MAYO CLINIC LABORATORIES

Test Definition: WARSQ

Warfarin Response Genotype, Varies

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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
MATCC	Maternal Cell Contamination, B	Yes	No

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.

Testing Algorithm

Cord blood:

For cord blood specimens that have an accompanying maternal blood specimen, maternal cell contamination studies will be performed at an additional charge.

Special Instructions

- Informed Consent for Genetic Testing
- Pharmacogenomic Association 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 2C9QT / Cytochrome P450 *2C9* Genotype, Varies, 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: *CYP*s *1A2, 2C9, 2C19, 2D6, 3A4, 3A5, 4F2, SLCO1B1,* and *VKORC1*. Order PGXQP / Focused Pharmacogenomics Panel, Varies if multiple pharmacogenomic genotype testing is desired.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant or liver transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a hematopoietic stem cell or liver transplant.

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 whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

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

DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)
Container/Tube:
Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab
Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

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:
-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:
-Neurology Specialty Testing Client Test Request (T732)
-Therapeutics Test Request (T831)
-Cardiovascular Test Request (T724)

Specimen Minimum Volume

See Specimen Required

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 & 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 (CYP) 2C9 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 overmedicating, driving the INR above the therapeutic target.

The second gene, *VKORC1* encodes vitamin K epoxide reductase complex subunit-1, 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 genetic variants in *VKORC1* that lead to warfarin resistance that are tested by this assay. These variants 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 2C9QT / Cytochrome P450 *2C9* Genotype, Varies.

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** (NM_000771.3)	**Effect on enzyme metabolism**
**1*	None (wild type)	Normal activity
**2*	c.430C>T	Reduced activity
**3*	c.1075A>C	No activity
**4*	c.1076T>C	Reduced activity
**5*	c.1080C>G	Reduced activity
**6*	c.818del	No activity
**8*	c.449G>A	Reduced activity
**9*	c.752A>G	Normal activity
**11*	c.1003C>T	Reduced activity
**12*	c.1465C>T	Reduced activity
**13*	c.269C>T	No activity
**14*	c.374G>A	Reduced activity
**15*	c.485C>A	No activity
**16*	c.895A>G	Reduced activity
**17*	c.1144C>T	Reduced activity
**18*	c.1190A>C	No activity
**25*	c.353_362del	No activity
**26*	c.389C>G	Reduced activity
**28*	c.641A>T	Reduced activity
**30*	c.1429G>A	Reduced activity
**33*	c.395G>A	No activity
**35*	c.374G>T;c.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.-1639G>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** (*VKORC1* NM_024006.5; *CYP4F2* NM_001082.4)	**Effect on enzyme metabolism**
VKORC1	c.-1639G>A	Warfarin sensitivity
VKORC1	c.85G>T	Warfarin resistance
VKORC1	c.106G>T	Warfarin resistance
VKORC1	c.121G>T	Warfarin resistance
VKORC1	c.134T>C	Warfarin resistance

VKORC1	c.172A>G	Warfarin resistance
VKORC1	c.196G>A	Warfarin resistance
VKORC1	c.358C>T	Warfarin resistance
VKORC1	c.383T>G	Warfarin resistance
*CYP4F2*3*	c.1297G>A	Warfarin resistance
rs12777823G>A*	N/A	Warfarin sensitivity

* 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. 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 non-leukocyte reduced blood transfusions or allogeneic hematopoietic stem cell transplantation. Results from samples obtained under these circumstances may not accurately reflect the recipient's genotype. For individuals who have received non-leukocyte reduced blood transfusions, the genotype usually reverts to that of the recipient within 6 weeks. For individuals who have received allogeneic hematopoietic stem cell 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 predict the patient's warfarin sensitivity.

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 altered CYP2C9 or CYP4F2 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, Bevans CG, Muller CR, Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin K cycle. Antioxid Redox Signal. 2006;8(3-4):347-353. doi:10.1089/ars.2006.8.347
2. Watzka M, Geisen C, Bevans 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. doi:10.1111/j.1538-7836.2010.04095.x
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(13):1745-1751. doi:10.1093/hmg/ddi180
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(7):2329-2333. doi:10.1182/blood-2005-03-1108
5. PharmVar: Pharmacogene Variation Consortium. Updated July 24, 2025. Accessed August 6, 2025. Available at 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;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(9894):790-796. doi:10.1016/S0140-6736(13)60681-9
8. McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol. 2009;75(6):1337-1346. doi:10.1124/mol.109.054833
9. Warfarin dosing. Washington University; Accessed August 6, 2025. Available at www.warfarindosing.org/Source/Home.aspx
10. U.S National Library of Medicine: DailyMed. National Institutes of Health; Accessed August 6, 2025. Available at https://dailymed.nlm.nih.gov/dailymed/index.cfm

Performance

Method Description

Genomic DNA is extracted from whole blood or saliva. Genotyping for the alleles is performed using a polymerase chain reaction (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.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Varies

Report Available
3 to 10 days

Specimen Retention Time
Whole blood: 28 days (if available); Extracted DNA: 3 months, Saliva: 30 days (if available)

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
0030U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WARSQ	Warfarin Response Genotype, V	93196-4

Result ID	Test Result Name	Result LOINC® Value
610175	Warfarin CYP2C9 Genotype	46724-1
610176	Warfarin VKORC1 Promoter Genotype	50722-8
610560	Warfarin CYP2C9 and VKORC1 Promoter Phenotype	54451-0
610177	Warfarin Resistance Variants	50722-8
614410	Warfarin VKORC1 Resistance Genotype	50722-8
610178	Warfarin CYP4F2 *3 Genotype	93197-2
610179	Warfarin rs12777823 Genotype	93198-0
610180	Interpretation	69047-9
610181	Additional Information	48767-8
610182	Method	85069-3

610183	Disclaimer	62364-5
610184	Reviewed by	18771-6