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

A. 510(k) Number:
k071867

B. Purpose for Submission:
New device

C. Measurand:
Genotype of Cytochrome P450 2C9 (CYP450 2C9) and Vitamin K epoxide reductase complex subunit 1 (VKORC1)

D. Type of Test:
Qualitative genetic test for single nucleotide polymorphism detection

E. Applicant:
ParagonDx, LLC

F. Proprietary and Established Names:
Rapid Genotyping Assay – CYP2C9 & VKORC1

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.3360 Drug Metabolizing Enzyme Genotyping Systems
   21 CFR §864.7750 Prothrombin time test
2. Classification:
   Class II
3. Product code:
   ODW Cytochrome P450 2C9 (CYP450 2C9) Drug Metabolizing Enzyme Genotyping System
   ODV Vitamin K epoxide reductase complex subunit 1 (VKORC1) Genotyping System
4. Panel:
   Toxicology (91), Hematology (81)

H. Intended Use:

1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:
   The *Rapid Genotyping Assay – CYP2C9 & VKORC1* is intended to be used as an *in vitro* diagnostic test kit that detects the presence of CYP2C9 *2 and *3 and VKORC1 1173 C>T alleles. This test is for use with EDTA-anticoagulated whole blood samples. Information about the CYP2C9 and VKORC1 genotypes may be used as an aid in the identification of patients with greater risk for warfarin sensitivity.

3. Special conditions for use statement(s):
   For Prescription Use Only.

The information provided from this test may supplement therapeutic decision-making and should only be used in conjunction with routine monitoring by a physician. Clinicians should use professional judgment in the interpretation of results from this type of test.

4. Special instrument requirements:
   Cepheid Smart Cycler Dx

I. Device Description:

Each *Rapid Genotyping Assay – CYP2C9 & VKORC1* kit contains reagents sufficient to complete 30test reactions. Each kit contains the following components:

• Two dispensers, each containing 15 lyophilized CYP2C9 beads. Each bead contains four individual primers and four individual probes to detect CYP2C9 *2 and CYP2C9 *3 wild-type and variant sequences. The primer/probe mixture is contained in HEPES-KCl buffer.

• Two dispensers, each containing 15 lyophilized VKORC1 beads. Each bead contains two individual primers and two individual probes to detect VKORC1 1173 C>T wild-type and variant sequences. The primer/probe mixture is contained in HEPES-KCl buffer.

• Three Cepheid SmartMix™ HM Master Mix dispensers, each containing 20 lyophilized beads. Each bead contains hot-start Taq polymerase, dNTP's and $MgCl_2$ in HEPES buffer.

• One CYP2C9 Variation Detection Control (50µL Human Genomic DNA at 10ng/µL) with a combined CYP2C9 genotype of CYP2C9 *2/*3 (individual genotypes of CYP2C9 *2/wt and CYP2C9 *3/wt) and one VKORC1 Variation Detection Control (50µL Human Genomic DNA at 10ng/µL) with a VKORC1 genotype of VKORC1 1173/wt. These controls (VDCs) are suspended in 10mM Tris-EDTA (pH 8.0) buffered solution containing 0.05% sodium azide.

• One No Template Control (NTC): 100 µL TE buffer with 0.05% sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Nanosphere Verigene Warfarin Metabolism Nucleic Acid Test
2. Predicate K number(s):
   k070804
3. Comparison with predicate:

Similarities		
Item	Predicate Device (k070804)	Proposed Device
Intended Use	As an aid in the identification of patients at risk for increased warfarin sensitivity.	Same

Similarities		
Item	Predicate Device (k070804)	Proposed Device
Sample type	Genomic DNA isolated from EDTA-anticoagulated whole blood	Same
Loci genotyped	*2 and *3 alleles of the CYP2C9 gene and a single-point polymorphism (C to T at position 1173) of the *VKORC1* gene	Same

Differences		
Item	Predicate Device (k070804)	Proposed Device
Thermal cycling step	No thermal cycling is required for DNA amplification.	Utilizes thermal cycling to amplify genomic DNA.
Technology	Reactions occur on a single glass slide using microfluidics, with up to 32 cartridges (slides) hybridized & enhanced simultaneously.	Reactions occur in two plastic PCR tubes designed specifically for the instrument.

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

None referenced.

L. Test Principle:

The *Rapid Genotyping Assay – CYP2C9 & VKORC1* is an *in vitro* diagnostic test kit that detects the presence of CYP2C9 *2 and *3 and VKORC1 1173 C>T alleles. The *Rapid Genotyping Assay – CYP2C9 & VKORC1* is based on real-time PCR amplification of target genomic DNA and simultaneous detection of the corresponding single-nucleotide polymorphisms (SNPs) using the Cepheid SmartCycler® Dx platform. The assay consists of two independent reaction mixes (i.e. one for CYP2C9 and one for VKORC1) containing wild-type and mutant detection probes for each allele. Each probe is dually-labeled with a reporter fluorophore on the 5'-end and a quencher dye on the 3' end. The level of fluorescence obtained from both the wild-type and mutant reactions is directly correlated to a sample genotype. Genotype values for CYP2C9 *2, *3 and VKORC1 1173 C>T are determined separately by manually performing calculations obtained from the Cycle Threshold (Ct) values, which is defined as the first amplification cycle at which the fluorescence

signal crosses the established threshold. Numeric Ct values taken from the sample results table in the Dx software are recorded for each fluorophore and the numeric difference between the wild-type and mutant Ct values (ΔCt) for a particular allele is calculated. Using instructions and interpretive flow diagrams provided in the package insert, the user is then able to determine the genotype values for each sample tested.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Inter-site reproducibility was demonstrated by testing five genomic DNA samples covering all possible genotypes for all three alleles in CYP2C9 and VKORC1. The DNA samples were extracted from EDTA-anticoagulated whole blood at ParagonDx, all genotypes were determined by bi-directional DNA sequencing and then samples were sent to the investigational sites. Three investigational sites (two external; one internal) performed testing. Testing was performed in triplicate for a period of three days. Two of the test sites had one operator perform the testing to yield a total of 45 test results per site, while the internal testing site had two operators perform the testing to yield a total of 90 test results. In order to demonstrate lot-to-lot reproducibility, each site also performed testing on three different manufactured lots of assay kits. One indeterminate call was observed at Site 1. Testing of the sample was repeated and the genotype call obtained agreed with bi-directional DNA sequencing.

Genotypes of samples in Reproducibility Panel:

Sample ID	CYP2C9 *2	CYP2C9 *3	VKORC1 1173
PDX-001	wt/wt	*3/*3	1173/wt
PDX-002	*2/*2	wt/wt	wt/wt
PDX-003	wt/wt	wt/wt	wt/wt
PDX-004	*2/wt	*3/wt	1173/wt
PDX-005	wt/wt	*3/wt	1173/1173

Summary of Reproducibility Results (sorted by genotype):

Site	Allele	# Total Tests	Correct Calls[a]	Incorrect Calls	No Calls[b]	% Correct Call Rate[c] (95% CI)
1	CYP2C9 *2	90	90	0	0	100.0% (96.5 - 100%)
	CYP2C9 *3	90	89	0	1[d]	98.9% (93.4 - 100%)
	VKORC1 1173	90	90	0	0	100.0% (96.5 - 100%)

Site	Allele	# Total Tests	Correct Calls[a]	Incorrect Calls	No Calls[b]	% Correct Call Rate[c] (95% CI)
2	CYP2C9 *2	45	45	0	0	100.0% (93.2 - 100%)
	CYP2C9 *3	45	45	0	0	100.0% (93.2 - 100%)
	VKORC1 1173	45	45	0	0	100.0% (93.2 - 100%)
3	CYP2C9 *2	45	45	0	0	100.0% (93.2 - 100%)
	CYP2C9 *3	45	45	0	0	100.0% (93.2 - 100%)
	VKORC1 1173	45	45	0	0	100.0% (93.2 - 100%)
Total		540	539	0	1	99.9% (98.9 - 100%)

[a] A sample with a correct call indicates the correct genotype at all three loci, based on comparison to bi-directional DNA sequencing. An incorrect or no call at one of the three loci is considered an incorrect or no call for the whole sample.

[b] *No Calls* are those test results that did not yield a genotype call (e.g., *indeterminate*, *no signal*, or *out of range* calls).

[c] % Correct Call Rate = (# Correct Calls)/(# Total Tests) x 100.

[d] One indeterminate call was observed at Site 1. The sample was repeated and the genotype call obtained agreed with bi-directional DNA sequencing.

Summary of Reproducibility Results for Individual Samples:

Sample ID	Site	[a]Tests per Site	[b]Correct Calls	[c]Incorrect Calls	[d]No Calls	% Correct Call Rate[e]
PDX-001	1	18	18	0	0	100%
	2	9	9	0	0	100%
	3	9	9	0	0	100%
PDX-002	1	18	18	0	0	100%
	2	9	9	0	0	100%
	3	9	9	0	0	100%
PDX-003	1	18	18	0	0	100%
	2	9	9	0	0	100%
	3	9	9	0	0	100%
PDX-004	1	18	17	0	1[f]	94.4%
	2	9	9	0	0	100%
	3	9	9	0	0	100%

Sample ID	Site	[a]Tests per Site	[b]Correct Calls	[c]Incorrect Calls	[d]No Calls	% Correct Call Rate[e]
PDX-005	1	18	18	0	0	100%
	2	9	9	0	0	100%
	3	9	9	0	0	100%
Total		180	179	0	1	99.4%

[a] Tests per Site, where a test represents genotype calls obtained for all three loci (CY2C9 *2, *3 and VKORC1 1173).

[b,c] Correct Calls are genotype calls that were concordant with genotypes determined using bi-directional sequencing. Incorrect calls are genotype calls that did not agree with bi-directional sequencing.

[d] *No Calls* are those test results that did not yield a genotype call (e.g., *indeterminate*, *no signal*, or *out of range* calls).

[e] % Correct Call Rate = (# Correct Calls)/(# Total Tests) x 100.

[f] An indeterminate call was obtained for one replicate test performed on this sample. The user repeated testing and obtained the correct genotype call.

An extraction method study was carried out in order to demonstrate that personnel at different laboratories can isolate genomic DNA starting from whole blood samples using standard purification kits and utilize that DNA in the Rapid Genotyping assay to generate the correct genotype calls. Each of the three investigation sites (one internal and two external) received eight whole blood samples representing a variety of CYP2C9 and VKORC1 genotypes. The sample genotypes are indicated in first table below. Prior to shipment, DNA isolated from an aliquot in each blood tube was analyzed at ParagonDx via bi-directional sequencing. Each site used a different validated extraction method to purify the DNA. A trained technologist purified each sample one time *on three separate days*, to give a total of twenty-four DNA samples. Each sample was then tested in the *Rapid Genotyping Assay*. The person running the assay was not necessarily the same personthat performed the purification (this reflects typical use since sample purification may occur in a separate department). For this arm of the study, each site performed testing on three different lots of manufactured assay kits (i.e., testing performed on each of the three days were performed using a different lot of material). These were the same three kit lots used for the Reproducibility Study. Each site used a different DNA isolation method. In total, there were 72 DNA samples tested in the extraction study, resulting in 216 genotype calls. There were no indeterminate or out of range calls made for the extraction method comparison study.

Genotypes for blood samples in Extraction Method Comparison Panel:

Sample ID	CYP2C9 *2	CYP2C9 *3	VKORC1 1173
PDX-006	*2/wt	wt/wt	wt/wt
PDX-007	*2/wt	wt/wt	1173/wt
PDX-008	wt/wt	wt/wt	wt/wt
PDX-009	*2/wt	wt/wt	1173/1173
PDX-0010	wt/wt	*3/wt	1173/wt
PDX-0011	*2/wt	wt/wt	wt/wt
PDX-0012	wt/wt	*3/wt	wt/wt
PDX-0013	wt/wt	*3/*3	1173/wt

Extraction Method Comparison Results (sorted by testing site):

Site	Allele	# Total Tests	Correct Calls[a]	Incorrect Calls	No Calls[b]	% Correct Call Rate[c] (95% CI)
1	CYP2C9 *2	24	24	0	0	100.0% (88.0 - 100%)
	CYP2C9 *3	24	23	1[d]	0	95.8% (78.1 - 100%)
	VKORC1 1173	24	24	0	0	100.0% (88.0 - 100%)
2	CYP2C9 *2	24	24	0	0	100.0% (88.0 - 100%)
	CYP2C9 *3	24	24	0	0	100.0% (88.0 - 100%)
	VKORC1 1173	24	24	0	0	100.0% (88.0 - 100%)
3	CYP2C9 *2	24	24	0	0	100.0% (88.0 - 100%)
	CYP2C9 *3	24	24	0	0	100.0% (88.0 - 100%)
	VKORC1 1173	24	24	0	0	100.0% (88.0 - 100%)
Total		216	215	1	0	99.5% (97.1 - 100%)

[a] A sample with a correct call indicates the correct genotype at all three loci, based on comparison to bi-directional DNA sequencing. An incorrect or no call at one of the three loci is considered an incorrect or no call for the whole sample.

[b] *No Calls* are those test results that did not yield a genotype call (e.g., *indeterminate*, *no signal*, or *out of range* calls).

[c] % Correct Call Rate = (# Correct Calls)/(# Total Tests) x 100.

[d] A miscalled genotype was observed for the CYP2C9 *3 allele for one replicate test performed on a CYP2C9 *3/wt sample (user obtained a result of CYP2C9 wt/wt). All repeat testing performed on this sample generated the correct genotype. The reason for this miscall is unknown.

Extraction Method Comparison Results (sorted by sample):

Sample ID	Site	# Total Tests	Correct Calls[a]	Incorrect Calls	No Calls[b]	% Correct Call Rate[c]
1	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
2	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
3	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
4	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
5	1	3	2	1[d]	0	66.7%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
6	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
7	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
8	1	3	3	0	0	100%
	2	3	3	0	0	100%
	3	3	3	0	0	100%
Total		72	71	1	0	98.6%

[a] A sample with a correct call indicates the correct genotype at all three loci, based on comparison to bi-directional DNA sequencing. An incorrect or no call at one of the three loci is considered an incorrect or no call for the whole sample.

[b] *No Calls* are those test results that did not yield a genotype call (e.g., *indeterminate*, *no signal*, or *out of range* calls).

[c] % Correct Call Rate = (# Correct Calls)/(# Total Tests) x 100.

[d] A miscalled genotype was observed for the CYP2C9 *3 allele for one replicate test performed on a CYP2C9 *3/wt sample (user obtained a result of CYP2C9 wt/wt). All repeat testing performed on this sample generated the correct genotype. The reason for this miscall is unknown.

One discrepant genotype call was obtained for sample PDX-010 during the second day of testing. Based on bi-directional DNA sequencing, the true genotype of this sample is CYP2C9 *3/wt, but the genotype reported was CYP2C9 wt/wt for the CYP2C9 *3 assay. The user repeated testing on the DNA sample in triplicate and all repeat results gave the correct result.
In order to rule out the extraction method being the cause of the discrepant genotype call, the DNA sample was re-extracted from blood and tested in triplicate; however, no additional miscalls were obtained for this sample, suggesting that the extraction method was not the cause of this discrepancy.

b. Linearity/assay reportable range:
Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Controls: Bidirectional sequencing of the Human Genomic DNA Reference Control (HGDRC) materials was used to validate the presence of mutant or

wild type sequence. The CYP2C9 Variation Detection Control (VDC) has a combined CYP2C9 genotype of CYP2C9 *2/*3 (individual genotypes of CYP2C9 *2/wt and CYP2C9 *3/wt). The VKORC1 Variation Detection Control (VDC) has a VKORC1 genotype of VKORC1 1173/wt. These control materials are derived from human β-lymphoblastoids obtained from properly consented donors.

Stability: Stability testing protocols for the control material, summary data and acceptance criteria was evaluated and found to be acceptable. Currently, the sponsor has 6 months stability data for each of the positive controls and 3 months individual stability data for the No Template Control (NTC). Real time stability testing is on-going to extend the stability claim.

Stability: The recommended storage condition for the Rapid Genotyping Kits is 2-8°C. Protocols for real time stability testing of closed and open kits and acceptance criteria were reviewed and found to be adequate. Currently, the sponsor has six months of data for closed kit and open kit stability.

d. Detection limit:

The limit of detection for the *Rapid Genotyping Assay – CYP2C9 & VKORC1* was determined by analysis of genomic DNA samples diluted to 1ng/µl, 10ng/µl, 100ng/µl, and 500ng/µl. The highest concentration tested was 500ng/µl, which is five times above the stated high limit of concentration of 100ng/µl. The volume of template DNA used in each real-time qPCR reaction was the standard 5µl. The concentration of the parent DNA samples was determined by UV/Vis spectrophotometry. A total of thirty replicates for each genotype were tested at each of the four DNA input concentrations, yielding a total of 360 data points for the study.

DNA Concentration (ng/µL)	Number of tests	Correct calls	Indeterminate calls	Wrong Calls	% Concordance (95% CI)
500	90	90	0	0	100% (96.5 - 100%)
100	90	90	0	0	100% (96.5 - 100%)
10	90	90	0	0	100% (96.5 - 100%)
1	90	90	0	0	100% (96.5 - 100%)

This study demonstrates that the input DNA concentration for the *Rapid Genotyping Assay* can range from 1 to 500ng/µl without any significant effect on the assay performance for all of the alleles provided in the kit. Final results

are reported as Delta Ct values and show that as the input template amount is increased, the reactions are efficient enough to maintain the same fluorescent profile in relation to the thresholds. In this study, 5µl of DNA at concentrations of 1ng/µl to 500ng/µl was added to each reaction, which is equivalent to a range of 5ng to 2500ng of total DNA per reaction. The results from these studies demonstrate that even at five times below or above the recommended DNA concentration limit, the assay still provides accurate genotype calls with 95% confidence.

e. Analytical specificity:

Three whole blood samples were tested:
Sample 1: CYP2C9 wt/wt & VKORC1 wt/wt
Sample 211: CYP2C9 *3/wt & VKORC1 wt/wt
Sample 214: CYP2C9 *2/wt & VKORC1 1173/1173

Each sample of whole blood was split into six different vials. Four of these vials were spiked with one of the following interfering substances: 20 mg/dL conjugated bilirubin, 20 mg/dL un-conjugated bilirubin, 1.67mg/mL human albumin, or 3000 mg/dL triglycerides. One vial was treated under extreme freeze-thaw conditions known to induce hemolysis in order to test for the interference of excessive heme. Finally, one vial was left untreated and served as the control for the interference study.

Each whole blood sample was spiked with the interfering substance one time and extracted one time to generate genomic DNA. Each genomic DNA sample was then tested in triplicate using the Rapid Genotyping Assay.

None of the substances tested interfered with the assay. Furthermore, none of the genotype calls fell close to any threshold cutoffs, suggesting that they were not at risk of being miscalled.

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 assay accuracy as compared to bi-directional DNA sequencing. Testing for this arm of the study was conducted at one site only (internal site). The test panel for the method comparison study consisted of 150 unique human genomic DNA samples isolated from EDTA-anticoagulated whole blood. One lot of *Rapid Genotyping Assay* kit was used in this arm of the study. The total assay correct call rate is 98.9% (445 genotype calls made/450 total possible genotype calls) with no incorrect calls.

CYP2C9 *2

*2 Genotype[a]	# Tests per Genotype	Correct Calls[b]	Incorrect Calls	No Calls[c]	% Correct Call Rate[d]
wt/wt	73	73	0	0	100.0% (95.7 - 100%)
***2/wt**	72	72	0	0	100.0% (95.7 - 100%)
***2/*2**	5	5	0	0	100.0% (59.9 - 100%)
Total	150	150	0	0	100.0% (97.9 - 100%)

CYP2C9 *3

*3 Genotype[a]	# Tests per Genotype	Correct Calls[b]	Incorrect Calls	No Calls[c]	% Correct Call Rate[d]
wt/wt	101	100	0	1[e]	99.0% (94.1 – 100%)
***3/wt**	47	45	0	2[e]	95.7% (85.0 – 100%)
***3/*3**	2	2	0	0	100.0% (59.9 - 100%)
Total	150	147	0	3	97.4% (94.0 – 99.6%)

VKORC1 1173 C>T

VKORC1 Genotype[a]	# Tests per Genotype	Correct Calls[b]	Incorrect Calls	No Calls[c]	% Correct Call Rate[d]
wt/wt	61	60	0	1[f]	98.4% (90.4 – 100%)
1173/wt	73	72	0	1[g]	98.6% (91.9 – 100%)
1173/wt	16	16	0	0	100.0% (59.9 - 100%)
Total	150	148	0	2	98.7% (95.0 – 100%)

[a] Genotypes determined through bi-directional DNA sequencing

[b] A sample with a correct call indicates the correct genotype at all three loci, based on comparison to bi-directional DNA sequencing. An incorrect or no call at one of the three loci is considered an incorrect or no call for the whole sample.

[c] *No Calls* are those test results that did not yield a genotype call (e.g., *indeterminate*, *no signal*, or *out of range* calls).

[d] % Correct Call Rate = (# Correct Calls)/(# Total Tests) x 100

[e] These tests yielded *out of range* calls.

[f] This test yielded an *indeterminate* call. The user reported a sample loading problem that likely was the result of this no call.

[g] This test yielded a *no signal* calls. The user reported a sample loading problem that likely was the result of this no call.

b. Matrix comparison:
Not applicable.

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:

Allele frequency across various ethnic groups:

Genotype	Literature frequency (Caucasian)	Literature Frequency (Afr Am)	Literature Frequency (Asian)
[1] CYP2C9 *2			
wt/wt	86.6%	96.9%	99.8%
***2/wt**	12.0%	3.0%	<0.1%
***2/*2**	1.4%	<0.1%	<0.1%
[1] CYP2C9 *3			
wt/wt	92.5%	98.4%	78.8%
***3/wt**	7.4%	1.5%	18.0%
***3/*3**	0.5%	<0.1%	3.2%
[2] VKORC1 1173 C>T			
wt/wt	49.3%	84.0%	<0.1%
1173/wt	37.0%	14.0%	10.9%
1173/1173	13.7%	2.0%	89.0%

[1] Reynolds KK, Valdes R, Hartung B, Linder MW. Individualizing warfarin therapy. *Personalized Medicine.* 2007 4:1:11-31.

[2] Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS *et al*. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *The New England Journal of Medicine* 2005, 352:2285-93.

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.