

Overview

Useful For

Identifying patients who may require warfarin dosing adjustments(3,4) including:

- Patients being started on a first prescription for warfarin
- Patients who have previously been prescribed warfarin and have required multiple dosing adjustments to maintain the international normalized ratio in the target range
- Patients with a history of thrombosis or bleeding when taking warfarin

Genetics Test Information

This test is used for assessing *CYP2C9*, *VKORC1*, *CYP4F2*, and rs12777823 for variants affecting the metabolism of warfarin (Coumadin). This assay should be ordered on patients who are receiving warfarin for the first time or who are experiencing difficulties in maintaining the international normalized ratio (INR) in the therapeutic range.

Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Pharmacogenomic Associations Tables](#)
- [Multiple Genotype Test List](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

Method Name

Real-Time Polymerase Chain Reaction (PCR) with Allelic Discrimination Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

If patient is using medications other than warfarin, the preferred test is 2C9GV / Cytochrome P450 2C9 Genotype, which tests for only the *CYP2C9* gene.

Testing is available as the single gene assay (this test) or as a part of a focused pharmacogenomics panel, which includes testing for the following genes: *CYPs 1A2, 2C9, 2C19, 2D6, 3A4, 3A5, 4F2, SLCO1B1*, and *VKORC1*. Order PGXFP / Focused Pharmacogenomics Panel if multiple pharmacogenomic genotype testing is desired.

Specimen Required

Multiple genotype tests can be performed on a single specimen after a single extraction. See [Multiple Genotype Test List](#) in Special Instructions for a list of tests that can be ordered together.

Submit only 1 of the following specimens:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.
2. Send specimen in original tube.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.

Supplies: Saliva Swab Collection Kit (T786)

Specimen Volume: 1 swab

Collection Instructions: Collect and send specimen per kit instructions.

Specimen Stability Information: Ambient

Specimen Type: DNA

Container/Tube: 2 mL screw top tube

Specimen Volume: 100mcL (microliters)

Collection Instructions:

1. The preferred volume is 100 mcL at a concentration of 50 ng/mcL.
2. Include concentration and volume on tube.

Specimen Stability Information: Frozen (preferred)/Ambient/Refrigerated

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:

-[Informed Consent for Genetic Testing](#) (T576)

-[Informed Consent for Genetic Testing-Spanish](#) (T826)

2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-[Cardiovascular Test Request](#) (T724)

-[Neurology Specialty Testing Client Test Request](#) (T732)

[-Therapeutics Test Request](#) (T831)

Specimen Minimum Volume

Blood: 0.4 mL

Saliva: 1 swab

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical and Interpretive

Clinical Information

Warfarin is a Coumarin-based drug commonly utilized in anticoagulation therapy to prevent thrombosis due to inherited and acquired hemostatic disorders. The drug is also used in a number of other medical conditions and treatments including atrial fibrillation and hip replacement surgery. Warfarin acts by interfering with the metabolism of vitamin K, which is necessary for production of key coagulation factors. Warfarin inhibits vitamin K recycling by blocking its metabolism at the vitamin K-epoxide intermediate; thereby decreasing the amount of available vitamin K. Warfarin has a narrow therapeutic window; undermedicating increases the risk for thrombosis and overmedicating increases the risk for cerebrovascular accidents. Warfarin therapy has one of the highest rates of severe adverse drug reactions.

Warfarin is dosed using nongenetic factors including gender, weight, and age, and is monitored by coagulation testing in order to maintain the international normalized ratio (INR) within specific limits. However, warfarin metabolism is highly variable and dependent upon genetic factors. Variants within 3 genes and 1 intragenic locus are known to affect the metabolism of warfarin and the dose needed to maintain the correct serum drug level and degree of anticoagulation.

The *CYP2C9* gene encodes the cytochrome P450 2C9 (*CYP2C9*) enzyme that primarily metabolizes the more active isomer of warfarin (S-warfarin) to inactive products. Some *CYP2C9* variants result in decreased enzymatic activity and may lead to increases in serum warfarin and over-medication, driving the INR above the therapeutic target.

The second gene (*VKORC1*) encodes vitamin K epoxide reductase complex subunit-1 (*VKORC1*), a small transmembrane protein of the endoplasmic reticulum that is part of the vitamin K cycle and the target of warfarin therapy.⁽¹⁾ Vitamin K epoxide, a by-product of the carboxylation of blood coagulation factors, is reduced to vitamin K by *VKORC1*. A *VKORC1* promoter variant leads to decreased expression of the gene, resulting in reduced availability of vitamin K. This may cause increases in serum warfarin and overmedication, driving the INR above the therapeutic target. In addition, there are variations in *VKORC1* that lead to warfarin resistance that are tested by this assay. These variations are rare.

CYP4F2 metabolizes reduced vitamin K to hydroxyl-vitamin K1, thus removing it from the pathways involved in the

activation of clotting factors impacted by warfarin. In individuals who self-identify as being of non-African ancestry, carriers of the *CYP4F2**3 (C.1297G->A; rs2108622) variant may need a small (5%-10%) warfarin dosage increase to achieve therapeutic goals.

The rs12777823G->A variant is located intragenic in the *CYP2C* locus on chromosome 10. The A allele has been associated with the need for a 10% to 15% decrease in dose in individuals who self-identify as being of African ancestry.

CYP2C9:

CYP2C9 metabolizes a wide variety of drugs including warfarin and phenytoin. (Note that if testing is desired for other CYP2C9 substrates, order 2C9GV / Cytochrome P450 2C9 Genotype.

A number of specific *CYP2C9* variants result in enzymatic deficiencies. The following information outlines the relationship between the variants detected in this assay and their effect on the activity of the enzyme (Table 1):

Table 1:

CYP2C9 Allele	cDNA Nucleotide Change	Effect on Enzyme Metabolism
*1	None (wild type)	Normal activity
*2	430C->T	Reduced activity
*3	1075A->C	No activity
*4	1076T->C	Reduced activity
*5	1080C->G	Reduced activity
*6	818delA	No activity
*8	449G->A	Substrate specific
*9	752A->G	Reduced activity
*11	1003C->T	Reduced activity
*12	1465C->T	Reduced activity
*13	269C->T	Minimal activity
*14	374G->A	Minimal activity
*15	485C->A	No activity
*16	895A->G	Minimal activity
*17	1144C->T	Reduced activity
*18	1190A->C	No activity
*25	353_362del	No activity
*26	389C->G	Minimal activity
*28	641A->T	Minimal activity
*30	1429G->A	Minimal activity
*33	395G->A	Minimal activity
*35	374G->T + 430C->T	No activity

VKORC1:

The c.-1639 promoter variant is located in the second nucleotide of an E-Box (CANNTG) and its presence disrupts the consensus sequence, reducing promoter activity. In vitro experiments show a 44% higher transcription level of the G versus the A allele.(1) The c.-1639 G->A nucleotide change results in decreased gene expression and reduced enzyme activity. This test also determines the genotype for multiple other loci within *VKORC1* that have been associated with warfarin resistance. The mechanism by which these variations cause warfarin resistance is not clearly understood.

Table 2: Additional Variants Tested

Gene/SNV	cDNA Nucleotide Change	Effect on Enzyme Metabolism
<i>VKORC1</i>	-1639G->A	Warfarin sensitivity
<i>VKORC1</i>	85G->T	Warfarin resistance
<i>VKORC1</i>	106G->T	Warfarin resistance
<i>VKORC1</i>	121G->T	Warfarin resistance
<i>VKORC1</i>	134T->C	Warfarin resistance
<i>VKORC1</i>	172A->G	Warfarin resistance
<i>VKORC1</i>	196G->A	Warfarin resistance
<i>VKORC1</i>	358C->T	Warfarin resistance
<i>VKORC1</i>	383T->G	Warfarin resistance
<i>CYP4F2</i> *3	1297G->A	Warfarin resistance
rs12777823G->A(a)		Warfarin sensitivity

a. rs12777823G->A is an intergenic single nucleotide variant (SNV)

Warfarin dosing may require adjustment depending on the genotypes identified and the predicted phenotype. Patients who have high warfarin sensitivity may benefit from greatly reduced warfarin dosage or by transitioning to another comparable medication.(2) Similarly, in rare instances, individuals with *VKORC1* warfarin resistance variants, may require a higher warfarin dose or may benefit from selection of an alternate medication.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided that includes assay information, genotype, and an interpretation indicating the patient's predicted warfarin response.

The *CYP2C9* and *CYP4F2* genotypes, with associated star alleles, are assigned using standard allelic nomenclature as published by the Pharmacogene Variation (PharmVar) Consortium.(5)

Individuals without a detectable alteration in *CYP2C9* or *CYP4F2* will be designated as *CYP2C9**1/*1 or *CYP4F2**1/*1

For additional information regarding pharmacogenomic genes and their associated drugs, see [Pharmacogenomic Associations Tables](#) in Special Instructions. This resource also includes information regarding enzyme inhibitors and inducers, as well as potential alternate drug choices.

Individuals who have variants in 1 or more gene tested by this assay may require more frequent monitoring of international normalized ratio (INR) to maintain the INR in the target range.

Drug-drug interactions and drug/metabolite inhibition must be considered when prescribing warfarin. Warfarin metabolism may be inhibited through drug-drug interactions, including amiodarone and some statins. It is important to interpret the results of testing and dose adjustments in the context of hepatic and renal function and patient age.

Cautions

Samples may contain donor DNA if obtained from patients who received heterologous blood transfusions or allogeneic blood or marrow transplantation. Results from samples obtained under these circumstances may not accurately reflect the recipient's genotype. For individuals who have received blood transfusions, the genotype usually reverts to that of the recipient within 6 weeks. For individuals who have received allogeneic blood or marrow transplantation, a pre-transplant DNA specimen is recommended for testing.

CYP2C9, *VKORC1*, *CYP4F2*, and rs12777823 genetic test results in patients who have undergone liver transplantation may not accurately reflect the patient's *CYP2C9* or *VKORC1* status.

This method may not detect all variants that impact warfarin sensitivity or resistance. Therefore, absence of a detectable variant does not rule out the possibility that a patient has an altered *CYP2C9* or *VKORC1* metabolism due to other variants that cannot be detected with this method. Furthermore, when 2 or more variants are identified, the cis-/trans- status (whether the variants are on the same or opposite chromosomes) is not always known.

Clinical Reference

1. Oldenburg J, Bevens CG, Muller CR, Watzka M: Vitamin K epoxide reductase complex subunit I (*VKORC1*): the key protein of the vitamin K cycle. *Antioxid Redox Signal* 2006;8(3-4):347-353
2. Watzka M, Geisen C, Bevens CG, et al: Thirteen novel *VKORC1* mutations associated with oral anticoagulant resistance: insights into improved patient diagnosis and treatment. *J Thromb Haemost* 2011;9(1):109-118
3. Yuan HY, Chen JJ, Lee MT, et al: A novel functional *VKORC1* promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Hum Mol Genet* 2005;14:1745-1751
4. Sconce EA, Khan TI, Wynne HA, et al: The impact of *CYP2C9* and *VKORC1* genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. *Blood* 2005;106:2329-2333
5. Pharmacogene Variation Consortium database. Accessed 04/27/2018. Available at <https://www.pharmvar.org/gene/CYP2C9>
6. Johnson JA, Caudle KE, Gong L, et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. *Clin Pharmacol Ther* 2017 Sep;102(3):397-404 doi:10.1002/cpt.668
7. Perera MA, Cavallari LH, Limdi NA, et al: Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. *Lancet* 2013;382,790-796
8. McDonald MG, Rieder MJ, Nakano M, et al: *CYP4F2* is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. *Mol Pharmacol* 2009;75,1337-1346
9. Warfarindosing.org website. Accessed June 2017. Available at <http://www.warfarindosing.org/Source/Home.aspx>
10. FDA approved drug label for warfarin. Accessed May 3, 2017. Available at <http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=558b7a0d-5490-4c1b-802e-3ab3f1efe760>

Performance

Method Description

Genomic DNA is extracted from whole blood or saliva. Genotyping for the alleles is performed using a PCR-based 5'-nuclease assay. Fluorescently labeled detection probes anneal to the target DNA. PCR is used to amplify the section of DNA that contains the variant. If the detection probe is an exact match to the target DNA, the 5'-nuclease polymerase degrades the probe, the reporter dye is released from the effects of the quencher dye, and a fluorescent signal is detected. Genotypes are assigned based on the allele-specific fluorescent signals that are detected. (User Guide: TaqMan SNP Genotyping Assay, Applied Biosystems Revision A.0 January 2014)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 10 days

Specimen Retention Time

Whole Blood/Saliva swab: 2 weeks; Extracted DNA: 2 months

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

0030U

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
WARSV	Warfarin Response Genotype	93196-4

Result ID	Test Result Name	Result LOINC Value
BA0251	CYP2C9 Genotype	46724-1
BA0248	VKORC1 Genotype	50722-8



Result ID	Test Result Name	Result LOINC Value
BA0232	Additional VKORC1 Variants	50722-8
BA0254	CYP4F2 *3 Genotype	93197-2
BA0257	rs12777823 Genotype	93198-0
BA0234	Interpretation	69047-9
BA0260	Additional Information	48767-8
BA0236	Method	49549-9
BA0237	Disclaimer	62364-5
BA0238	Reviewed by	18771-6