Background Information
Warfarin (Coumadin) is a commonly prescribed oral anticoagulant to prevent or treat clotting disorders, including those associated with venous thrombosis, pulmonary embolism, atrial fibrillation, cardiac valve replacements, stroke and acute myocardial infarction. Warfarin has a narrow therapeutic range, which can influence the optimal dose for individual patients, ranging from < 2 mg/day to > 10 mg/day. While underdosing can result in the formation of blood clots, overdosing can increase the risk for bleeding.

The current approach to determining an appropriate warfarin dose for an individual considers age, gender, weight, height, current medications and diet and monitors the patient’s prothrombin time (PT) and international normalized ratio (INR). It is a lengthy process with an increased risk for ADE.

Genetic Test for Warfarin Therapy
Alternatively, molecular diagnostic tests based on the patient’s genetic information can be used to determine an appropriate dosing strategy to achieve a stable maintenance dose. Using this technique, recommended dosing and risk level can be determined quickly, providing safer, more effective warfarin therapy (www.warfarindosing.org).

Clinical Indication
Warfarin is a vitamin K antagonist composed of S- and R-isomers. The more potent S-warfarin is metabolized by an enzyme cytochrome P450 2C9 (CYP2C9), encoded by the CYP2C9 gene. Warfarin exerts its anticoagulant effect by inhibiting its target enzyme, Vitamin K epoxide reductase (VKOR), encoded by the Vitamin K epoxide reductase complex subunit 1 (VKORC1) gene. VKOR is required to recycle oxidized vitamin K to reduced vitamin K, a co-factor necessary for the production of activated clotting factors II, VII, IX and X.

Regulation of Warfarin Activity

- **Warfarin**
  - R-warfarin
  - S-warfarin

- **Inactive Metabolites**
  - Inactive Factors II, VII, IX, X
  - Protein C, S, Z

- **CYP2C9**
  - CYP2C9*2
  - CYP2C9*3

- **Inactive Factors**
  - II, VII, IX, X
  - Protein C, S, Z

- **Oxidized Vitamin K**
  - (VKORC1 -1639G>A)
  - A Allele - Promoter Activity
  - mRNA

- **Reduced Vitamin K**

- **Active Factors**
  - II, VII, IX, X
  - Protein C, S, Z
Genetic variations in the *CYP2C9* and *VKORC1* genes can affect warfarin's efficacy and the dose required to achieve stable INR. Specifically, two variants in the *CYP2C9* gene (*CYP2C9*+2 and *CYP2C9*+3) result in an enzyme with reduced activity, leading to increased active warfarin levels. A variant in the *VKORC1* gene (*VKORC1*- 1639/3673 G>A) can lead to reduced gene expression, resulting in decreased level of VKOR. Together, these three variants can account for 40 to 60% of the variability in warfarin dosage. These allelic variations of individual patients can be factored in while determining the warfarin dose to reduce the risk of ADE.

This genetic test is used to identify patients at risk for ADE due to impaired warfarin metabolism and sensitivity by:

- Detecting two genetic variants in the warfarin metabolizing gene *CYP2C9* (*CYP2C9*+2 and *CYP2C9*+3)
- Detecting one genetic variant in the warfarin target gene *VKORC1* (-1639G>A).

**Interpretation**

Interpretation of the assay is based on the presence of specific genetic variants of *CYP2C9* and *VKORC1*:

<table>
<thead>
<tr>
<th>Genetic Variant</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CYP2C9</em> +1/*1</td>
<td>Extensive (normal) metabolizer</td>
</tr>
<tr>
<td><em>CYP2C9</em> +1/*2</td>
<td>Intermediate metabolizer</td>
</tr>
<tr>
<td><em>CYP2C9</em> +1/*3</td>
<td>Slow metabolizer</td>
</tr>
<tr>
<td><em>CYP2C9</em> +2/*2</td>
<td>Slow metabolizer</td>
</tr>
<tr>
<td><em>CYP2C9</em> +2/*3</td>
<td>Slow metabolizer</td>
</tr>
<tr>
<td><em>CYP2C9</em> +3/*3</td>
<td>Very slow (poor) metabolizer</td>
</tr>
<tr>
<td><em>VKORC1</em> -1639 GG</td>
<td>Low sensitivity to warfarin</td>
</tr>
<tr>
<td><em>VKORC1</em> -1639 AG</td>
<td>Medium sensitivity to warfarin</td>
</tr>
<tr>
<td><em>VKORC1</em> -1639 AA</td>
<td>High sensitivity to warfarin</td>
</tr>
</tbody>
</table>

1. Individuals with *CYP2C9* variant alleles designated *2 and *3 have reduced enzyme activity compared to *1 (wild-type) individuals, leading to increased levels of the active S-warfarin, typically require reduced maintenance doses of warfarin and take longer to achieve steady state.

2. *VKORC1* variants are alleles designated G/G, A/G, and G/G that alter the activity of VKOR enzymatic activity, leading to differences in warfarin dose required and time to achieve steady state. Individuals with the A/A genotype have decreased VKOR activity leading to increased inhibition to warfarin and are at increased risk of bleeding complications from anticoagulant overdose. They typically require reduced doses of warfarin to achieve therapeutic INR sooner compared with A/G individuals.

3. Prevalence of gene variations in *CYP2C9* and *VKORC1* differs with ethnicity.

**Ordering Recommendations**

- This test should be ordered prior to starting warfarin, or within the first week to help guide dosing.
- This test is not recommended for patients on stable warfarin therapy.

**Limitations of the Assay**

Analysis for specific genetic variants detected in this test does not rule out the possibility of the presence of other variant alleles that may influence drug effect and metabolism. *CYP2C9* variant alleles are important in the metabolism of drugs other than warfarin, such as phenytoin, glyburide, glimepiride, tolbutamide, sulfamethoxazole and certain nonsteroidal anti-inflammatory agents (NSAIDs). A +2 or +3 result for *CYP2C9* is associated with a poor metabolizer phenotype for all drugs metabolized by *CYP2C9*. 
Methodology
An array-based diagnostic test kit employing Infiniti analyzer (AutoGenomics Inc., CA) is used for genotyping CYP2C9-VKORC1 variants. The assay involves a multiplex PCR amplification of genomic DNA followed by allele-specific (AS) primer extension using fluorescently labeled dCTP and hybridization onto a microarray coated with capturing oligonucleotides (zipcodes), which are specific for complementary oligonucleotides (anti-zipcodes) linked to the allele specific primer-extended products.

A built-in confocal microscope is used to capture fluorescent signal from the pre-determined hybridization spots corresponding to specific products and genotypes deciphered from the signal ratio.

References


**Test Overview**

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Warfarin Sensitivity Genotyping Genetic test for warfarin therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Range</td>
<td>An interpretive report will be provided.</td>
</tr>
<tr>
<td>Specimen Requirements</td>
<td>Testing Volume/Size: 5 mL; Type: Whole blood; Tube/Container: EDTA (Lavender); Coll. Temperature: Refrigerated</td>
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<td>Ordering Mnemonic</td>
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<td>Billing Code</td>
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<td>CPT Codes</td>
<td>83891, 83900, 83901, 83914 x6, 83912, 83912-26</td>
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