



Factor V Leiden Detection by PCR

Marcy L. Hoffmann, Ph.D. and William A. Dittman, Jr., M.D.

PAML AND SHMC ARE NOW OFFERING a polymerase chain reaction (PCR)-based test to detect the most common hereditary defect predisposing to venothromboembolic disease, *Factor V Leiden*.

Factor V Leiden is the name given to the mutation in the coagulation Factor V gene that is the most common cause of inherited predisposition to thrombosis.¹ Factor V Leiden occurs in 3%-5% of the general population and in 20%-40% of patients with venous thromboembolic disease.² In contrast, abnormalities of protein C, protein S and antithrombin III are identified in approximately 5% of patients with thrombotic disease. Individuals who are heterozygous for the Factor V Leiden defect have a 5-10 fold increased risk of thrombosis compared to the general public, while homozygotes have a 50-100 fold increased risk.³

Factor V Leiden is a single base mutation in the gene for coagulation Factor V.⁴ This results in a substitution of glutamic acid for an arginine at amino acid 506 in the Factor V protein molecule. Because this is a normal cleavage site for activated protein C to inactivate Factor V, the net effect of the substitution is relative increased Factor V activity with increased coagulation. The biological effect of Factor V Leiden is relative resistance to activated protein C, the basis of assays for activated protein C resistance (APCR), an alternative means of diagnosing this hypercoagulable state.

Nearly all activated protein C resistance as diagnosed by APCR assays is due to Factor V Leiden. APCR testing cannot distinguish between heterozygotes and homozygotes with Factor V Leiden, and since treatment may differ for the two genotypes, it is appropriate to test all patients with a low APCR ratio (i.e., increased APC resistance) for the Factor V Leiden mutation. Some physicians prefer to use DNA based testing initially and will order Factor V Leiden by PCR in all patients with suspected inherited hypercoagulable states instead of APCR. Additionally, there are occasional patients whose APCR testing is not interpretable due to inhibitors such as lupus inhibitors or heparin; therefore, these patients may be more easily evaluated by PCR methodology.

After isolation of DNA from patient cells, Factor V Leiden is identified by PCR using DNA sequences specific for the normal and abnormal gene. In normal individuals, no Factor V Leiden allele is detected. Heterozygotes express one normal and one abnormal allele, and homozygotes express two abnormal alleles.

References

1. Price, D.T. and Ridker, P.M. *Factor V Leiden mutation and the risks for thromboembolic disease: a clinical perspective*. Ann Intern Med, 1997. **127**(10): p. 895-903.
2. Dahlback, B., *Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism*. Blood, 1995. **85**(3): p. 607-14.
3. Rosendaal, F.R., et al., *High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance)*. Blood, 1995. **85**(6): p. 1504-8.
4. Bertina, R.M., et al., *Mutation in blood coagulation factor V associated with resistance to activated protein C*. Nature, 1994. **369**(6475): p. 64-7.

Test Information

DESCRIPTION	FACTOR V LEIDEN BY PCR
METHOD	PCR
WORKPAR	FVPCR
CPT CODE	83890, 83894, 83901×2, 83912
SPECIMEN	5 mL EDTA whole blood (lavender-top tube). Store and transport at room temperature. If delayed more than 72 hours, store and transport refrigerated. Do not freeze specimen.
COMMENTS	<i>Minimum amount:</i> 1 mL or a full EDTA microtainer. <i>Other acceptable specimens:</i> sodium citrate or ACD whole blood (blue or yellow-top tube). <i>Unacceptable conditions:</i> heparinized whole blood, serum, grossly hemolyzed, frozen specimens, or specimens over 5 days old. Also, specimens in leaking containers. <i>Stability:</i> 72 hours at room temperature, 5 days refrigerated, unstable frozen.
SCHEDULE	Variable
TURNAROUND	Variable
RANGES	Factor V Leiden by PCR Negative Interpretation Comment

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