
Molecular Diagnostics August 2008

Starting August 19, 2008, **Factor V Leiden** and **Prothrombin Nucleotide 20210 (Factor II G20210A)** genotyping will be performed in the Molecular Diagnostics Laboratory at Methodist Hospital instead of being sent to our reference laboratory, ARUP. The test results will be available in 1 to 2 days. The tests will be performed Monday through Friday (The specimen must be received by 10 a.m. Friday to be included in the Friday run).

Specimen Requirements:

- One lavender top EDTA tube containing a minimum of 2 mLs of whole blood. Both genetic tests can be ordered with one tube (2 mLs) of blood.
- If the specimen will be stored for more than 1 hour before reaching the Molecular Diagnostics Laboratory, it should be refrigerated but not frozen.

Cerner Orderables:

- Leiden Factor V PCR – MH or
- Factor V PCR – MH

- Prothrombin Nucleotide 20210 – MH or
- Factor II mutation - MH

Thrombophilia:

Factor V Leiden and Factor II G20210A genetic tests are two of the many tests available for use in the evaluation of thrombophilia (hypercoagulable state) and these mutations are the most common mutations associated with thrombophilia. A clinical history and physical examination of the patient are the first steps in the evaluation. The ideal initial test for suspected inherited thrombophilia is APC-resistance. The absence of APC-resistance rules out Factor V Leiden. **It is recommended that individuals who test POSITIVE for APC-Resistance have Factor V Leiden and Factor II G20210A mutation testing.** Factor II G20210A mutation testing may also be indicated in patients who lack APC-resistance. Factor V Leiden and Factor II G20210A may also be co-inherited, which further amplifies thrombogenic risk. Testing for other less common causes of thrombophilia may be

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indicated in the absence of Factor V Leiden or Factor II G20210A mutation. Tests for these abnormalities may be affected by active thrombosis and / or anticoagulant therapy.

The American College of Medical Genetics (ACMG) and the College of American Pathologists (CAP) agree that testing for inherited thrombophilia should be offered to:

1. Individuals with venous thromboembolism (VTE) under age 50.
2. Recurrent VTE.
3. VTE at any age with a strong family history of thrombotic disease (i.e. several affected relatives or relatives with VTE under age 50).
4. VTE in an unusual vascular site (such as the hepatic, mesenteric, portal and cerebral veins) at any age.
5. Women with VTE who are pregnant or post-partum or taking oral contraceptives.
6. There is general agreement that women with pregnancy loss that is either recurrent or late in pregnancy should be offered testing for thrombophilia.
7. It is less clear whether testing should be offered in cases of VTE in the setting of post-menopausal hormonal therapy or unprovoked VTE over age 50. Whether or not women with other gestational vascular complications should be offered an evaluation is also controversial.

Screening laboratory tests may include some or all of the following:

1. CBC
2. Prothrombin time (PT)
3. PTT
4. Thrombin time and reptilase time
5. Lupus anticoagulant panel
6. Cardiolipin and beta 2 glycoprotein antibodies
7. Activated Protein C (APC)-resistance ratio
8. Fibrinogen, soluble fibrin monomer complex and D-dimer
9. Factor V Leiden mutation genotyping
10. Prothrombin G20210GA mutation genotyping
11. Homocysteine, Total

Factor V Leiden-associated activated protein C (APC) resistance is regarded as the most prevalent inherited coagulation factor mutation associated with venous thrombosis, occurring in about 5% of Caucasians. The Factor II (Prothrombin) G20210A mutation is present in 1 to 2% of the general population and its involvement in venous thromboembolism is well established.

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Counter-Indications for Factor V Leiden and Factor II G20210A Testing:

Testing is not recommended in certain scenarios:

1. General population screening: for all individuals, before or during oral contraceptive or other hormone therapy, during pregnancy, or in the newborn period (for asymptomatic newborns), or in patients with heart attack/stroke.
2. In healthy individuals with known risk factors for VTE (such as surgery, trauma, paralysis, or cancer).
3. Venous thromboembolism (VTE) in patients over age 50 who have known risk factors.
4. Asymptomatic individuals prior to adolescence. However, some exceptions exist—such as testing of siblings of children who have had arterial or venous thrombosis in childhood.

The results of the Factor V Leiden and Factor II G20210A genotyping assays are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

Patient counseling and informed consent are recommended for genetic testing. In Nebraska, informed consent is required for testing presymptomatic individuals such as family members of patients with thrombophilia. These regulations can be found at the following website: <http://law.justia.com/nebraska/codes/s71index/s7101104001.html> Model consent forms for presymptomatic (predictive) genetic testing developed from the regulations referred to above can be found at the following website (Attachment C): http://www.sos.state.ne.us/rules-and-regs/regsearch/Rules/Health_and_Human_Services_System/Title-181/Chapter-5.pdf

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References:

1. Ridker, PM, *et al.* (1997) Ethnic Distribution of Factor V Leiden in 4047 Men and Women: Implications for Venous Thromboembolism screening. *JAMA*, **277**: 1305-1307.
2. Grody, WW, *et al.* (2001) American College of Medical Genetics Consensus Statement on Factor V Leiden Mutation Testing. *Genetics in Medicine*, **3**, Vol.2:139-148.

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3. Press, RD, et al. (2003) Clinical Utility of Factor V Leiden (R506Q) Testing for the Diagnosis and Management of Thromboembolic Disorders. CAP Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia.

Further Details about the Assays:

Verification studies showed that the Factor V Leiden assay and the Factor II (Prothrombin) G20210A assay both detected the respective mutations with 100% accuracy and 100% precision. The performance characteristics of these modified FDA-approved tests were verified by the Methodist Hospital Molecular Diagnostics Laboratory. Assay verification and modification validation details are available upon request. The Methodist Hospital Molecular Diagnostics Laboratory is authorized under Clinical Laboratory Improvement Amendments (CLIA), by the College of American Pathologists (CAP) and by the state of Nebraska to perform high-complexity testing.