CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

DETECTION OF A1691G MUTATION IN FV LEIDEN GENE

AMPLI FV LEIDEN REAL TIME Cat 1.311RT

In 1994 a point mutation (substitution A-G) in the position 1691 of the gene encoding for the coagulation factor has been linked to the resistance to the degradation of activated Protein C(1-4). This functional alteration can be correlated to an increased venous thrombosis risk.
The functional assay of rAPC (resistance to activated Protein C) is based on the detection of aPTT in plasma of patient with and without a fixed concentration of activated Protein C. This assay is affected by a lot of factors, as citrate used as anticoagulative, calcium chlo- ride, the kind of activator, the purity of activated protein C. Reduced levels of F II and X can distort the results (false normal results). It is impossible to perform the functional assay of activated Protein C during the oral anticoagulant therapy (5), so only the genetic analysis of DNA allows to detect the mutation.
The resistance to activated Protein C is a dominant autosomal transmission.
The mutation has been described in 2-4% of a Dutch control group (6), and in 3-5% of a Britain group (7).The mutation in heterozygosis is linked to an increase of 5-10% of thrombosis risk, whereas homozygosis is joined to an increase of 50%
As shown in fig 1, the detection of the polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites (1). The probe is labelled with a fluorescent reporter dye bound to the 5' and quencher on the 3' end. (2). Due to the 5' nuclease activity of Taq pol during extension, the cleavage of the probe causes an increase of the reporter dye signal (3), and the fluorescent intensity is proportion- al to the amount of amplicon produced (4) (real time quantitative PCR).
In the kit for the detection of A1691G polymorphism, the probe matching the wild type sequence is labelled to the FAM dye reporter, whereas the probe matching the mutation sequence is labelled to the VIC dye reporter

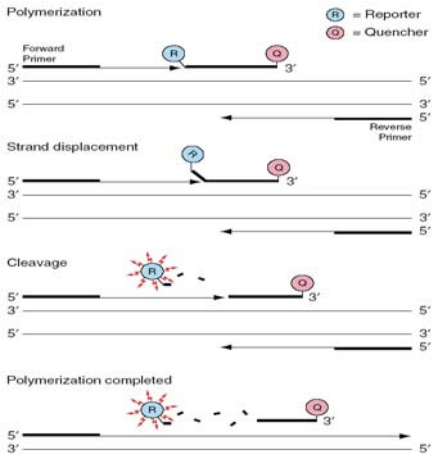


Fig. 1

Principle of method: A) extraction of genomic DNA B) amplification C) detection using real time PCR instrument
Applicability: Genomic DNA extracted and purified by whole blood samples
Number of reactions: 96.

REAGENTS AND STORAGE

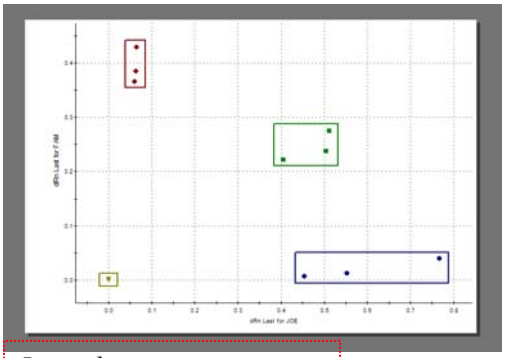
AMPLIFICATION	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X	-20°C in the dark
WT Control	-20°C
Mutant Control	-20°C
Eterozygosis positive control	-20°C

Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS

After an AD post-read run, the software analyzes raw data using the AD specific program. Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.

Allelic discrimination FV LEIDEN



Legend:
Red: allele A
Green: A and G alleles
Blue: allele G

References:
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Dahlback B et al. Thromb Haemost. 1995; 73:739-42 Roger M et al. Nature 1994; 369:64-67
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