



INFINITI[®] CYP450 2C9-VKORC1

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use



FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue, Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI CYP450 2C9-VKORC1 is an *in vitro* diagnostic test for the detection and genotyping of the *2, *3, *4, *5, *6, and *11 CYP2C9 genetic variants and the VKORC1 3673 (-1639), 6009, 6484 (1173), 6853, 7566, 8773 and 9041 (3730) intronic variants in genomic deoxyribonucleic acid (DNA) obtained from human blood samples. The INFINITI CYP450 2C9-VKORC1 is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

The INFINITI CYP450 2C9-VKORC1 is indicated for use to identify individuals at risk for sensitivity to warfarin.

BACKGROUND INFORMATION

The detection of genetic variations of CYP450 2C9 and VKORC1 aids in determining variability in the response to certain therapeutic agents.

Vitamin K antagonists such as warfarin, acenocoumarol, and phenprocoumon are commonly used for the prevention and treatment of venous and arterial thrombosis. The vitamin K epoxide reductase complex subunit 1 (VKORC1) was found to play a significant role in individuals with combined deficiency of vitamin K-dependent clotting factors, or with warfarin resistance. It was found that more than 25 percent of the variance in warfarin dose is due to this one gene, and is possibly the single biggest contributor to variability in people’s responses to the drug, and could be a central factor in setting the initial dose.

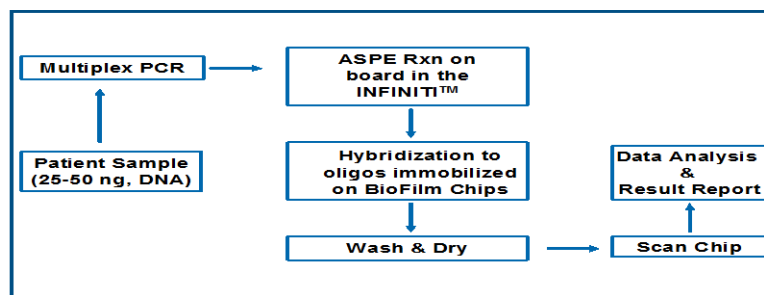
The other significant genotype polymorphism in a gene encoding the CYP2C9 enzyme that metabolizes warfarin accounts for about 10 percent of the difference in responses to warfarin. However, tests for these genetic variations are not routinely performed.

TEST PRINCIPLE / ASSAY OVERVIEW

The INFINITI CYP450 2C9-VKORC1 is designed to simultaneously detect the *2, *3, *4, *5, *6, and *11 CYP2C9 genetic variants and the VKORC1 3673 (-1639), 6009, 6484 (1173), 6853, 7566, 8773 and 9041 (3730) intronic variants. 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.

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 CYP450 2C9-VKORC1, 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 *2, *3, *4, *5, *6, and *11 CYP2C9 genetic variants and the VKORC1 3673 (-1639), 6009, 6484 (1173), 6853, 7566, 8773 and 9041 (3730) intronic variants in genomic deoxyribonucleic acid (DNA) obtained from human blood samples.

The INFINITI CYP450 2C9-VKORC1 is comprised of the BioFilmChip[®] Microarray, the Intellipac[®] Reagent Module, and the Amplification Mix.

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 CYP450 2C9-VKORC1 uses a microarray chip (R-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 four 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 **Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Four vials each containing 250µl of the PCR reaction master mix are packaged in a box.

The INFINITI CYP450 2C9-VKORC1 should be run using the AutoGenomics **INFINITI Analyzer or INFINITI PLUS Analyzer**. The Analyzers are instruments used for clinical multiplex systems intended to measure and sort multiple signals from a clinical sample. The Analyzers are designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays. The Analyzers 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 Analyzer and INFINITI PLUS Analyzer are CE marked.

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.
- Safety data Sheets (SDS) 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: 24 months Refrigerated (2 to 8°C)

Intellipac Reagent: 12 months Refrigerated (2 to 8°C)

Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four 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 (EACH PACKAGE IS SUFFICIENT FOR 48 TESTS)

- AutoGenomics Product Number 03-1040-02 INFINITI CYP450 2C9-VKORC1 BioFilmChip® Microarray Magazine: 4 magazines per package

- AutoGenomics Product Number 03-2040-02 INFINITI CYP450 2C9-VKORC1 Intellipac[®] Reagent Module
2 modules per package; 24 tests per module. Each Intellipac module contains
 - 1.1ml ASPE Master Mix:
 - dNTPs
 - Labeled –dCTP
 - Allele Specific Primers
 - PCR Extension Buffer
 - 2.6ml Hybridization Buffer
 - SSC
 - Sodium Azide
 - Hybridization Positive Control
- AutoGenomics Product Number 03-3040-02 INFINITI CYP450 2C9-VKORC1 Amp Mix. Each package contains
4 x 250µl vials of AMP Mix containing:
 - dNTPs
 - dCTP
 - PCR Primer Mix
 - MgCl₂
 - PCR Reaction Buffer
- : Product Number 12-0010-020: Wash buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI CYP450 2C9-VKORC1 can detect 2C9 and VKORC1 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 CYP450 2C9-VKORC1 has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Distilled Water (DNase and RNase free)
- Titanium Taq DNA Polymerase (see AutoGenomics Product catalog for recommended Titanium Taq DNA Polymerase and supplier)

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.

DNA Control

It is required to run known positive controls and a negative control should also be included in each test run.

PCR Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents 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.

Note:

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

1. Prepare the PCR master mix.

Amplification mix	17.75 µl
Titanium Taq DNA polymerase	0.25 µl
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Total	18.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the well plate.

3. Add 2 µl of sample DNA (30 ng/µl) to each well.

PCR master mix	18.0 µl
Sample DNA	2.0 µl
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Total volume of amplification reaction	20.0 µl

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Multiplex 2 Tm PCR Conditions

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

Note: After each cycle in step 2b the temperature is decreased by 0.5°C. When using an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour and 40 minutes (\pm 10 min). If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading - INFINITI Analyzer or INFINITI PLUS Analyzer

- 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 (Catalog # 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) (Catalog # 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 Wash buffer.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)

INFINITI PLUS Analyzer Operator's Manual (Part Number 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.

It is recommended that a positive (heterozygous and/or homozygous for the three genotypes) sample for each mutation, a negative control (a sample that does not contain the mutation of interest, i.e., a wild type sample); and a "Non Template Control" (Molecular Grade water 1XTE) be included with each test run. Please contact AutoGenomics for recommendations.

All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Note: The thermal cycler used should be regularly maintained and calibrated with an external temperature standard, according to the laboratory's regulatory and QC requirements.

LIMITATIONS

The results obtained from the INFINITI CYP450 2C9-VKORC1 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.

INTERPRETATION OF RESULTS

Results from the INFINITI CYP450 2C9-VKORC1 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 2C9*2, *3, *4, *5, *6, and *11 and VKORC1 3673 (-1639), 6009, 6484 (1173), 6853, 7566, 8773 and 9041 (3730).

A blank page with a message is displayed when the assay is not completed, and no genotype call is made. The assay has to be repeated. The message will indicate the reason why no genotype call was made. If errors occur during the assay, "Test Error" message (e.g., "low DNA") is shown. An Error Log is generated which identifies the problem. When an error message occurs, please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual. Depending on the error message/problem, the assay may have to be repeated only after the problem/error is corrected.

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 only the 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 CYP450 2C9-VKORC1. The study established the minimum DNA concentration for the INFINITI System Assay for CYP450 2C9-VKORC1 to be 1ng DNA. The recommended DNA concentration for the INFINITI CYP450 2C9-VKORC1 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 CYP450 2C9-VKORC1.

Percent Agreement vs. Bi-directional Sequencing

The results of the comparison studies conducted in three clinical sites comparing the INFINITI CYP450 2C9-VKORC1 to bi-directional sequencing demonstrated:

97.3% agreement for 2C9 genotypes as compared with bi-directional sequencing on 1 st run
97.7% agreement for VKORC1 genotypes as compared with bi-directional sequencing on 1 st run

A summary of the method comparison results is provided in Table 1.

Table 1 INFINITI CYP450 2C9-VKORC1 vs. Bi-Directional DNA Sequencing

Genotype	No. of Samples Tested	First Time Run		
		No. of Correct calls	No. of Invalid Calls*	Agreement with Bi-directional Sequencing
2C9				
*2	150	147	3	98.0%
*3	150	145	5	96.7%
*4	150	147	3	98.0%
*5	150	144	6	96.0%
*6	150	147	3	98.0%
*11	150	146	4	97.3%
2C9 (all)	900	876	24	97.3%
VKORC1				
861	150	147	3	98.0%
3673 (-1639)	150	147	3	98.0%
5808	150	147	3	98.0%
6009	150	146	4	97.3%
6484 (1173)	150	146	4	97.3%
6853	150	146	4	97.3%
7566	150	146	4	97.3%
8773	150	147	3	98.0%
9041 (3730)	150	147	3	98.0%
VKORC1 (all)	1,350	1,319	31	97.7%

* 2 incorrect calls for 2C9 and 4 incorrect calls for VKORC1; 22 no results for 2C9 and 27 no results for VKORC1

Assay Precision and Reproducibility

- Within Chip** - The average %CV within a chip ranges from 4.5 to 7.7. All genotype calls were 100% correct.
- Chip-to-chip** - Based on two-way ANOVA of the mean RFU readings, p-values for chip-to-chip reproducibility were:
 - p = 1.77904E-43 (F-test)
 - p = 3.73325E-71 (F-test)
 - p = 1.31994E-29 (F-test)
 All genotype calls were 100% correct.
- Lot-to-lot** - Three lots of reagents were tested in duplicate two times, each time using a different Coriell DNA sample. Two-way ANOVA indicates no significant difference (p>0.05) between the lots. All genotype calls were 100% correct.
- Run-to-run (intra-instrument)** - Based on two-way ANOVA of the mean RFU readings, p-values for run-to-run reproducibility were:
 - p = 0.247696 (F-test)
 - p = 0.000289 (F-test)
 - p = 0.001747 (F-test).
 All genotype calls were 100% correct.

- Instrument-to-instrument** - Based on two-way ANOVA of the mean RFU readings, p-values for inter-scanner reproducibility were:
 - R10-C33 p = 2.457E-16 (F-test)
 - R10-C34 p = 1.945E-09 (F-test)
 - R10-D20 p = 0.003648 (F-test)
 - R10-D24 p = 2.41E-18 (F-test)
 - R10-D52 p = 4.5766E-12 (F-test)

All genotype calls were 100% correct.

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 CYP450 2C9-VKORC1.

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 CYP450 2C9-VKORC1 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 CYP450 2C9-VKORC1 did not affect the results of the INFINITI Assay for Factor II & Factor V, and vice versa.

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