability testing of the Oragene Dx collection devices involves storage at three temperatures: -20°C, 6°C and room temperature (23°C). Two sets of endpoints: chemistry endpoints (evaluating the chemical parameters of the device reagents, including pH and conductivity) and DNA endpoints (DNA concentration, yield and  $A_{260}/A_{280}$  ratio) are evaluated at each time point. At designated time-points, devices are removed from the storage conditions; pH and conductivity of the reagents are evaluated. The devices are used to collect saliva samples from 12 donors, followed by DNA extraction and evaluation of the DNA endpoints. Stability testing protocols and sponsor’s acceptance criteria were reviewed and found to be acceptable.

Current real-time data supports a shelf-life 24 months at ambient temperature (recommended), and 12 months at -20 °C and 6 °C.

Stability of samples post-saliva collection: A study was performed to evaluate the stability of samples after saliva collection. Thirty donors each self-collected four saliva samples in each format (OGD-500, OYD-500 and OXD-525). (OGD-500 and OGD-575 have identical chemical properties; therefore OGD-575 was not included in this evaluation.) Three lots of devices in each format were used with 10 donors per lot. Each of the four devices per donor were stored at a different storage temperature (room temperature, 6°C, -20°C and 50°C) for up to 3 years (studies are on-going). At each time point, an aliquot of sample was extracted and the DNA was analyzed for concentration, yield and  $A_{260}/A_{280}$  ratio.

For OGD-500 and OYD-500 devices, all specimens from samples stored at -20°C, 6°C or 24°C for 12 months yielded a DNA concentration of 2 ng/μL or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010 μg). For OXD-525 devices, all specimens from samples stored at 24°C for 3 months yielded a DNA concentration of 2 ng/μL or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10

ng (0.010 µg). Therefore, the claimed sample stability is 12 months for OGD-500, OGD-575 and OYD-500 at ambient temperature and is 3 months for OXD-525.

For all formats, all specimens from samples stored at 50°C for 1 and 3 months yielded a DNA concentration of 2 ng/µL or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010 µg).

A sub-population of samples (chosen from the four Oragene Dx formats, different storage time points and conditions) were tested on the eSensor® Warfarin Sensitivity Test. Eight donors used the collection device, followed by DNA extraction and evaluation on the genotyping test.

In first-pass results, there was 98.0% agreement (400/408 tests) of the eSensor® Warfarin Sensitivity Test results to bidirectional sequencing. After re-testing, there was 99.8% agreement (407/408 tests) between the eSensor® Warfarin Sensitivity Test results and DNA sequencing. First-pass failures were a result of seven no-call results and one incorrect call<sup>2</sup>.

Freeze-thaw study: Environmental conditions experienced during shipping were simulated by subjecting samples to freeze-thaw cycles (samples stored at high temperatures that could be experienced during shipping were evaluated above). After pooling samples for the post-collection stability study above, one aliquot was removed from each pooled sample and subjected to three freeze - thaw cycles. DNA was extracted from each sample and tested for concentration, yield and  $A_{260}/A_{280}$  ratio. 30 samples per format (OGD-500, OYD-500 and OXD-525) were evaluated for a total of 90 samples. All specimens yielded a DNA concentration of 2 ng/µL or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010 µg).

*d. Detection limit:*

Sample Volume Tolerance: The OGD-500 collection device is intended for the collection of 2 mL of saliva. A study was conducted to evaluate the effect of over or under filling into an OGD-500 collection device.

Individuals spit into four different devices with varying fill-to lines (1 mL, 1.5 mL, 2.0 mL and 3.0 mL). The actual amount of saliva collected was examined as well as the impact of delivering less and more saliva (the volume of stabilizing liquid each device was unchanged) on DNA yield, purity, concentration and testing on a genotyping device.

A total of 60 donors were asked to collect saliva using the 4 devices with differing fill to lines. A total of 240 DNA samples were analyzed for DNA concentration, yield and  $A_{260}/A_{280}$  ratio. In addition, all 240 samples were processed on the eSensor® Warfarin Sensitivity Test.

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<sup>2</sup> One sample from donor 5 was incorrectly called \*1/\*2; this sample was extracted from a device stored for one month time point at -20C. Investigation by the sponsor concluded this was most likely due to operator error, resulting from contamination of donor 5's sample as it was aliquoted after a sample that was \*1/\*2.

When collected saliva volumes were examined, it was apparent that donors successfully delivered saliva corresponding to the fill to line. The median amount of saliva collected was typically just above the fill to line (1.12 mL [1 mL target], 1.65 mL [1.5 mL target], 2.25 mL [2 mL target], 3.04 mL [3 mL target]) This study demonstrated that as the fill to line increases between 1 mL, 1.5 mL, 2 mL, and 3 mL, the concentration of DNA also trends up with medians of 26.6 ng/μL, 36.0 ng/μL, 42.1 ng/μL, 47.9 ng/μL, respectively. When the fill to line was set at 2 mL for the OGD-500 collection device, the DNA concentration range was 7.66 – 168.12 ng/μL, with 95% of samples having a minimum concentration of 11.49 ng/μL.

This study also has demonstrated that as the fill to line increases between 1 mL, 1.5 mL, 2 mL, and 3 mL, the total DNA yield also trends up with medians of 18.0 μg, 25.9 μg, 36.1 μg, 48.6 μg, respectively. When the fill to line was set at 2mL the minimum amount of DNA collected was observed to be 6.4 μg, with 95% of samples above 9.3 μg, 91.7% of samples had a minimum yield of 10 μg.

DNA quality as assessed by  $A_{260}/A_{280}$  ratio remains constant. The study has demonstrated that as the fill to line increases between 1 mL, 1.5 mL, 2 mL, and 3 mL, the  $A_{260}/A_{280}$  ratios remain constant with medians of 1.7, 1.7, 1.7, 1.8, respectively.

Within this range of collected saliva 100% agreement (after re-testing) was observed between bi-directional sequencing and the eSensor® Warfarin Sensitivity Test:

**Number (%) of Genotypes of Each Allele Tested in the Sample Volume Tolerance Study**

n=60	<b>CYP2C9*2</b>	<b>CYP2C9*3</b>	<b>VKORC1</b>
<b>WT</b>	47 (78%)	57 (95%)	22 (37%)
<b>HET</b>	12 (20%)	3 (5%)	28 (47%)
<b>MUT</b>	1 (2%)	0	10 (17%)
Compound Het (*2/*3) = 0			

First test results by sample volume:

Fill to line (mL)	Range of collected Saliva Volume (mL)	Samples tested	Correct Calls	Incorrect Calls	No-Calls	% Agreement	95% LCB
1	0.61–1.83	60	60	0	0	100%	95.13%
1.5	0.58–2.56	60	60	0	0	100%	95.13%
2	0.69–3.42	60	60	0	0	100%	95.13%
3	1.81–3.64	60	59	0	1	98.3%	92.34%

After re-testing:

Fill to line (mL)	Range of collected Saliva volume (mL)	Samples tested	Correct Calls	Incorrect Calls	No-Calls	% Agreement	95% LCB
1	0.61–1.83	60	60	0	0	100%	95.13%
1.5	0.58–2.56	60	60	0	0	100%	95.13%
2	0.69–3.42	60	60	0	0	100%	95.13%
3	1.81–3.64	60	60	0	0	100%	95.13%

Test results by genotype:

Mutation	WT calls by sequencing				HET calls by sequencing				MUT calls by sequencing			
	Correct Calls	Incorrect Calls	No-Calls	% Agreement	Correct Calls	Incorrect Calls	No-Calls	% Agreement	Correct Calls	Incorrect Calls	No-Calls	% Agreement
2C9*2	187	0	1	99.5%	48	0	0	100%	4	0	0	100%
2C9*3	228	0	0	100%	11	0	1	91.7%	0	0	0	N/A
VKORC1	88	0	0	100%	112	0	0	100%	39	0	1	97.5%

*e. Analytical specificity:*

Effect of Endogenous Interfering Substances: Interfering substances including salivary  $\alpha$ -amylase, hemoglobin, immunoglobulin A (IgA) and total protein were spiked into saliva samples at the highest amounts found in literature. 10 donors provided five saliva samples each which were each spiked with one of the four interfering substances. A control sample was included. All samples were evaluated for effect on DNA concentration, purity,  $A_{260}/A_{280}$  ratio and testing on a genotyping device.

All specimens spiked with endogenous substances yielded a DNA concentration of 2 ng/ $\mu$ L or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010  $\mu$ g).

Three independent DNA extractions were performed on each sample. There was 100% agreement between the eSensor® Warfarin Sensitivity Saliva Test results and bidirectional DNA sequencing for all test substances in first pass, demonstrating no effect of any interfering substances on genotyping.

Effect of Exogenous Interfering Substances: Potentially interfering exogenous substances (eating, drinking, chewing gum, using mouthwash and smoking) introduced into saliva samples through various activities were tested. Each activity group was composed of five donors who each provided three samples - a baseline/control sample prior to the activity, and samples collected immediately after the activity and then 30 minutes after the activity. (It is recommended that sample collection only be performed at least 30 minutes after one of these activities.) Three samples per donor were tested.

All samples were evaluated for effect on DNA concentration, purity,  $A_{260}/A_{280}$  ratio and testing on a genotyping device.

All specimens collected after one of the various activities listed above yielded a DNA concentration of 2 ng/ $\mu$ L or better, DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3 and total DNA yield of at least 10 ng (0.010  $\mu$ g) (even collection

immediately after the activity).

There was 100% agreement between the eSensor® Warfarin Sensitivity Saliva Test results and bidirectional DNA sequencing for all activities tested in first pass, demonstrating no effect of any interfering substances on genotyping.

*f. Assay cut-off:*

Not applicable.

2. Comparison studies:

*a. Method comparison with predicate device:*

A method comparison study was performed in order to determine the accuracy of the genotype obtained on the eSensor® Warfarin Sensitivity Saliva Test (k110786) using saliva samples collected by the Oragene•Dx collection device as compared to bi-directional DNA sequencing. The total assay correct call rate for this study (316 samples) is 99.05% and a 95% confidence lower bound of 97.56%; see k110786.

*b. Matrix comparison:*

A study was performed to evaluate the four formats of the Oragene•Dx collection device, OGD-500, OYD-500, OXD-525, and OGD-575.

A total of 45 donors were asked to collect saliva using the four different collection devices. Only 43 OGD-575 samples were returned. A total of 178 DNA samples were analyzed for DNA concentration, yield and A260/A280 ratio and 178 tests were performed on the eSensor® Warfarin Sensitivity Saliva Test. The samples obtained using the OGD-500 device were used as the control.

All samples collected from the four formats yielded a DNA concentration of 2 ng/μL or better and total DNA yield of at least 10 ng (0.010 μg). All samples collected using the OGD-500, OYD-500 and OGD-575 formats had DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3; 97.8% of the samples collected with the OXD-525 sample had DNA purity ( $A_{260}/A_{280}$ ) between 1.2 and 2.3.

After first pass genotyping tests on the eSensor Warfarin Sensitivity Saliva Test, three samples from the OGD-575 model generated a no-call on the eSensor® Warfarin Sensitivity Test for a total percent agreement of 98.3%. The three no-calls originated from a single run and were due to an eSensor® Warfarin Sensitivity Test control failure and not an issue with sample signal. Re-testing was performed as per the eSensor® Warfarin Sensitivity Test product insert and all three samples got a correct genotyping result, for a final total percent agreement of 100%.

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable.

- b. Clinical specificity:*  
Not applicable.
  - c. Other clinical supportive data (when a. and b. are not applicable):*  
Not applicable.
- 4. Clinical cut-off:  
Not applicable.
- 5. Expected values/Reference range:  
Not applicable for this device type.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.