

Molecular Predisposition Testing

1. Prothrombin G20210A Mutation Detection by PCR

The prothrombin G20210A mutation is an autosomal co-dominant mutation within coagulation factor II (prothrombin). This mutation involves a single nucleotide substitution of adenine for guanine at nucleotide position 20210 of the prothrombin gene located on chromosome 11 (p11-q12). Carriers of this mutation are associated with elevated levels of prothrombin in plasma, which, in turn, predisposes the patient to thrombotic episodes such as strokes and deep-vein thromboses.

The prevalence of the prothrombin G20210A mutation in the general population is estimated to be 3-6%. However, for patients with a history of familial thrombosis, there is an estimated 18% incidence of carrying at least one copy of this mutation.

About the test

The Polymerase Chain Reaction (PCR) is used to amplify a 164bp region of the factor II gene containing nucleotide position 20210. Following amplification, the PCR products are digested with the restriction endonuclease *Hind* III (exploiting the fact that the nucleotide substitution creates a *Hind* III restriction site). All digested PCR products are resolved by gel electrophoresis and viewed under ultraviolet light following staining of the DNA with a fluorescent dye in order to determine genotype.

Specimen Requirements

EDTA-, ACD-, or sodium citrate-anticoagulated whole blood (min. 2 ml) or cells can be used for this test. Specimens should be shipped cold, but not frozen.

Order Code	F2PCR
CPT Codes	83891, 83892, 83894, 83898 (x2) and 83912
Routine TAT	Performed Thursday. Reported Friday after 16:00.

2. Factor V Leiden (A1691G) Mutation Detection by PCR

Factor V Leiden is an autosomal co-dominant mutation within coagulation factor V. This mutation involves a single nucleotide substitution of adenine for guanine at nucleotide position 1691 of the factor V gene located on chromosome 1 (q2.23). This substitution results in a subsequent change in the amino acid sequence such that amino acid 506 codes for glutamine instead of the wild type arginine. This change in the amino acid sequence eliminates one of the cleavage sites for activated Protein C, prolonging the procoagulant effect of factor V. The inability of activated Protein C to cleave, and thus, inactivate the Factor V Leiden protein, increases the risk of thrombotic episodes such as strokes and deep-vein thromboses.

The prevalence of the Factor V Leiden gene in the general population is estimated to be 3-5%. However, for patients with a history of familial thrombosis, estimates run as high as 50% for the presence of at least one copy of the gene.

About the test

The Polymerase Chain Reaction (PCR) is used to amplify a 220bp region of the Factor V Leiden gene containing nucleotide position 1691. Following amplification, the PCR products are digested with the restriction endonuclease *Mnl* I (exploiting the fact that the nucleotide substitution also eliminates a *Mnl* I restriction site). All digested PCR products are resolved by gel electrophoresis and viewed under ultraviolet light following staining of the DNA with a fluorescent dye in order to determine genotype.

Specimen Requirements

EDTA-, ACD-, or sodium citrate-anticoagulated whole blood (min. 2 ml) or cells can be used for this test. Specimens should be shipped cold, but not frozen.

Order Code	F5LPCR
CPT Codes	83891, 83892, 83894, 83898 (x2) and 83912
Routine TAT	Performed Thursday. Reported Friday after 16:00.

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3. **Methylenetetrahydrofolate Reductase (MTHFR) C677T Mutation Detection by PCR**

MTHFR C667T is an autosomal recessive mutation within the MTHFR gene that results in the production of a thermolabile enzyme with decreased activity for methylating homocysteine. This mutation involves a single nucleotide substitution of thymidine for cytosine at nucleotide position 667 of the MTHFR gene. Carriers of this mutation are associated with elevated levels of homocysteine in plasma, which, in turn, increases the risk of arterial disease and venous thrombosis.

The prevalence of the MTHFR C667T mutation in the general population is estimated to be 10-15%. However, for patients with a history of familial hyperhomocysteinemia, there is an estimated 28% incidence of homozygosity for this mutation. MTHFR C667T heterozygosity has a reported incidence of approximately 45% in the Caucasian population. This genotype, however, is not reported to increase homocysteine levels to those that are symptomatic.

About the test

The Polymerase Chain Reaction (PCR) is used to amplify a 198bp region of the MTHFR gene containing nucleotide position 677. Following amplification, the PCR products are digested with the restriction endonuclease *Hinf* I (exploiting the fact that the nucleotide substitution creates a *Hinf* I restriction site). All digested PCR products are resolved by gel electrophoresis and viewed under ultraviolet light following staining of the DNA with a fluorescent dye in order to determine genotype.

Specimen Requirements

EDTA-, ACD-, or sodium citrate-anticoagulated whole blood (min. 2 ml) or cells can be used for this test. Specimens should be shipped cold, but not frozen.

Order Code	MTHFR
CPT Codes	83891, 83892, 83894, 83898 (x2) and 83912
Routine TAT	Performed Thursday. Reported Friday after 16:00.

Note: The Polymerase Chain Reaction (PCR) process is covered by U.S. patents owned by Hoffman-LaRoche Inc. These tests are performed under a licensed agreement with Roche Molecular Systems Inc.