AutoGenomics

INFINITI® Warfarin Assay

(2C9 & VKORC1 Multiplex Assay)

Directional Package Insert

For *In Vitro* Diagnostic Use

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INTENDED USE

The INFINITI Warfarin Assay is an *in vitro* diagnostic test for the detection and genotyping of the *2 and *3 CYP2C9 genetic variants and the VKORC1 3673 (-1639) intronic variant in genomic deoxyribonucleic acid (DNA) obtained from EDTA-anticoagulated whole blood samples. The INFINITI Warfarin Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

The INFINITI Warfarin Assay is indicated for use to identify individuals at risk for sensitivity to Warfarin.

BACKGROUND INFORMATION

Warfarin is a widely used anticoagulant prescribed for patients with venous thrombosis and pulmonary embolism, chronic atrial fibrillation and prosthetic heart valves. FDA estimates that two million persons start taking Warfarin in the United States every year to prevent blood clot[2].

Individual differences such as the intake of vitamin K, illness, age, gender, concurrent medication and body surface area, and genetic differences may affect a patient's response to warfarin.

Warfarin acts by interfering with the recycling of vitamin K, which leads to reduced activation of several clotting factors. Two genes affecting the pharmacokinetic and pharmacodynamic parameters of Warfarin are CYP2C9 (cytochrome P_{450} 2C9) and *VKORC1* (vitamin K epoxide reductase complex subunit 1). These two genes, together with other factors such as age, body surface area, and gender, partly explain the inter-individual variation in warfarin response[1]. Research suggests that some of the inter-patient variability in response to warfarin may depend on a patient's variants of the genes CYP2C9 and VKORC1[2].

CYP2C9

Warfarin is supplied as a racemic mixture of *R*- and *S*-isomers; the *S*-isomer has 3-5 times the potency of the *R*-form. After oral administration, free Warfarin is taken up by the liver where it is metabolized by cytochrome $P_{450.}$ The *S*-isomer is metabolized by cytochrome $P_{450,}$ subfamily IIc, polypeptide 9 protein (CYP2C9) and eventually excreted in the bile[3].

CYP2C9*2 and CYP2C9*3 are among the CYP2C9 variants that have been shown to exhibit decreased enzymatic activity[5,9]. The *2/*2 homozygous variant leads to a reduction to approximately 12% of wild-type CYP2C9 activity and the *3/*3 homozygous variant has a <5% of wild type CYP2C9 activity[4]. In a retrospective association study, individuals with one or both genetic variants were reported to require a lower maintenance dose of Warfarin compared with patients without these variations[5,7,8].

VKORC1

Warfarin's anticoagulant activity results from inhibition of vitamin K epoxide reductase important for the activation of various coagulation factors. Studies suggest that single nucleotide polymorphisms (SNPs) in the C1subunit of the vitamin K 2,3 epoxide reductase complex (VKORC1) may influence Warfarin sensitivity.

Polymorphisms of the VKORC1 gene tend to occur in haplotype blocks with particular combinations of polymorphisms that exhibit strong linkage disequilibrium. The identification of one member of a haplotype block is often predictive for the overall haplotype.[7] The identification of the VKORC1 3673 (-1639) polymorphism, which is common to the H1 and H2 haplotypes, could be predictive of an individual's response to Warfarin.

The following table provides the allele frequency across ethnic groups for the CYP2C9 and VKORC1 variants which are measured by this test:

Allele	Ethnic Group		
	Caucasian	African	Asian
CYP2C9*2	0.9 - 20%[6]	0.8 - 7%[6]	0%[6]
CYP2C9*3	0 - 14.5%[6]	0.4 - 3%[6]	0 - 8.2%[6]
VKORC1 3673 (-1639)	37%[7]	14%[7]	89%[7]

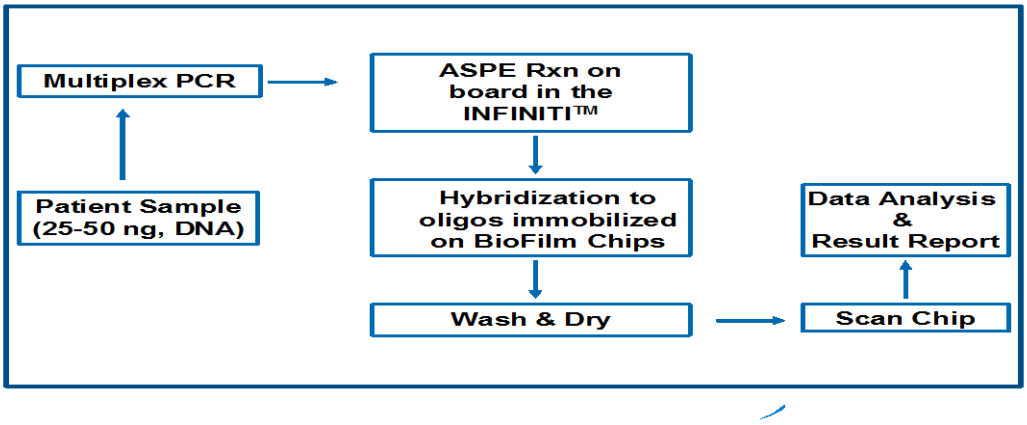
TEST PRINCIPLE / ASSAY OVERVIEW

The INFINITI Warfarin Assay is designed to simultaneously detect the *2 and *3 CYP4502C9 genetic variants and the VKORC1 3673 (-1639) intronic variant. The assay protocol is based on the following major processes:

(a) DNA extraction
(b) PCR amplification of purified DNA from human genomic DNA
(c) Labeling of the amplified product (allele specific primer extension)
(d) Hybridization of the labeled amplified product to a microarray by signature Tag/Capture probe hybridization under isothermal conditions.
(e) Scanning of the microarray
(f) Signal detection and analysis [determination of the 2C9*2, 2C9*3 and VKORC1 3673 (-1639) genotypes]

Steps (c) through (f) are automated by the INFINITI Analyzer and the INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI Warfarin Assay, an *in vitro* diagnostic device, utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for the detection and genotyping of the 2C9*2, 2C9*3, and VKORC1 3673 (-1639) mutations from human whole peripheral blood samples.

The INFINITI Warfarin Assay is comprised of the BioFilmChip® Microarray, and the Intellipac® Reagent Module.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The microarrays are designed to be assay specific. The INFINITI Warfarin Assay uses a microarray chip which contains unused Capture

Probes which could potentially be used for certain specific assays. Therefore, multiple assays can be developed using the same microarray.

The **Intellipac Reagent Module** which acts as a communication link contains up to eight reservoirs that house the test reagents and has an integrated memory chip. Information on the reagent such as lot number, expiration date and volume usage is archived in the memory chip.

The **INFINITI Analyzer** (including the modified version **INFINITI PLUS Analyzer** is an instrument used for clinical multiplex systems intended to measure and sort multiple signals from a clinical sample. The Analyzer is designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays. The Analyzer automates the 2C9 and VKORC1 assays and integrates all the discrete processes of sample (PCR amplicon) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and the spots are read by the built-in confocal microscope. Results are analyzed and presented as genotype calls. The INFINITI Warfarin Assay should be run using the AutoGenomics INFINITI Analyzer or INFINITI PLUS Analyzer.

Instructions on how to use the Analyzers are provided in the Operator's Manuals.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with whole blood collected in EDTA. Do not freeze / thaw blood samples. Specimens should be assayed as soon as possible.
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Store samples at the specified conditions.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- Do not pool / mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations
- Material Safety data Sheets (MSDS) are available upon request from AutoGenomics Customer Service

Sample Preparation

- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer and INFINITI PLUS Analyzer

- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray:	12 months Refrigerated (2 to 8°C)
Intellipac Reagent:	12 months Refrigerated (2 to 8°C)
	Note: Do not use after Intellipac has been opened for 4 weeks.
Amplification Mix:	18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label

SPECIMEN COLLECTION AND STABILITY

- Peripheral blood drawn in an EDTA (purple-top) tube.
- Do not freeze / thaw blood samples. Specimens should be assayed as soon as possible.

MATERIALS PROVIDED (SUFFICIENT FOR 48 TESTS)

- AutoGenomics Product Number 03-1140-01: INFINITI Warfarin Assay BioFilmChip Microarray Magazine: 4 magazines per package
- AutoGenomics Product Number 03-2140-01: INFINITI Warfarin Assay Intellipac Reagent Management Module: 2 modules per package. Each Intellipac module contains
  - 1.4ml ASPE master mix:
    - d[AGT]TP Mix
    - Cy5 –dCTP
    - ASPE Primer Mix
    - PCR Buffer
  - 2.6ml Hybridization Buffer
    - SSC
    - Sodium Azide
    - Hybridization Positive Control (Pos C-PTAG10, Cy5)
- AutoGenomics Product Number 03-3140-01: INFINITI Warfarin Assay Amplification Mix. Each package contains 4 x 250µl vials of AMP Mix and 1 x 30µl vial of Taq DNA Polymerase-1.
  - Amp Mix contains:
  - d[AGT]TP Mix
  - dCTP
  - PCR Primer Mix
  - $MgCl_2$
  - PCR Buffer
- **FOR INFINITI Analyzer***:* Product Number 12-0010-00: Wash buffer

  OR

  FOR INFINITI PLUS Analyzer*:* Product Number 12-0330-00: Buffer Solution BF1

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI Warfarin Assay can detect 2C9*2, 2C9*3, and VKORC1 3673 (-1639) genetic variations using genomic DNA isolated from blood with sufficient purity, i.e., with the ratio of absorbance at 260nm to absorbance at 280nm of 1.7 to 2.0. Any DNA extraction method that meets this specification may be used. The INFINITI Warfarin Assay has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Distilled Water (DNAse and RNAse free)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
    - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
    - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
    - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
    - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
    - AutoGenomics Product Number 11-0100-00: 48-Well Plates
    - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)

ASSAY PROCEDURE

DNA Extraction

Follow the instructions provided with the DNA extraction kit used.

PCR Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling specimens and controls.
- The PCR product cannot be stored prior to testing. Use immediately**.**

- Setting up the PCR reaction in the micro-well plate is recommended to minimize sample handling in subsequent steps.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Thaw and bring to room temperature the tube of PCR master mix and keep Taq DNA polymerase on ice.

2. Vortex the PCR master mix tube 2 to 5 sec to mix reagents and centrifuge briefly to bring the content to the bottom of the tubes. Include one negative control in every PCR reaction set up.

10X PCR Buffer	17.75µl
Taq DNA polymerase-1	0.25µl
Template DNA (25 ng/µl)	2.0µl
Total	20.0µl

For the PCR negative control, add 1µl of DNase free water.

3. Vortex tubes for 2 to 5 seconds to mix and then centrifuge briefly.

4. Place tubes in the thermocycler and cycle using the following conditions

Multiplex 2 Tm PCR Conditions

Step No.	Temperature °C	Time	No. of Cycles
1	94	2 minutes	
2 (touch down)	94 60 (-0.5 °C/cycle) 72	20 seconds 30 seconds 30 seconds	12
3	94 54 72	20 seconds 30 seconds 30 seconds	30
4	72	2 minutes	1
5	4	Hold	

Note:

- Total time for the PCR should be 1 hour and 38 minutes ± 5 Minutes. If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All PCR reaction volumes should be about 20µl. Otherwise, do not proceed with the assay.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.

2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Product No. 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Product No. 11-0110-00, reusable).

3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash Buffer should be placed in the INFINITI bottle holders. The Wash Buffer goes in the left holder (near the magazine).
   - **FOR INFINITI PLUS Analyzer***:*
     Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Buffer solution BF1.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Product No. EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Product No. EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from the INFINITI Warfarin Assay should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are taken.

In the VKORC1 gene, additional polymorphisms other than 3673G>A have been observed. Similarly, 2C9 variants other than the *2 and *3 have been observed. This test does not report those variations.

INTERPRETATION OF RESULTS

Results from the INFINITI Warfarin Assay are reported to the user as a genotype "call", indicating which genotype was detected in the sample, i.e., Wild Type, Homozygous, or Heterozygous for the 2C9*2, 2C9*3 and VKORC1 3673 (-1639) variants. An "Indeterminate" result means no genotype call was made.

Important: All results with an "Indeterminate" or no call require a repeat assay.

When an error message occurs, refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

Limit of Detection (analytical sensitivity)

Serial dilutions (200, 100, 50, 25, 10, 1, 0.1ng DNA) were prepared from a known purified DNA sample. Each serial dilution was assayed three times using the INFINITI Warfarin Assay. The study established the minimum DNA concentration for the INFINITI System Assay for 2C9-VKORC1 to be 1ng DNA. The recommended DNA concentration for the INFINITI Warfarin Assay is 25ng/µl. The assay requires 2µl of DNA sample or the equivalent of 50ng per test.

In addition, the same study demonstrated that DNA concentrations of 100ng and 200ng, which were in excess of the recommended concentration (50ng), did not interfere with the INFINITI Warfarin Assay. The following table provides a summary of the results of the study.

Limit of Detection

Sample				Run 1				Run 2				Run 3			
ng DNA	Genotype			Genotype Calls			Result	Genotype Calls			Result	Genotype Calls			Result
	2C9		VKORC1 3673 (-1639)	2C9		VKORC1 3673 (-1639)		2C9		VKORC1 3673 (-1639)		2C9		VKORC1 3673 (-1639)	
	*2	*3		*2	*3			*2	*3			*2	*3		
200	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
100	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
50	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
25	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
10	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
1	W	W	H	W	W	H	Pass	W	W	H	Pass	W	W	H	Pass
0.1	W	W	H	No call	W	H	Fail	W	W	H	Pass	No call	W	W	Fail

Percent Agreement vs. Bi-directional Sequencing

The results of the comparison studies conducted in three clinical sites comparing the INFINITI Warfarin Assay to bi-directional sequencing demonstrated

98.0% agreement for 2C9*2 as compared with bi-directional sequencing on 1st run
97.3% agreement for 2C9*3 as compared with bi-directional sequencing on 1st run
98.0% agreement for VKORC1 3673 (-1639) as compared with bi-directional sequencing on 1st run

The results of the comparison studies are summarized in Table 1a and Table 1b.

Table 1a: Agreement between INFINITI Warfarin Assay and Bi-Directional DNA Sequencing

Genotype[a]	Number Tested	Replicates per Sample	Number of Correct Genotype Calls[b]	Number of Incorrect Calls	No Calls	Agreement	95% One-Sided Confidence Lower Limit
2C9*2 *1/*2	35	1	34	0	1	97.1%	88.82%
2C9*2 *2/*2	2	1	2	0	0	100.0%	50%[d]
2C9*2 *1/*1	113	1	111	0	2	98.2%	94.91%
Total for 2C9*2	150	1	147	0	3	98.0%	95.09%
2C9*3 *1/*3	19	1	19	0	0	100.0%	80.45%
2C9*3 *3/*3	1	1	1	0	0	100.0%	0%[d]
2C9*3 *1/*1	130	1	126	1[c]	3	96.9%	94.30%
Total for 2C9*3	150	1	146	1	3	97.3%	94.09%
VKORC1 3673 (-1639) GA	63	1	62	0	1	98.4%	93.74%
VKORC1 3673(-1639) AA	27	1	25	0	2	92.6%	79.01%
VKORC1 3673 (-1639) GG	60	1	60	0	0	100.0%	98.33%
Total for VKORC1 3673 (-1639)	150	1	147	0	3	98.0%	95.09%
Total for Assay	450	1	440	1	9	97.8%	96.86%

[a] Genotype determined through bi-directional DNA sequencing
[b] Calls produced on first run
[c] Initial INFINITI results (*1/*1 for 2C9*2, ***1/*3 for 2C9*3*** and GG for VKORC1 3673) did not match bi-directional sequence results (*1/*1 for 2C9*2, ***1/*1 for 2C9*3*** and GG for VKORC1 3673). The same INFINITI results were obtained on repeat run. Reason unknown.
[d] For sample sizes 1 and 2, and 100% agreement, SE(p2-p1)=0. Pure sample size correction for sample size 2 is 50% and for sample size 1 is 100%, therefore, the 95% One –Sided Confidence Limits are 50% (n=2) and 0% (n=1).

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Table 1b: Agreement between INFINITI Warfarin Assay and Bi-Directional DNA Sequencing (by Sample Type)

Sample Description Genotype[a]			# Samples Tested	First Time Run					Final Result[d]				
2C9		VKORC1 3673 (-1639)		Samples with Genotype Calls made by INFINITI[b]	Samples with Correct Calls[c]	Samples with No Calls	Samples with Incorrect Calls	Correct Call Rate[e] (%)	Samples with Genotype Calls made by INFINITI[b]	Samples with Correct Calls[c]	Samples with No Calls	Samples with Incorrect Calls	Correct Call Rate[e] (%)
*2	*3												
*1/*1	*1/*1	AA	20	18	18	2	0	90.0	20	20	0	0	100
*1/*1	*1/*1	GA	34	34	34	0	0	100	34	34	0	0	100
*1/*1	*1/*1	GG	43	43	42	0	1[f]	97.7	43	42	0	1	97.7 (42/43)
*1/*2	*1/*1	AA	4	4	4	0	0	100	4	4	0	0	100
*1/*2	*1/*1	GA	15	14	14	1	0	93.3	15	15	0	0	100
*1/*2	*1/*1	GG	12	12	12	0	0	100	12	12	0	0	100
*1/*1	*1/*3	AA	2	2	2	0	0	100	2	2	0	0	100
*1/*1	*1/*3	**GA**	10	10	10	0	0	100	10	10	0	0	100
*1/*1	*1/*3	GG	3	3	3	0	0	100	3	3	0	0	100
*2/*2	*1/*1	GA	1	1	1	0	0	100	1	1	0	0	100
*2/*2	*1/*1	GG	1	1	1	0	0	100	1	1	0	0	100
*1/*2	*1/*3	AA	1	1	1	0	0	100	1	1	0	0	100
*1/*2	*1/*3	GA	2	2	2	0	0	100	2	2	0	0	100
*1/*2	*1/*3	GG	1	1	1	0	0	100	1	1	0	0	100
*1/*1	*3/*3	GA	1	1	1	0	0	100	1	1	0	0	100
Total			150	147	146	3	1	97.3	150	149	0	1	99.3 (149/150)

a Genotype determined through bi-directional DNA sequencing
b Excludes samples with indeterminate/no calls
c A sample with correct call indicates a correct call at all three loci. One incorrect or no call at one out of the three loci for the sample is considered an incorrect call for the whole sample
d Final results reflect one time repeat of samples with indeterminate (no) calls
e Correct call rate = # samples with correct calls/# samples tested
f Initial INFINITI results (*1/*1 for 2C9*2, ***1/*3 for 2C9*3** and GG for VKORC1 3673) did not match bi-directional sequence results (*1/*1 for 2C9*2, ***1/*1 for 2C9*3** and GG for VKORC1 3673). The same INFINITI results were obtained on repeat run. Reason unknown.

Assay Inter-Laboratory Reproducibility

A three-site study was conducted to demonstrate the reproducibility of the INFINITI Warfarin Assay. The study involved three lots of the INFINITI Warfarin Assay. The sites ran identical samples comprised of seven genomic DNA samples and five whole blood samples. The sites were blinded to sample identity. Each site used a different DNA extraction method. At each site, each sample was run in duplicate per day / operator for six days. Three operators were required for each site. Results of the inter-laboratory reproducibility study are summarized in Table 2a and Table 2b.

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Table 2a: Inter-Laboratory Reproducibility of the INFINITI Warfarin Assay by Genotype calls

Genotype	Samples Tested	Tests Per Site	Site	Genotype Calls	First Time Run					Final Result*				
					Correct Calls	Incorrect Calls	No Calls	% Correct Calls	95% One-Sided Confidence Lower Limit	Correct Calls	Incorrect Calls	No Calls	% Correct Calls	95% One-Sided Confidence Lower Limit
2C9*2 *1/*2	3	36	1	36	36	0	0	100		36	0	0	100	
			2	36	34	0	2[b]	94.4		36	0	0	100	
			3	36	36	0	0	100		36	0	0	100	
			total	108	106	0	2	98.15	95.14%	108	0	0	100	99.53%
2C9*2 *2/*2	1	12	1	12	12	0	0	100		12	0	0	100	
			2	12	11	0	1[b]	91.7		12	0	0	100	
			3	12	12	0	0	100		12	0	0	100	
			total	36	35	0	1	97.22	90.47%	36	0	0	100	98.61%
2C9*2 *1/*1	8	96	1	96	95	0	1[a]	99.0		96	0	0	100	
			2	96	96	0	0	100		96	0	0	100	
			3	96	96	0	0	100		96	0	0	100	
			total	288	287	0	1	99..65	99.13	288	0	0	100	99.83
Total for 2C9*2				432	428	0	4	99.07	98.06%	432	0	0	100	99.88
2C9*3 *1/*3	2	24	1	24	24	0	0	100		24	0	0	100	
			2	24	22	0	2[b]	91.7		24	0	0	100	
			3	24	24	0	0	100		24	0	0	100	
			total	72	70	0	2	97.22	92.73%	72	0	0	100	99.30%
2C9*3 *3/*3	2	24	1	24	24	0	0	100		24	0	0	100	
			2	24	24	0	0	100		24	0	0	100	
			3	24	23	0	1[c]	95.8		24	0	0	100	
			total	72	71	0	1	98.61	95.21%	72	0	0	100	99.30%
2C9*3 *1/*1	8	96	1	96	95	0	1[a]	99.0		96	0	0	100	
			2	96	95	0	1[b]	99.0		96	0	0	100	
			3	96	96	0	0	100		96	0	0	100	
			total	288	286	0	2	99.31	98.17%	288	0	0	100	99.83%
Total for 2C9*2				432	427	0	5	98.84	97.72%	432	0	0	100	99.88%
VKORC1 3673 (-1639) GA	5	60	1	60	60	0	0	100		60	0	0	100	
			2	60	59	0	1[b]	98.3		60	0	0	100	
			3	60	60	0	0	100		60	0	0	100	
			total	180	179	0	1	99.44	98.08%	180	0	0	100	99.72%
VKORC1 3673 (-1639) AA	2	24	1	24	24	0	0	100		24	0	0	100	
			2	24	22	0	2[b]	91.7		24	0	0	100	
			3	24	24	0	0	100		24	0	0	100	
			total	72	70	0	2	97.22	92.73	72	0	0	100	99.30%
VKORC1 3673 (-1639) GG	5	60	1	60	59	0	1[a]	98.3		60	0	0	100	
			2	60	60	0	0	100		60	0	0	100	
			3	60	60	0	0	100		60	0	0	100	
			total	180	179	0	1	99.44	98.08%	180	0	0	100	99.72%
Total for VKORC1 3673 (-1639)				432	428	0	4	99.07	98.06%	432	0	0	100	99.88%

AutoGenomics

Genotype	Samples Tested	Tests Per Site	Site	Genotype Calls	First Time Run					Final Result*				
					Correct Calls	Incorrect Calls	No Calls	% Correct Calls	95% One-Sided Confidence Lower Limit	Correct Calls	Incorrect Calls	No Calls	% Correct Calls	95% One-Sided Confidence Lower Limit
Total per Site			1	432	429	0	3	99.3	98.41%	432	0	0	100	99.88%
			2	432	423	0	9	97.9	96.45%	432	0	0	100	99.88%
			3	432	431	0	1	99.8	99.31%	432	0	0	100	99.88%
Total for Assay				1296	1283	0	13	98.99	98.42%	1296	0	0	100	99.96%

* Results after one repeat of samples

a Site 1: no results - no tips for one sample; repeat OK

b Site 2: no results - tip sensor did not sense liquid due low volume of wash buffer on one sample; correct test result achieved on second test.
no calls on two samples - reason unknown; correct test result achieved on second test

c Site 3: one sample had no call; correct test result achieved on second test

Table 2b: Reproducibility Study Results by Sample Type

Sample ID	Genotype			# Samples Tested	First Time Run					Final Result[c]				
	2C9		VKORC1 3673 (-1639)		Samples with Genotype Calls made by INFINITI[a]	Samples with Correct Calls[b]	Samples with No Calls	Samples with Incorrect Calls	Correct Call Rate[d] (%)	Samples with Genotype Calls made by INFINITI[a]	Samples with Correct Calls[b]	Samples with No Calls	Samples with Incorrect Calls	Correct Call Rate[d] (%)
	*2	*3												
1	*1/*2	*1/*3	AA	36	34	34	2	0	94.4	36	36	0	0	100
2	*1/*1	*1/*1	GG	36	36	36	0	0	100	36	36	0	0	100
3	*2/*2	*1/*1	GA	36	35	35	1	0	97.2	36	36	0	0	100
4	*1/*1	*3/*3	GA	36	35	35	1	0	97.2	36	36	0	0	100
5	*1/*2	*1/*1	GG	36	36	36	0	0	100	36	36	0	0	100
6	*1/*1	*1/*1	GG	36	36	36	0	0	100	36	36	0	0	100
7	*1/*1	*1/*1	GG	36	36	36	0	0	100	36	36	0	0	100
8	*1/*1	*1/*3	GA	36	36	36	0	0	100	36	36	0	0	100
9	*1/*1	*1/*1	GG	36	35	35	1	0	97.2	36	36	0	0	100
10	*1/*1	*3/*3	GA	36	36	36	0	0	100	36	36	0	0	100
11	*1/*2	*1/*1	GA	36	36	36	0	0	100	36	36	0	0	100
12	*1/*1	*1/*1	AA	36	36	36	0	0	100	36	36	0	0	100
Total				432	427	427	5	0	98.8	432	432	0	0	100

a Excludes samples with indeterminate / no calls

b A sample with correct call indicates a correct call at all three loci. One incorrect or no call at one out of the three loci for the sample is considered an incorrect call for the whole sample

c Final results reflect one time repeat of samples with indeterminate (no) calls

d Correct call rate = # samples with correct calls / # samples tested (excludes samples with indeterminate/no calls)

Drug Interference
Evaluation of potential interference from bilirubin, cholesterol, and heparin demonstrated that presence of these compounds in concentrations of 8mg/dl bilirubin, 70mg/dl cholesterol and 133v/dl heparin does not interfere with the INFINITI Warfarin Assay.

Sample Carry-Over
No sample carry-over was detected when 300ng of a positive sample was followed by 10ng of a second positive sample, and when 300ng of a positive sample was followed by a "No Template Control" or water. All genotype calls were 100% correct.

Assay Interference
Running the INFINITI Warfarin Assay and the INFINITI Assay for Factor II & Factor V on the same instrument did not affect the results of the assays, i.e., the INFINITI Warfarin Assay did not affect the results of the INFINITI Assay for Factor II & Factor V, and vice versa.

REFERENCES

1 Wadelius M et al "Pharmacogenetics of warfarin: current status and future challenges" The Pharmacogenomics Journal (2007) 7, 99-111

2 "FDA Approves Updated Warfarin (Coumadin) prescribing Information" FDA News, August 16, 2007

3 Gage B "Pharmacogenetics-Based Coumarin Therapy" Hematology 2006

4 Caldwell M et al "Evaluation of Genetic factors for Warfarin Dose prediction" Clinical medicine & Research (2007) Volume 5, Number 1:8-16

5 Cho H et al "Factors affecting the interindividual variability of warfarin dose requirement in adult Korean patients" Pharmacogenomics (2007) 8(40, 329-337

6 Lee CR, Goldstein JA, Peiper JA "Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data" Pharmacogenetics 2002; 12: 251-261-263

7 Rieder M et al "Effect of VKORC1 Haplotypes on Transcriptional Regulation and Warfarin Dose" The New England Journal of Medicine (June 2, 2005) 352;22

8 Takahashi H et al "Pharmacogenetics of CYP2C9 and interindividual variability in anticoagulant response to warfarin" The Pharmacogenomics Journal (2003) 3, 202-214

9 Takahashi H et al "Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans" Pharmacogenetics and Genomics 2006, Vol. 16 No 2