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FACTOR V LEIDEN AND FACTOR II (PROTHROMBIN) G20210A KITS FROM ROCHE DIAGNOSTICS

Real-Time PCR Tests Detect and Identify Thrombophilia Genetic Disorders

The **Roche Diagnostics (Basel, Switzerland)** thrombophilia genetic disorder tests represent a significant advancement in genotyping technology. Genetic identification of Factor V Leiden,[1] Factor II Prothrombin[1] and MTHFR[2] can be accomplished using the polymerase chain reaction (PCR): a molecular amplification technique, using biological patient samples. Roche Diagnostics' **LightCycler® Factor II (Prothrombin) G20210A** and **LightCycler® Factor V Leiden Kits** were the first FDA-cleared human DNA genetic marker tests. These tests are performed with the **LightCycler® 1.2** or **2.0** instruments with either manual or automated specimen preparation using the High Pure PCR Template Preparation Kit or the **MagNA Pure LC** instrument. The LightCycler® instrument is the standard for rapid, sensitive and accurate real-time PCR. Combined with **Melting-curve analysis,** the LightCycler® instrument offers laboratories detection of PCR products as well as genotyping.

The LightCycler® Factor II (Prothrombin) G20210A Kit and the LightCycler® Factor V Leiden Kit allow the detection and genotyping of a single point variation (G to A at position 20210, and G to A at position 1691, respectively) of the human Factor II and Factor V genes from DNA isolated from human whole blood collected in an EDTA tube. Real-time PCR helps clinical molecular diagnostic laboratories deliver highly sensitive tests that can quickly determine if a patient carries these specific variations; and, the testing will aid in the evaluation and diagnosis of suspected thrombophilia.

Hypercoagulation disorders occur with abnormalities in the clotting process and are associated with an increased risk of deep vein thrombosis (DVT).[3] These disorders can be inherited or acquired.[4] DVT can restrict blood flow from the extremities and undissolved clots that form can pass through the circulatory system and block blood flow in the lungs, resulting in pulmonary embolism (PE).[3] Certain risk factors can contribute to the development of thrombosis in individuals with an inherited hypercoaguable disorder including, but not limited to: cancer, age, obesity, hospitalization, major surgery, restricted mobility caused by long-distance travel, trauma, use of birth control pills, or postmenopausal hormone replacement therapy.[5,6] The Factor V Leiden mutation is also associated with a somewhat increased risk of miscarriage, and some research suggests that it may also increase the risk of other obstetric complications.[7] These complications may include preeclampsia, slow fetal growth, and placental abruption. As such, there is a growing consensus that Factor V Leiden testing may be considered for individuals who include:[8]

The LightCycler® 2.0 from Roche Diagnostics.

- Selected women with unexplained severe preeclampsia, placental abruption, or a fetus with intrauterine growth retardation;
- A first venous thromboembolism (VTE) related to

the use of tamoxifen or other selective estrogen receptor modulators (SERMs);

• Asymptomatic female family members of probands with known Factor V Leiden thrombophilia who are pregnant or are considering oral contraceptive use or pregnancy;

• Women with recurrent unexplained first-trimester pregnancy losses with or without second- or third-trimester pregnancy losses.

Testing is not recommended during routine initial pregnancy tests.[8] It is important to note that most women with the Factor V Leiden mutation have normal pregnancies.[7]

Arundhati Rao, MD, PhD, Section Chief, Technical Pathology and Molecular Genetics, Scott & White Health System (Temple, TX), points out that gynecologists need to be aware of the importance of the thrombophilia genetic disorder tests. "A DNA-based PCR test directly identifies the gene mutation that codes for the aberrant protein. Medications such as warfarin and/or autoantibodies such as the lupus anticoagulant do not interfere with these DNA-based tests. The tests may also distinguish between heterozygous and homozygous individuals. While DNA genotype testing is not the only method by which the defect can be discovered, DNA methods have the advantage of determining the exact mutation. Though other functional tests use an indirect method and can show that the APC anticoagulant pathway is impaired, these results are optimally confirmed by a DNA-based direct testing method. Roche's PCR based FDA-cleared DNA test can find the defect in the DNA that codes for Factor V Leiden and Factor II Prothrombin, the mutations involved in the coagulation pathway. Relative risk for thrombosis is higher when there is more than one genetic mutation."

"What is so nice about this technology is that the amplification of the DNA and identification of the end product takes place in real time, thus shortening the time it takes to get a result . . . This feature is unique compared to other testing platforms."

Arundhati Rao, MD, PhD

Dr. Rao continues, "Once a physician knows the exact defect, they are able to better advise their patients and their patient's families who may not be symptomatic. I think it's very important to understand the family history and perform appropriate screening for family members." Dr. Rao further notes that Roche has a great deal of experience with the LightCycler® real-time PCR technology. "What is so nice about this technology is that the amplification of the DNA and identification of the end product takes place in real time, thus shortening the time it takes to get a result. We are able to see and monitor the test every step of the way. This feature is unique compared to other testing platforms.

More than 3 million cases of DVT overall are reported in the United States each year and the number is steadily increasing.[5] Almost anyone can have a DVT event, although certain risk factors increase its likelihood. If left untreated, DVT can lead to PE and death.[5] According to the National Heart, Lung and Blood Institute, PE is the third most common cause of death in hospitalized patients, with at least 300,000 cases each year in the United States.[5] If not treated, approximately 30% of patients with PE will die, often within the first few hours of the event. Early recognition and appropriate treatment of DVT and PE can save many lives.

To Learn More

For further information, please visit the Web site at www.roche-diagnostics.us or www.roche.us.

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