



eQ-PCR™ LC Warfarin Genotyping Kit

For *In Vitro* Diagnosis Use

Package Insert v1.5

INTENDED USE

The eQ-PCR™ LC Warfarin Genotyping kit is an **in vitro diagnostic test** for genotyping single nucleotide polymorphisms (SNPs) in the cytochrome P450 enzyme gene CYP2C9 known as CYP2C9*2 (C430T), CYP2C9*3 (A1075C), and a SNP in the vitamin K epoxide reductase complex 1 gene (VKORC1) known as VKORC1 (-1639G>A). The test uses genomic DNA extracted from whole blood and the genotype detection is performed on Roche Real-Time PCR System. The eQ-PCR™ LC Warfarin Genotyping kit is designed for use in clinical laboratories upon prescription by the attending physician.

The eQ-PCR™ LC Warfarin Genotyping kit is a qualitative genotyping test indicated for use as an aid in identifying patients, adults of age 18 or older, who may be at risk of warfarin sensitivity. The kit is not indicated for use in fetal diagnosis or monitoring. This kit is also not indicated for stand-alone diagnostic purposes.

BACKGROUND INFORMATION

Warfarin, a coumarin derivative, is a commonly prescribed oral anticoagulant used for the prevention and treatment of individuals with thromboembolytic problem. However, the drug has a narrow therapeutic range and the management of warfarin medication is difficult due to wide individual variation. The inter-individual variability in response to warfarin is mainly affected by age, gender, genetic variations, body mass index (BMI), and use of concomitant medications. Blood tests (prothrombin, PT test) are frequently required during the first several weeks of treatment to establish stable therapeutic levels. A standard International Normalized Ratio (INR) is used to standardize the different sensitivities of various thromboplastin reagents across laboratories.

Pharmacogenetics in Warfarin Metabolism

Genetic-based tests have the potential to identify sources of inter-individual variability in drug response and the ability to individualize therapy with the intent of maximizing effectiveness and minimizing risks.

Two genes have been identified as having effects on the metabolism of warfarin:

- **CYP2C9** is a member of the cytochrome P450 enzyme superfamily that is involved in drug metabolism. CYP2C9*2 and CYP2C9*3 are among the CYP2C9 variants that have been shown to affect the function of the enzyme that regulates warfarin catabolism.
- **VKORC1** is vitamin K epoxide reductase. Variants of this gene are associated with variable warfarin requirements. In particular, the variation at the VKORC1 3673 (-1639) has a significant impact on warfarin catabolism.

Many studies have indicated that CYP2C9*2 and CYP2C9*3 variants decrease the enzyme activity of warfarin metabolism and patients with these variant alleles may have a greater risk of bleeding compared to the patients carrying only wild-type alleles^{1,2,3,4,5}. The single nucleotide polymorphisms of VKORC1 3673 (-1639G>A) have clinical significance and have been shown to be useful in predicting the risk of warfarin complications^{6, 7 8,9}. Warfarin drug treatment may have different impact on the patients with the VKORC1 3673 (-1639>A) variant compared to those patients with wild type allele^{10, 11,12, 13}.

The allele frequency of each gene varies among different ethnic groups. The expected frequency of each SNP across ethnic groups is summarized below.

Expected Genotypic Frequency of Different Ethnic Groups			
	Caucasian^{†1}	African^{†2,3}	Asian^{†1}
2C9*2			
*1/*1	78.7%	87.0%	100%
*1/*2	20.4%	8.7%	0%
*2/*2	0.9%	0%	0%
2C9*3			
*1/*1	88.0%	N/A	92.7%
*1/*3	11.6%	4.3%	7.3%
*3/*3	0.4%	0%	0%
VKORC1			
GG	39.1%	79.2%	1.9%
GA	46.7%	20.8%	18.3%
AA	14.2%	0%	79.8%

[†] The data presented are based on the genotypic frequencies in the following 3 publications:

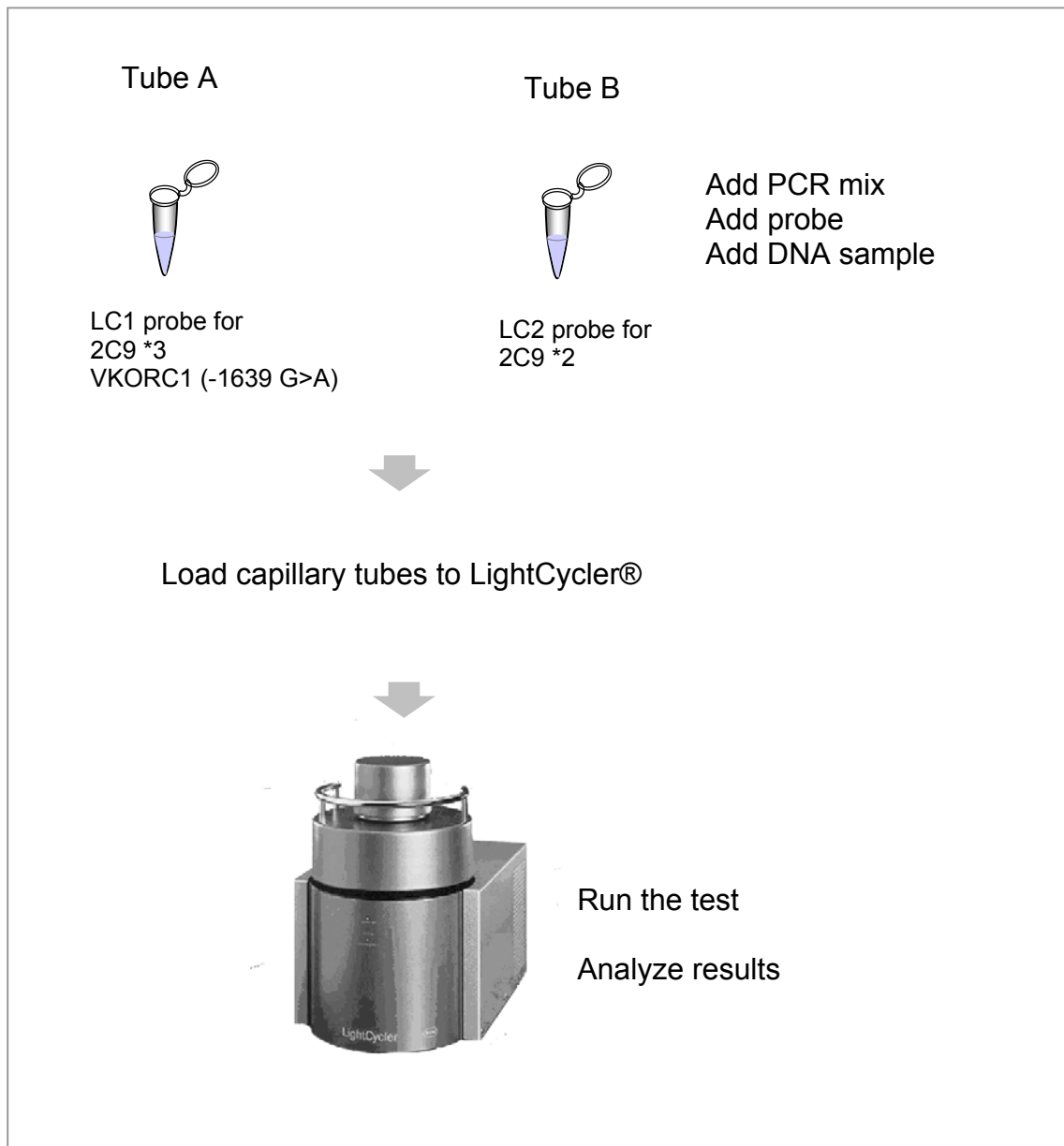
1. Yuan et al., A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Human Molecular Genetics* 14(13): 1745-1751, 2005.
2. Kirchheiner and Brockmoller. Clinical Consequences of Cytochrome P450 2C9 Polymorphisms. *Clinical Pharmacology & Therapeutics* 77(1):1-16, 2005.
3. Schelleman et al., Warfarin response and Vitamin K Epoxide Reductase Complex in African Americans and Caucasians. *Nature* 81(5): 742-747, 2007

TrimGen eQ-PCR LC Warfarin Genotyping kit is dedicated to provide accurate genotype information of these SNPs.

DEVICE DESCRIPTION

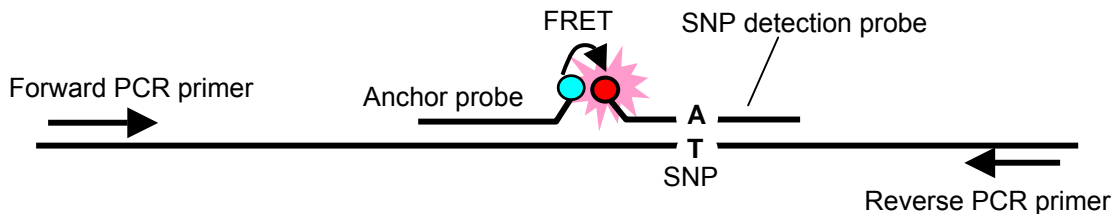
The eQ-PCR LC Warfarin Genotyping kit includes all reagents required for genotyping 2C9 *2, 2C9 *3 and VKORC1 (-1639 G>A) polymorphisms. The test uses DNA extracted from patient whole blood samples. After the DNA sample is mixed with the reagents, the genotyping test is performed in a closed-tube system using Roche Diagnostics LightCycler® Real-Time PCR System. The LightCycler® Real-Time PCR System automatically proceeds to target amplification and SNP detection. This walk-away test eliminates post-PCR handling contamination and also reduces labor.

TEST OVERVIEW

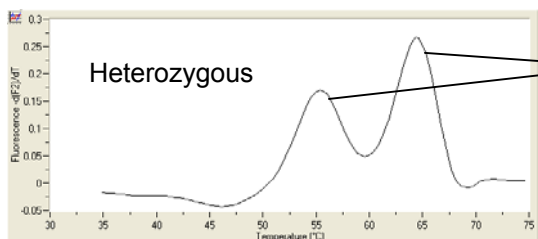
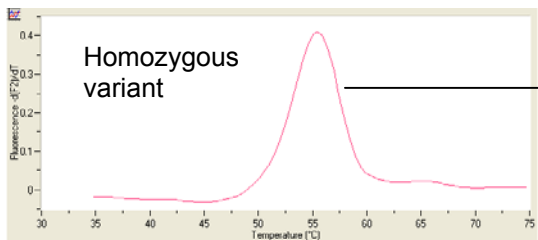
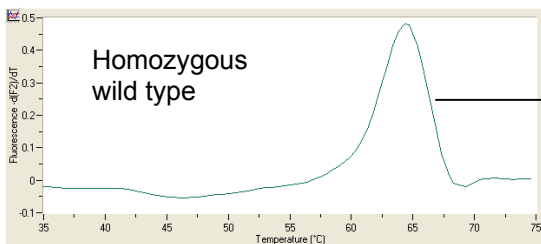


TEST PRINCIPLE

The kit uses PCR technology to amplify target DNA and signal detection is based on fluorescence resonance energy transfer (FRET) technology. Two probes are designed for each SNP and labeled with different fluorophores. The anchor probe selectively hybridizes to the sequence flanking the SNP site and the SNP detection probe recognizes the SNP and hybridizes to the sequence containing the SNP site. During FRET, the fluorophore on the anchor probe is excited by the light source of the LightCycler and the excitation energy is transferred to the fluorophore on the SNP detection probe. The emitted fluorescence is then measured at the respective wavelength.



The SNP detection is performed by melting curve analysis, a method that discriminates different allelic forms of DNA by melting temperature. The SNP detection probe is designed to have a unique melting temperature to the wild type and variant. When the temperature increases, the probe dissociates from the target sequence at a specific melting temperature depending on the allelic type of the SNP. The SNP type is then detected as different melting curves as follows:



WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use

Sample Handling

All patient specimens should be handled following **CLSI guidelines**.

Test Precautions

- **The capillary tube is easily broken and must be handled with care.** Practice tube handling and loading before starting the test.
- Do not pool / mix reagents from different lots.
- Do not use expired kits or reagents.
- Store the kits according to the product label.

Product Safety and Liabilities

When working with the kit reagents, always wear a suitable lab coat, disposable gloves, and protective goggles. TrimGen Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the misuse, the results of use, or the inability to use this product.

Limited Product Warranty

It is imperative that the users strictly adhere to this manual. Failure to do so will void TrimGen's guarantee of this product. TrimGen Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose.

Waste Handling

Follow the instruction of federal, state and local regulations to dispose unused reagents and waste.

Material Safety Data Sheet is available upon request.

REAGENTS

The eQ-PCR™ LC Warfarin Genotyping kit contains pre-packaged reagents for 32 reactions.

Tube Label	Contents	Quantity
PCR Mix	PCR Buffer, DNA polymerase, and dNTPs	One tube (500 µl)
LC1	Primers and probes for genotyping 2C9*3 (A1075C) and VKORC1 (-1639 G>A)	One tube (55 µl)
LC2	Primers and probes for genotyping 2C9*2 (C430T)	One tube (55 µl)
NF Water	Nuclease-free water for the no template control	One tube (500 µl)

***Note:** The Probe Mixes are **light sensitive**. Keep these reagents protected from direct light.

MATERIALS REQUIRED BUT NOT PROVIDED:

- LightCycler® Color Compensation Kit (Roche Diagnostics, Catalog number 12158850001) for the discrimination of different genotypes.
- **Whole blood DNA extraction kit**
Commercially available whole blood DNA extraction kits are acceptable for use and customer should validate the product prior to testing.
- **Control DNA**
Genomic DNA controls for the 2C9 or VKORC1 genotype **are not provided** with the kit. Genotype controls should be obtained separately. It is required to run positive and no template control DNA at each run.

We recommended that a positive (heterozygous and/or homozygous for the three genotypes) sample for each variant, a negative control (a sample that does not contain the mutation of interest, i.e., a wild type sample); and a “Non-Template Control” be included with each test run. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations.

EQUIPMENT REQUIRED BUT NOT PROVIDED:

- Roche LightCycler® Real-Time PCR System 1.2
- LightCycler® capillary tubes, 20.0 µl volume
- LightCycler® cooling block, with centrifuge adapters from the refrigerator
- LightCycler® capping tool
- Mini centrifuge for 2.0 ml microcentrifuge tubes
- Pipets
- Sterile filter tips

REAGENT STORAGE INSTRUCTIONS

The eQ-PCR™ LC Warfarin Genotyping kit is shipped to the laboratory on dry ice. Upon receipt of the kit, store all reagents at -20°C and protect them from light. The kit is stable at -20°C for one year. After first use, store all reagents at 2-8°C for up to three months and protect the reagents from light.

SPECIMEN COLLECTION, HANDLING, AND PREPARATION

This kit is validated for use with DNA from whole blood. Reagents for DNA extraction are **not provided** with the eQ-PCR™ LC Warfarin Genotyping kit.

For whole blood samples, EDTA-anticoagulated whole blood is required. Any commercially available DNA extraction kit is acceptable, however, the user should validate the DNA extraction method prior to use.

For use with DNA from other types of samples, user should validate the product in their complete system as required by CLIA regulations in the U.S., or by other equivalents in other countries.

Adjust the final DNA concentration to 10-40 ng/µl.

Refer to the DNA extraction method's package insert for the collection, handling, storage and preparation of specimens for analysis.

CALIBRATION

To discriminate different genotypes, a color compensation test is required for the first use of the kit. Follow the instructions of the Roche LightCycler® User Manual and the LightCycler® Color Compensation Kit (Roche Diagnostics, Catalog number 12158850001) to perform the color compensation test.

LIGHTCYCLER® PROGRAM SETUP

Please see Appendix A

TEST PROCEDURE

Before starting, you need to:

- ✓ Pre-chill the cooling block with centrifuge adapters and capillaries in the refrigerator (2-8°C) for at least 4 hours.
- ✓ Set up the PCR parameters on the LightCycler® following Appendix A in the user manual.

NOTE

The kit contains reagents that are sufficient for detection of a maximum of 14 patient samples (1 for no template control, 1 for positive control and 14 for patient samples).

A. Reaction Preparation

Thaw all reagents at 2-8°C and protect them from light. Do not re-freeze (the reagents are stable up to three months at 2-8°C). Spin all tubes prior to use.

- A.1.** Use the following formula to calculate the total number of capillary tubes (not provided) needed:

$\left(\frac{\text{Sample \#}}{\quad} \times 2 \right) + 4^* = \text{tubes}$

*For the no template control (NTC) and the positive control.

- A.2.** Using the form provided in Appendix B, fill in the sample ID to eQ-PCR™ LC Warfarin Genotyping Worksheet (see example below). Each sample requires two capillary tubes, one tube (**A**) for VKORC1 (-1639G>A) and 2C9*3 (A1075C), and the other tube (**B**) for 2C9*2 (C430T).

Capillary A#	Tube A Set (VKORC1 and 2C9*3)	Capillary B#	Tube B Set (2C9*2)
A1	A1-NTC	B1	B1-NTC
A2	A2-Pos	B2	B2-Pos
A3	A3-Sample #1	B3	B3-Sample #1
A4	A4-Sample #2	B4	B4-Sample #2
A5	More samples...	B5	More samples...

A.3. Collect and label capillary tubes according to the diagram below.

	<u>Set A</u>	<u>Set B</u>
No template control (NTC) capillaries	Ⓐ1	Ⓑ1
Positive control (Pos) capillaries	Ⓐ2	Ⓑ2
Sample #1 capillaries	Ⓐ3	Ⓑ3
Sample #2 capillaries	Ⓐ4	Ⓑ4
Sample #3 capillaries	Ⓐ5	Ⓑ5

Keep all capillaries in LightCycler® cooling block at 2-8°C.

A.4. Set up two 2 ml tubes. Label as **A** and **B**.

A.5. Prepare the **Master Mix A** (Tube A):

Tube **A**: **Master Mix A**

PCR mix = $(12.5 \times \frac{\quad}{\text{Sample \#}} + 2^*) \times 1.1^{**} = \quad \mu\text{l}$

LC1 = $(2.5 \times \frac{\quad}{\text{Sample \#}} + 2^*) \times 1.1^{**} = \quad \mu\text{l}$

Transfer the reagents to tube **A** and mix the reagents by flicking the tube. Keep the tube at 2-8°C and protected from direct light.

* for controls ** for pipetting error

Prepare the **Master Mix B** (Tube B):

Tube **B**: **Master Mix B**

PCR mix = $(12.5 \times \frac{\quad}{\text{Sample \#}} + 2^*) \times 1.1^{**} = \quad \mu\text{l}$

LC2 = $(2.5 \times \frac{\quad}{\text{Sample \#}} + 2^*) \times 1.1^{**} = \quad \mu\text{l}$

Transfer the reagents to tube **B** and mix the reagents by flicking the tube. Keep the tube at 2-8°C and protected from direct light.

* for controls ** for pipetting error

- A.6. Remove the cooling block with centrifuge adapters and capillaries from the refrigerator (at 2-8 °C).
- A.7. Aliquot **15 µl** of **Master mix A** to each Set A capillary tube.
- A.8. Aliquot **15 µl** of **Master mix B** to each Set B capillary tube.
- A.9. Add **5µl** of **NF Water** into the A1 and B1 capillary tubes for the NTC (no template control).
- A.10. Add **5µl** of genomic control DNA (10-40 ng/µl or 50-200 ng per reaction tube, **genomic DNA controls are not provided**) into the A2 and B2 capillary tubes for the Positive Control.
- A.11. Add **5µl*** of the **sample DNA** (10-40 ng/µl or **50-200 ng per reaction** tube) into each respective **A** and **B** set of capillary tubes (Ref. diagram in Step A3).
It is important to confirm the sample ID with the labeling on each capillary tube.
- A.12. Using the capping tool, cap each capillary.
- A.13. Pulse spin the capillaries in pre-chilled centrifuge adapters, and then carefully place the capillaries (**Caution: tubes are fragile and can be easily broken**) on the LightCycler® carousel following the order on the sample worksheet. Place the carousel on the LightCycler® instrument.
It is important to confirm the sample tube ID with each capillary number on the carousel.

B. Run the PCR

- B.1. Double-click on the LightCycler® icon on the screen.
- B.2. At the main menu, click “Run.”
- B.3. At the next screen, run the “self-test” if not already performed for the day of instrument use.
- B.4. Go to the “Experiment” dialog box (at the top right corner of the screen), click on “Import.”
- B.5. Select and open pre-designated protocol (See *Appendix A*) in the “Protocol” folder.
(Note: Refer to the Roche LightCycler® User Manual for instructions on setting up a protocol and/or running an existing protocol.)
- B.6. Select all the cycle programs and click “OK.”
- B.7. Check the parameters for the PCR reaction.
- B.8. Click the “Edit Samples” button.
- B.9. Input the sample names and the total number of tubes. Click “Done.”
- B.10. Click on the green “Run” button on the screen.

B.11. Type in the data file name and click “Save” (this file will be used for data analysis).

C. Data collection

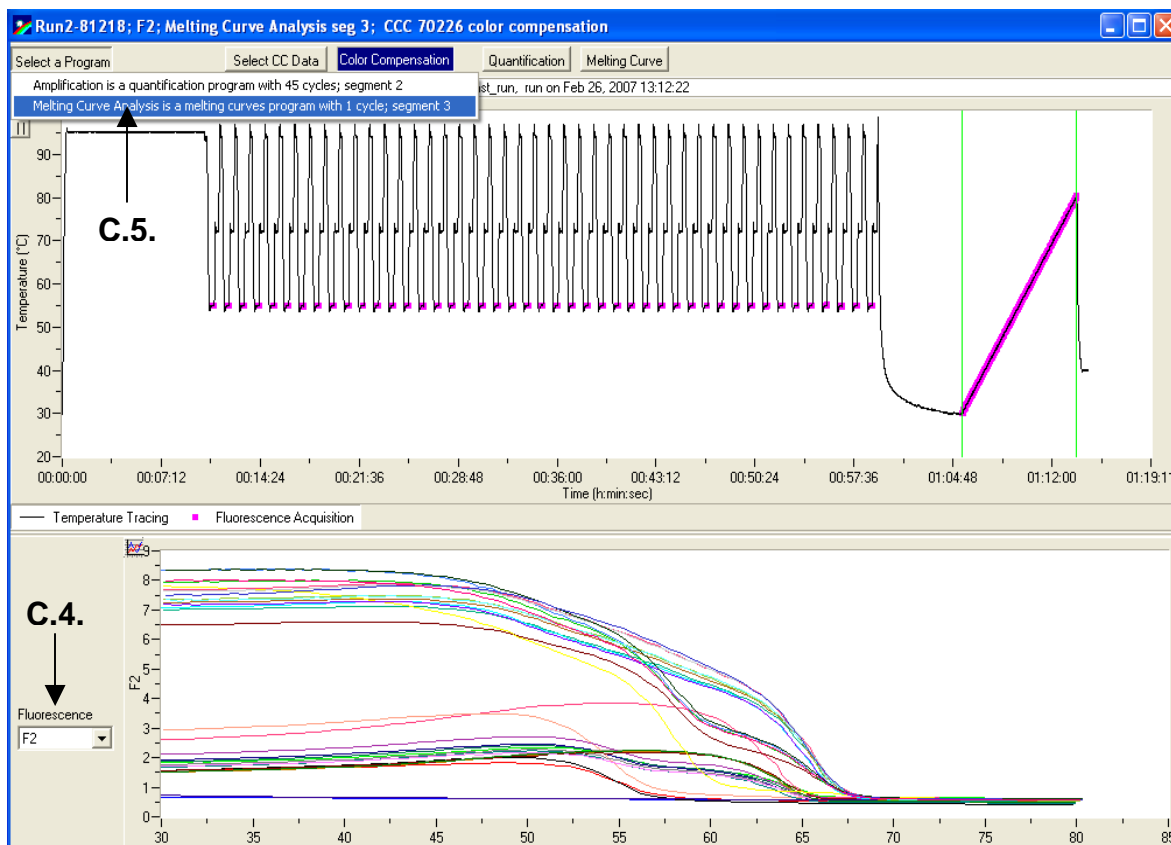
C.1. After the instrument has finished running, go to main menu. Click on “**Data Analysis.**”

C.2. At the next screen, scroll through the files to find the data file, select it and click “**Open.**”

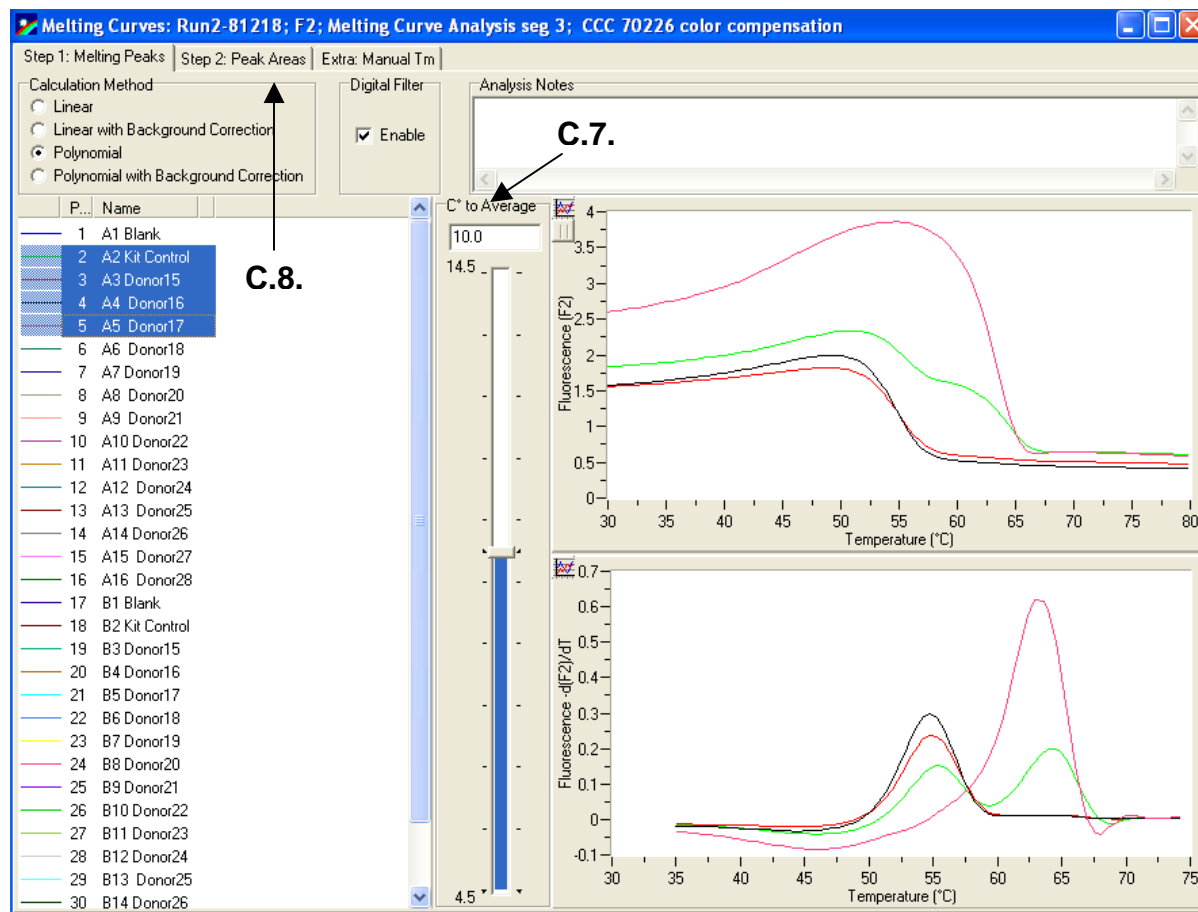
C.3. In the LightCycler® Data Analysis Window, click “**Select CC Data**” and choose a valid color compensation file corresponding to the specific channels used for this assay.

C.4. To view the melting curve of VKORC1 or 2C9*2, select F2 in the “**Fluorescence**” box at the lower window. To see the melting curve of 2C9*3, select F3 in the “**Fluorescence**” box.

C.5. Then, go back to the upper window. Click “**Select a Program**” at the top left of the pull down menu. Choose “**Melting Curve Analysis is a melting curve program with 1 cycle.**”



- C.6. Click “Melting Curve” button at the top right of the pull down menu. This will bring you to the “Step1: Melting Peaks” window.
- C.7. Set the “°C to average” to 10. Each sample can have one or two peaks depending on the genotype.



- C.8. To view the Tm values of each sample, click “Step 2: Peak Areas” and choose either “one” or “two” in the “Number of Peaks” dialog box depending upon the number of peaks shown in the right side graphic panel. When encountering a problem, use the Tm listed in the following Tables (Tables 1, 2, and 3) as a reference for the determination of number of peaks.

D. Interpretation of Results

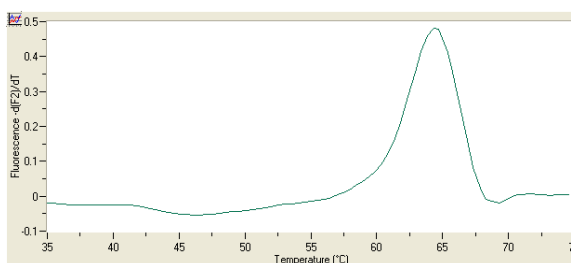
The T_m value for each SNP is listed in Tables 1, 2, and 3. Use the T_m value as a reference to determine the genotype of the unknown samples.

Select **F2 channel** to view the melting peaks and the T_m of the melting peaks for SNP VKORC1 (Table 1) and CYP2C9*2 (Table 2).

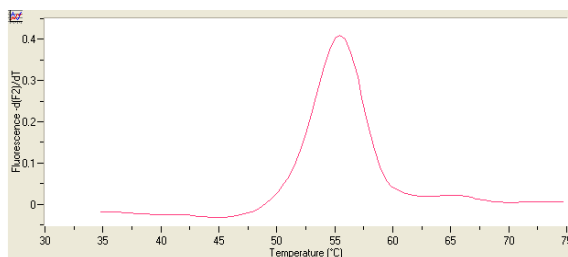
Table 1. F2 channel, Tube A Set – VKORC1 genotype

CTL Genotype	Number of peaks	T_m of melting peaks
Homozygous Wild type (-1639G)	1	63.57 ± 1.95
Homozygous Variant (-1639A)	1	54.94 ± 2.40
Heterozygous (-1639G & -1639A)	2	63.57 ± 1.95 54.94 ± 2.40

**VKORC1 (-1639)
Homozygous
Wild type**



**VKORC1 (-1639)
Homozygous
Variant**



**VKORC1 (-1639)
Heterozygous**

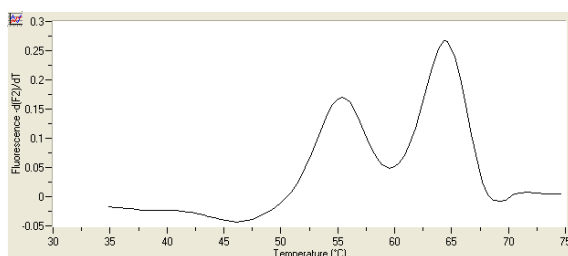
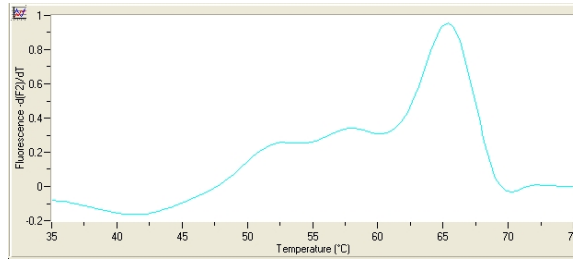


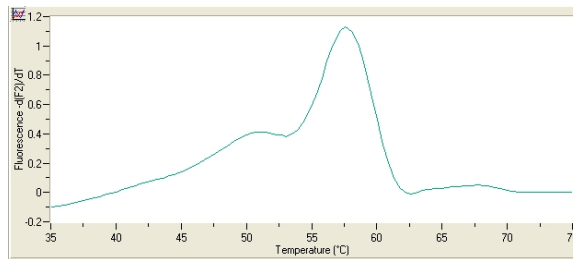
Table 2. F2 channel, Tube B Set – CYP2C9*2 genotype

CTL Genotype	Number of peaks	Tm of melting peaks
Homozygous Wild type (430C)	1	65.58 ± 1.77
Homozygous Variant (430T)	1	57.34 ± 2.70
Heterozygous (430C & 430T)	2	65.58 ± 1.77 57.34 ± 2.70

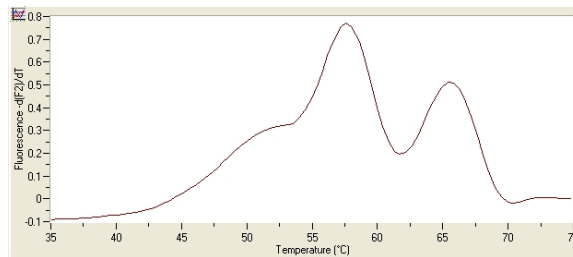
**CYP 2C9*2
Homozygous
Wild type**



**CYP 2C9*2
Homozygous
Variant**



**CYP 2C9*2
Heterozygous**

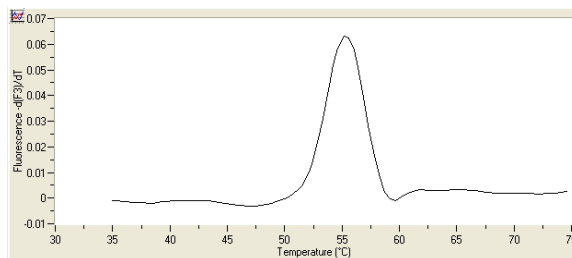


Select F3 channel to view the melting peaks and the Tm of the melting peaks For SNP CYP2C9*3.

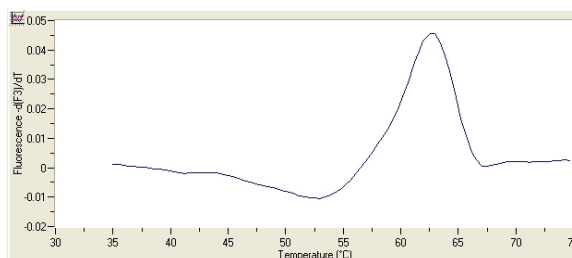
Table 3. F3 channel, Tube A Set – CYP2C9*3 genotype

CTL Genotype	Number of peaks	Tm of melting peaks
Homozygous Wild type (1075A)	1	55.23 ± 2.70
Homozygous Variant (1075C)	1	61.89 ± 2.58
Heterozygous (1075A & 1075C),	2	55.23 ± 2.70 61.89 ± 2.58

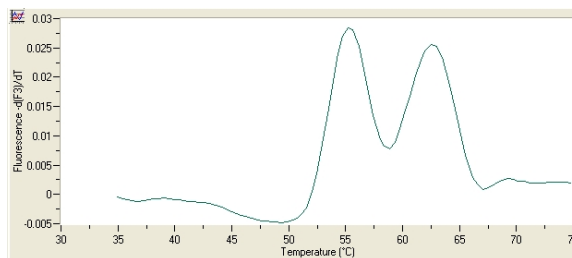
**CYP 2C9*3
Homozygous
Wild type**



**CYP 2C9*3
Homozygous
Variant**



**CYP 2C9*3
Heterozygous**



E. Limitations of the Procedure

The eQ-PCR™ LC Warfarin Genotyping kit is limited to detecting single nucleotide polymorphisms (SNP) in the cytochrome P450 enzyme gene CYP2C9 known as CYP2C9*2 (C430T) and CYP2C9*3 (A1075C), and a SNP in the vitamin K epoxide reductase complex 1 gene (VKORC1), known as VKORC1 (-1639G>A). The kit does not apply to detecting any other SNPs in CYP2C9 or VKORC1.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Specificity studies were conducted during assay development. PCR primer specificity was determined by agarose gel and sequencing the amplicon. The hybridization probe specificity was determined by hybridization assays with verified PCR products.

Method Comparison Studies

The results of the comparison studies were conducted at three clinical sites comparing the TrimGen eQ-PCR LC Warfarin Genotyping kit to bi-directional sequencing. The results can be seen in the table below

Genotype Call Rate Compared with Bi-directional Sequencing

Allele	Genotype*	# samples	# genotype calls	# correct calls**	# incorrect calls	# no calls	Correct call rate (%)	95% One-sided Confidence Lower Limit
2C9*2	*1/*1	126	126	123	0	3 [†]	97.6	94.40%
	*1/*2	28	28	27	1 [‡]	0	96.4	87.68%
	*2/*2	5	5	5	0	0	100	47.98%
	Sub-total	159	159	155	1	3	97.5	94.59%
2C9*3	*1/*1	140	140	138	0	2 [†]	98.6	96.09%
	*1/*3	13	13	12	0	1 [†]	92.3	75.32%
	*3/*3	6	6	5	1 [‡]	0	83.3	54.28%
	Sub-total	159	159	155	1	3	97.5	96.40%
VKORC 1	G/G	79	79	77	0	2 [†]	97.5	93.16%
	G/A	63	63	62	0	1 [†]	98.4	94.32%
	A/A	17	17	17	0	0	100	80.52%
	Sub-total	159	159	156	0	3	98.1	95.54%
Total for Assay			477	466	2	9	97.7	96.18%

*Genotype determined by bi-directional sequencing.

**Genotype determined by TrimGen eQ-PCR LC Warfarin Genotyping kit

[†] Three samples were no-calls. The no-call was caused by a system failure of the instrument during the test. The genotypes for the 1st no-call sample were *1/*1 for 2C9*2, *1/*1 for 2C9*3 and GA for VKORC1. The genotypes for the 2nd sample were *1/*1 for 2C9*2, *1/*1 for 2C9*3 and GG for VKORC1. The genotypes for the 3rd sample were *1/*1 for 2C9*2, *1/*3 for 2C9*3 and GG for VKORC1.

[‡] The incorrect call sample was a sample in dispute. Both the bi-directional sequencing and LightCycler results showed this sample has an unpublished rare genotype - *1/*2 for 2C9*2 and *3/*3 for 2C9*3. We were unable to further analyze the entire 2C9 gene sequence for this questionable sample due to limited sample resource. The dispute sample is reported as incorrect call.

Limit of Detection

Serial dilutions of 0.1ng to 600ng DNA were prepared from known purified DNA samples. Each serial dilution was repeatedly tested 26 times. The data is summarized below.

DNA Limitation Study

Input DNA Amount (ng)	Genotype	# samples	# repeats	# tests	# genotype calls	# correct calls	# incorrect calls	# no calls	Correct call rate (%)
0.1	2C9*2	6	26	156	468	466	0	2	99.6
	2C9*3	6	26	156					
	VKORC1	6	26	156					
1	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					
10	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					
50*	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					
100*	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					
200*	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					
300	2C9*2	2	3	6	18	18	0	0	100
	2C9*3	2	3	6					
	VKORC1	2	3	6					
400	2C9*2	2	3	6	18	18	0	0	100
	2C9*3	2	3	6					
	VKORC1	2	3	6					
600	2C9*2	6	26	156	468	468	0	0	100
	2C9*3	6	26	156					
	VKORC1	6	26	156					

* Equal to the recommended sample concentrations in the package insert (5 μ l of 10- 40ng/ μ L DNA).

Inter-laboratory Reproducibility Study

The study was performed at three testing sites. Each site received the same blood samples and the DNA was extracted by different DNA extraction methods. The samples were repeatedly tested five times on non-consecutive days. Results of the reproducibility study are summarized below.

Reproducibility Results - site by site

	Genotype	# samples tested	Genotyping calls made	# correct calls	# incorrect calls	# no calls	Correct call rate %
Site 1	2C9*2	155	153	153	0	2	98.7
	2C9*3	155	155	155	0	0	100
	VKORC1	155	155	155	0	0	100
Site 2	2C9*2	155	153	153	0	2	98.7
	2C9*3	155	154	154	0	1	99.3
	VKORC1	155	154	154	0	1	99.3
Site 3	2C9*2	155	155	155	0	0	100
	2C9*3	155	155	155	0	0	100
	VKORC1	155	155	155	0	0	100

Expected Values

Please see the table in BACKGROUND INFORMATION (page 3) for the expected frequency of each SNP in a population.

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- **Date of issuance of package insert (or revision letter)**

Revision: EP11-V1.5 February 4, 2009

APPENDIX A

	1. UNG Treatment (optional)	2: Denaturation	3: Amplification			4: Melting Curve Analysis			5: Cooling
Cycle Program Data	<i>Value</i>								
Cycles	1	1	45			1			1
Analysis Mode	None.	None.	Quantification.			Melting Curves.			None.
Temperature Targets	<i>Segment</i>								
	1	1	1	2	3	1	2	3	1
Target Temperature (°C)	50	95	94	55	72	95	30	80	40
Incubation Time (hh:mm:ss)	2:00	10:00	10	15	15	0	01:00	0	30
Temperature Transition Rate (°C/s)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	0.1	20.0
Second Target Temperature (°C)	0	0	0	0	0	0	0	0	0
Step Size (°C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Step Delay (Cycles)	0	0	0	0	0	0	0	0	0
Acquisition Mode	None	None	None	Single	None	None	None	Cont.	None

Appendix B **eQ-PCR™ LC Warfarin Genotyping Worksheet**
Use a copy of this sheet for your test

Capillary #	Tube A Set (VKORC1 and 2C9*3)	Capillary #	Tube B Set (2C9*2)
A1		B1	
A2		B2	
A3		B3	
A4		B4	
A5		B5	
A7		B7	
A8		B8	
A9		B9	
A10		B10	
A11		B11	
A12		B12	
A13		B13	
A14		B14	
A15		B15	
A16		B16	