

UCLA DIAGNOSTIC MOLECULAR PATHOLOGY LABORATORY
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Factor V Mutation Analysis

↑CPT

83890; 83898; 83896 (x2); 83903; 83912

↑Related Information

↑Synonyms

Leiden Mutation

↑Test Includes

Detection of Factor V Leiden (G1691A) mutation

↑Laboratory

Molecular Pathology

↑Availability

Monday-Friday, 0700-1700

↑Turnaround Time

3-14 days

↑Specimen

Whole blood

↑Volume

4 mL

↑ Minimum Volume

1 mL

↑ Container

Lavender top (EDTA) tube

↑ Storage Instructions

All specimens should be sent to the Laboratory immediately after collection, preferably by overnight delivery. Specimens should be kept at room temperature or refrigerated but not frozen.

↑ Causes for Rejection

Blood samples frozen and thawed will yield low quality DNA; specimens inadequately identified

↑ Reference Range

No G1691A mutation detected

↑ Use

Venous thrombosis has become a severe health problem affecting 1 in 1000 individuals per year. Fifty percent of patients with thromboembolism have a strong family history for this disease, yet only 5% to 10% of these cases can be explained by inherited deficiencies in protein C, protein S, and antithrombin III. Recently, a novel mechanism for thrombophilia was discovered which entails inherited resistance to the anticoagulant protease activated protein C (APC). This defect has been shown to occur in 60% to 70% of inherited thrombophilia. A single point mutation in the factor V gene (Arg506 to Gln506) has been shown to be responsible for 94% of APC resistance. This makes the factor V mutation the single most prevalent genetic defect in thromboembolic disease. Heterozygous carriers (3% to 7% of the population) for the factor V mutation have a life-long 5- to 10-fold increased risk for thromboembolism; individuals homozygous for the mutation have a life-long 50- to 100-fold increased risk for thromboembolism. With the factor V genotype in hand, the clinician will be able to determine the etiology of a thromboembolic event and offer prophylactic anticoagulation therapy to at-risk individuals for prevention of recurrence, as appropriate.

↑ Limitations

The factor V gene mutation, as any analyte used as a diagnostic adjunct, must be interpreted carefully with the overall clinical presentation and other supportive tests. The factor V Leiden gene product's resistance to degradation by activated protein C (APC) is

a recent finding (Dahlback, et al, 1993). Subsequent studies by other investigators indicate that this risk factor is at least 10 times more common than any of the other genetic defects associated with thrombosis (ie, deficiencies of antithrombin III, protein C, and protein S). However, other investigators have found this defect to be prevalent in the normal population (4% to 8%) and a large percent remain asymptomatic for thrombosis. This suggests that other factors may be required for the overt manifestations of thrombosis. Furthermore, studies on families with recurrent thrombosis have shown that family members may have APC resistance and not carry this factor V mutation. In his review of factor V gene mutation (*J Intern Med*, 1995), Dahlback estimates that the combined deficiencies of protein C, protein S, antithrombin III (also elevated plasma homocysteine), and inherited APC resistance account for only 60% to 70% of the cases of familial thrombophilia. The remaining 30% to 40% are unexplained and need further investigation. Whether or not a factor V gene mutation is identified in this test, investigation of the other factors known to predispose to thrombosis is indicated, especially since they may coexist in the same patient. Also, for this reasons, factor V Leiden testing is recommended primarily for work-up of thromboembolic events in individuals under age 50, rather than for presymptomatic testing in healthy individuals and at-risk relatives (Grody *et al*, *Genet. Med.* 2001).

↑ Methodology

The Roche Factor V Leiden Kit is performed on the LightCycler® Instrument utilizing Polymerase Chain Reaction (PCR) for the amplification of Factor V DNA recovered from clinical samples. A 222 bp fragment of the Factor V gene is amplified from human genomic DNA using specific primers. The amplicon is detected by fluorescence using a specific pair of hybridization probes. The hybridization probes consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle. The hybridization probes are also used to determine the genotype by performing a melting curve analysis after the amplification cycles are completed.
