Overview

Useful For

Genetic confirmation of factor V deficiency with the identification of an alteration in the *F5* gene known or suspected to cause the condition

Carrier testing for close family members of an individual with a factor V deficiency diagnosis

This test is **not intended** to evaluate for the factor V Leiden mutation.

This test is **not intended for** prenatal diagnosis.

Genetics Test Information

This test detects pathogenic alterations in the *F5* gene to delineate the underlying molecular defect in a patient with a laboratory diagnosis of coagulation factor V deficiency. This test may also detect rare alterations in *F5* that cause activated protein C resistance, resulting in thrombophilia.

The gene target for this test is:

Gene name (transcript): *F5* (GRCh37 [hg19] NM_000130)

Chromosomal location: 1q23-24

Testing Algorithm

If bleeding is the reason for testing, genetic testing for factor V deficiency should only be considered if there is an isolated reduction of factor V activity in plasma using a specific prothrombin -based factor V assay(1) and acquired causes of a low factor V are excluded.

Genetic testing for F5D is indicated if:

-Factor V activity is reduced and acquired causes of FV deficiency have been excluded

-The FVIII activity is normal (low factor V levels with concurrently low factor VIII levels suggest combined deficiency of

factor V and FVIII [F5F8D], a condition with a genetic etiology different from that of F5D)

If the patient appears to have thrombophilia, the results of an activated protein C (APC) resistance assay indicate presence of resistance to APC, and the factor V Leiden genotype does not correlate with the severity of thrombophilia or clinical presentation, full-gene sequencing of *F5* may be warranted.

For more information of specific testing scenarios, see Ordering Guidance.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Rare Coagulation Disorder Patient Information

Method Name

Custom Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing when appropriate

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

If the patient appears to have a bleeding disorder, the clinical workup for factor V deficiency (F5D) begins with special coagulation testing for factor V activity. Order FACTV / Coagulation Factor V Activity Assay, Plasma.

If thrombophilia is the indication for testing, the vast majority (approximately 95%) of individuals with thrombophilia due to hereditary activated protein C resistance have a specific point alteration in the *F5* gene called factor V Leiden (c.1601G>A, p.Arg534Gln; historically known as "R506Q" or "1691G>A"), which can be assayed directly and more cost-effectively by targeted alteration testing. If testing for factor V Leiden is desired instead of full-gene sequencing, order F5DNA / Factor V Leiden (R506Q) Mutation, Blood.

Shipping Instructions

Ambient and refrigerate specimens **must** arrive within 7 days of collection, and frozen specimens must arrive within 14 days.

Collect and package specimens as close to shipping time as possible.

Necessary Information

Rare Coagulation Disorder Patient Information **is required**. Testing may proceed without the patient information, however, the information aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD) or light-blue top (3.2% sodium citrate)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Stability: Ambient (preferred)/Refrigerated/Frozen

Specimen Type: Extracted DNA

Container/Tube: 1.5- to 2-mL tube

Specimen Volume: Entire specimen

Collection Instructions:

1. Label specimen as extracted DNA and source of specimen.

2. Provide indication of volume and concentration of the DNA.

Specimen Stability: Frozen (preferred)/Refrigerated/Ambient

Forms

1. **Rare Coagulation Disorder Patient Information (T824) is required**.

2. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available.

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

3. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK

Specimen Minimum Volume

Blood: 1 mL

Extracted DNA: 100 mcL at 50 ng/mcl concentration

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)	7 days	
	Frozen	14 days	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Factor V is a critical cofactor of Xa in the conversion of prothrombin to thrombin. Factor V protein has both procoagulant and anticoagulant properties, and molecular defects in it may result in either bleeding or clotting.

Factor V deficiency (F5D, also known as parahemophilia) causes mild to severe bleeding problems, including nosebleeds, bruising, soft tissue and joint bleeds, menorrhagia, umbilical stump bleeding and post-operative bleeding. Intracranial bleeding has been reported in neonates, but bleeding episodes in the central nervous system and in the GI tract, in general, are reported to occur only rarely.(2,3) F5D is a rare with a prevalence of 1 per 1 million in the general

population. The *F5* gene encodes Factor V. Alterations in the *F5* gene that reduce the amount of plasma factor V or disrupt its functional procoagulant activity cause F5D. Unlike some other factor deficiencies, there is no strict correlation between FV levels and disease state severity, as some patients with severe deficiency do not have severe bleeding despite low FV levels. However, it has been estimated that the minimum level of factor V needed to prevent symptoms is at least 10% of normal.(4) Hereditary factor V deficiency is considered to be an autosomal recessive disorder. Individuals homozygous or compound heterozygous for pathogenic F5D alterations usually have factor V plasma activity levels lower than 10%.(1) Heterozygous individuals typically have around 50% plasma factor V levels and are usually asymptomatic.(1) Causes of acquired (non-genetic) F5D that should be excluded prior to genetic testing include the development of inhibitors directed against factor V after exposure to bovine thrombin or in association with risk factors like surgical procedures, antibiotic administration, blood transfusions, cancers, and autoimmune disorders.(1) In addition, liver disease or consumptive coagulopathy may result in low factor V levels.

Defects in factor V may be associated with an increased risk of thrombosis. Activated protein C (APC) reduces the thrombotic activity of factor V by proteolytically cleaving certain sites in the protein. Point alterations at these cleavage sites make factor V resistant to this inactivation (ie, cause APC resistance), abnormally prolonging its procoagulant properties and increasing the risk for thrombosis. The vast majority of individuals with familial APC resistance have a specific point alteration in the *F5* gene called factor V Leiden (historically known as "R506Q" or "1691G>A"). Five percent of factor V Leiden heterozygotes develop thromboembolism by 65 years of age. Other far less common factor V alterations also cause APC resistance and have different thrombotic risks. The coinheritance of both a pathogenic APC-resistant *F5* gene alteration and a *F5* gene alteration that causes F5D (ie, "pseudohomozygosity") also results in an increased risk for thrombosis. The hereditary thrombophilia that results from *F5* alterations causing APC-resistance is inherited in an autosomal dominant manner that is incompletely penetrant (ie, the presence of a pathogenic variant increases the risk for but does not guarantee manifestation of disease).

These risks are further influenced by additional risk factors, such as oral contraceptive use, hyperhomocysteinemia, pregnancy, blood type, and the inheritance of other molecular defects in genes associated with heritable thrombophilia (eg, deficiencies in protein S and protein C). Still other factors can impair activity of activated protein C such as increased factor VIII, increased estrogen levels, antiphospholipid antibodies, cancer, elevated BMI, and smoking. The prevalence of alterations in *F5* that cause APC resistance other than factor V Leiden is unknown. Full gene sequencing of *F5* may be warranted if APC resistance assay (see APCRV / Activated Protein C Resistance V [APCRV], Plasma) suggests abnormal resistance to activated protein C and factor V Leiden genotype does not correlate with the severity of thrombophilia or clinical presentation. During testing for APC resistance, care should be taken to avoid, when possible, certain preanalytical conditions of the patient and blood specimen that may interfere with results. Examples include the presence of lupus-like anticoagulants and specific coagulation factor inhibitors, excessive exposure to estrogen, and markedly elevated levels of factor VIII.

Reference Values

An interpretive report will be provided

Interpretation

An interpretive report will be provided.

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Consultations with the Mayo Clinic Special Coagulation Clinic, Molecular Hematopathology Laboratory or Thrombophilia Center are available for DNA diagnosis cases. This may be especially helpful in complex cases or in situations where the diagnosis is atypical or uncertain.

Cautions

Clinical:

Some individuals may have a variant that is not identified by the methods performed. The absence of a variant, therefore, does not eliminate the possibility of factor V deficiency or a related disorder such as combined factor V and VIII deficiency ,which occurs due to alterations in *LMAN1* (ERGIC-53) or *MCFD2*. This assay does not distinguish between germline and somatic alterations, particularly with variant allele frequencies significantly lower than 50%. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Technical Limitations:

Next-generation sequencing (NGS) may not detect all types of genetic variants. Additionally, rare variants (ie, polymorphisms) may be present that could lead to false-negative or false-positive results. Therefore, test results should be interpreted in the context of activity and antigen measurements, clinical findings, family history, and other laboratory data. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If multiple alterations are identified, NGS is not able to distinguish between alterations that are found in the same allele ("in cis") and alterations found on different alleles ("in trans"). This limitation may complicate diagnosis or classification and has implications for inheritance and genetic counseling. To resolve these cases, molecular results must be correlated with clinical history, activity and antigen measurements, and family studies.

Unless reported or predicted to cause disease, alterations found deep in the intron or alterations that do not result in an amino acid substitution are not reported. These and common alterations (ie, polymorphisms) identified for this patient are available upon request.

Reclassification of Variants Policy:

At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

Clinical Reference

1. Lippi G, Favaloro EJ, Montagnana M, et al: Inherited and acquired factor V deficiency. Blood Coagul Fibrinolysis. 2011 Apr;22(3):160-166

2. Asselta R, Peyvandi F: Factor V deficiency. Semin Thromb Hemost. 2009 Jun;35(4):382-389

3. Mumford AD, Ackroyd S, Alikhan R, et al: Guideline for the diagnosis and management of the rare coagulation disorders: a United Kingdom Haemophilia Centre Doctors' Organization guideline on behalf of the British Committee for Standards in Haematology. Br J Haematol. 2014 Nov;167(3):304-326

4. Palla R, Peyvandi F, Shapiro AD: Rare bleeding disorders: diagnosis and treatment. Blood, 2015 Mar;125(13):2052-2061

5. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH: High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood. 1995 Mar;85(6):1504-1508

6. Van Cott EM, Khor B, Zehnder JL: Factor V Leiden. Am J Hematol. 2016 Jan;91(1):46-49

7. Dahlback B: Pro- and anticoagulant properties of factor V in pathogenesis of thrombosis and bleeding disorders. Int J Lab Hematol. 2016 May;38 Suppl 1:4-11

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed.

Regions of homology, high guanine-cytosine (GC)-rich content, and repetitive sequences may not provide accurate sequence. Therefore, all reported alterations detected by NGS in these regions are confirmed by an independent reference method. However, this does not rule out the possibility of a false-negative result in these regions.

Sanger sequencing is used to confirm alterations detected by NGS when appropriate.(Unpublished Mayo method)

PDF Report
No

Specimen Retention Time
Whole Blood: 2 weeks; DNA: Indefinitely

Performing Laboratory Location
Rochester

Fees & Codes

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81479

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
F5NGS	F5 Gene, Full Gene NGS	94236-7

Result ID	Reporting Name	LOINC®
113027	F5NGS Result	50397-9
113021	Alterations Detected	82939-0
113020	Interpretation	69047-9
113022	Additional Information	48767-8
113023	Method	85069-3
113024	Disclaimer	62364-5
113025	Panel Gene List	21669-7
113026	Reviewed By	18771-6