Identification of polymorphism CYP2C9*2 e CYP2C9*3 of gene CYP2C9 e -1639G>A in the VKORC1 gene

AMPLI-CYP2C9-VKORC1 Cat. n.2.001

Oral Anticoagulant Therapy (OAT) is prescribed following the manifestation of a thrombotic event and its dosage should be carefully monitored to avoid overdosing with an increased risk of bleeding, or underdosing with consequent ineffectiveness in thrombotic prophylaxis. The metabolism of the drug warfarin is highly variable and often subjective and depends on the presence of certain polymorphisms. The presence and/or combination of polymorphisms in the gene for cytochrome P450 2C9 gene and the vitamin K-epoxide reductase (VKORC1) determine important changes in therapeutic response to warfarin. CYP2C9, a member of the family of cytochrome P-450, is responsible for the metabolism of approximately 16% of drugs currently on the market, and has the function of oxidation and elimination of endogenous and exogenous substances. The CYP2C9 gene (10q24) has several genetic variants. In particular, the polymorphism 430C> T (CYP2C9*2) determines, in position 144, replacement of an arginine residue with a cysteine, while the polymorphism 1075A> C (CYP2C9*3) is characterized by an isoleucine at position 359 instead of a leucine residue. These polymorphisms are associated with reduced metabolism of warfarin, which therefore has a longer half-long and requires a lower dose to avoid exposing those with bleeding complications.
The VKORC1 gene (16p11.2) encoding subunits of the enzyme vitamin K 1-epoxide reductase involved in reducing and recycling of vitamin K are essential for the carboxylation of K-dependent coagulation factors (factor II, VII, IX and X), carboxylation, which is essential for their biological activity. VKORC1 is highly polymorphic. In particular, the polymorphism-1639G> A is associated with lower expression and activity of the enzyme. The presence of this polymorphism is considered predictive of the variability of response to treatment with warfarin, because it alters their sensitivity to the drug. The kit allows AMP-CYP2C9-VKORC1 to identify, through the use of PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) genetic variants 430C> T (CYP2C9*2) and 1075A> C (CYP2C9*3) of 'enzyme cytochrome P-450 2C9 and the variant-1639G> A of the vitamin K epoxide reductase.

Principle of the method: a) extraction of genomic DNA; b) amplification; c) enzymatic digestion; d) detection on agarose gels.
Applicability: of genomic DNA extracted and purified from whole blood samples.
Number of tests: 24x3 (72 reactions)

KIT CONTENTS AND STORAGE

AMPLIFICATION	
Mix PCR CYP2C9*2	-20°C
Mix PCR CYP2C9*3	-20°C
Mix PCR -1639G>A	-20°C
H_2O DNase/RNase-free	-20°C
Taq Polymerase (5U/□l)	-20°C
Controllo DNA	-20°C
ENZYMATIC DIGESTION	
Enzima *Ava*II (20U/□l)	-20°C
Enzima *Kpn*I (20U/□l)	-20°C
Enzima *Msp*I (20U/□l)	-20°C
Buffer 10X *Ava*II	-20°C
Buffer 10X *Kpn*I	-20°C
Buffer 10X *Msp*I	-20°C
BSA 100X	-20°C
H_2O RNase/DNase-free	-20°C

Stability: more than 18 months if properly stored.
References:

Aithal GP, et al., Lancet (1999) 353: 717-19
Klein TE, et al., N Engl J Med (2009) 360:753-64
Rieder MJ, et al, N Engl J Med. (2005) 352:2285-93
Limdi NA, et al., Blood (2010) 115:3827-34

ANALYSIS OF RESULTS

The test determines the presence of allelic variants of CYP2C9 and VKORC1 genes. Based on combinations of these variants is possible to distinguish the genotypes associated with different responses and / or ability to metabolize warfarin

			Fragments after digestion	
Mix PCR	**PCR in bp**	**Enzima restriction**	**wild type**	**mutate**
CYP2C9*2	**301**	***AvaII***	**168** **133**	**301**
CYP2C9*3	**105**	***KpnI***	**105**	**85** **20**
-1639G>A	**290**	***MspI***	**290**	**168** **122**

In clinical terms, the presence of mutant alleles results in a high risk of bleeding even low doses of warfarin. So VKORC1/CYP2C9 knowledge of the genotype of the patient plays an important role in the choice of drug doses to be administered.

VKORC1	CYP2C9	Risk bleeding
G/G	*1/*1	Low
A/G	*1/*1	Middle
G/G	*1/*2 *1/*3	
A/A	*1/*1	High
A/G	*1/*2 *1/*3	
G/G	*2/*2 *2/*3 *3/*3	