Test Definition: F2NGS

F2 Gene, Full Gene NGS

Overview

Useful For

Ascertaining a causative alteration in *F2* and the affected region of prothrombin protein in an individual clinically diagnosed with factor II deficiency

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

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

Genetics Test Information

This test detects pathogenic alterations in the *F2* gene to delineate the underlying molecular defect in a patient with a laboratory diagnosis of factor II (prothrombin) deficiency.

The gene target for this test is:

Gene name (transcript): *F2* (GRCh37 [hg19] NM_000506)

Chromosomal location: 11p11.2

Testing Algorithm

This genetic test should only be considered if clinical and family history, initial coagulation screens, and initial factor II (FII) tests (activity and antigen) indicate a diagnosis of factor II deficiency.

Genetic testing for F2D is indicated if:

-Prothrombin (factor II) activity is reduced (less than 80% of normal)

-Acquired causes of factor II deficiency have been excluded (eg, vitamin K deficiency, warfarin anticoagulation use, liver disease, etc)

Prothrombin antigen testing, to distinguish between type I and type II deficiencies, may be helpful in cases where genetic testing results yield variants of uncertain significance.

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

The clinical workup for factor II deficiency (F2D) begins with special coagulation testing for factor II. Order F_2 / Coagulation Factor II Activity Assay, Plasma.

This test is not intended to evaluate for the *F2* c.*97G>A alteration (historically known as G20210A) associated with prothrombin-related thrombophilia. If testing for the *F2* c.*97G>A alteration (G20210A) is desired instead of full-gene sequencing, order PTNT / Prothrombin G20210A 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 specimen 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 II (FII) deficiency (F2D) is a bleeding diathesis. Symptoms include subcutaneous and muscle hematomas, prolonged post-injury bleeding, bleeding into joint spaces, and mucosal tract bleeds.

Hereditary factor II deficiency is thought to be extremely rare, with an estimated prevalence of 1 in 2 million. If genetic in origin, F2D is inherited in an autosomal recessive manner. Both males and females may be affected if homozygous or compound heterozygous for pathogenic alterations in *F2*. Heterozygotes are typically asymptomatic, although both post-trauma excessive bleeding and post-operative bleeding have been described in carriers.

Factor II is also known as prothrombin and is produced by the *F2* gene. Prothrombin is proteolytically cleaved to form thrombin during the coagulation cascade. Thrombin has multiple roles in the hemostatic response to injury. These roles include the stimulation of platelets to form a platelet plug, the cleavage of fibrinogen to form fibrin clot, the activation of factors V and VIII by the excision of their central domains, and the activation of protein C and protein S to start the inhibition of the coagulation process. A significant deficiency (less than 1% to 5%) in the amount of functional prothrombin can cause abnormal spontaneous or post traumatic bleeding. It has been estimated that the minimum level of functional prothrombin needed to prevent these symptoms is 10% to 20% of normal.(1) Alterations in the *F2* gene that interfere with the production or function of prothrombin disrupt the coagulation cascade and can lead to bleeding complications.

FII deficiency is classified into 2 types. Mutations in the *F2* gene that interfere with the production of prothrombin lead to lower levels of the protein in blood causing type I F2D, or hypoprothrombinemia. Type I F2D may be classified as mild, moderate or severe based on the factor level in plasma. A factor level of less than 5% is considered a severe deficiency and is characterized by severe bleeding symptoms with bleeding typically occurring spontaneously. Moderate deficiency is defined as 5% to 10% activity and mild deficiency is greater than 10%. Individuals who are heterozygous for a pathogenic *F2* alteration typically have factor levels of 30% to 60%.

Mutations in *F2* that create a dysfunctional protein that is produced in normal amounts but isn't as active cause type II F2D, or dysprothrombinemia. Individuals with type II F2D alterations have bleeding of variable severity that is typically less severe than in type I F2D. Cases of compound heterozygosity for both a hypoprothrombinemia mutation and a dysprothrombinemia mutation in the same person have been reported. Additionally, a complete absence of prothrombin is thought to be incompatible with life.

Genetic testing is indicated if a coagulation screen shows prolonged prothrombin time ,prolonged activated partial thromboplastin time , normal thrombin time , and reduced levels of prothrombin (factor II) activity. Prothrombin antigen testing helps to distinguish between type I and type II deficiencies.

Causes of acquired (non-genetic) factor II deficiency that should be excluded prior to genetic testing include long-term use of antibiotics, impaired vitamin K absorption, liver disease, the obstruction of bile, and warfarin anticoagulation. Cases of an acquired factor II inhibitor can occur in the presence of a lupus anticoagulant, autoimmune disorders or during infection or lymphoma.(2) A small number of cases are suspected to have been drug induced (quinidine in one case and phenytoin in another).

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

2. Mulliez SM, De Keyser F, Verbist C, et al: Lupus anti-coagulant-hypoprothrombinemia syndrome: report of two cases and review of the literature. Lupus. 2015;94(4):713-715

3. Lancellotti S, Basso M, De Cristofaro R: Congenital Prothrombin Deficiency: An Update. Semin Thromb Hemost 2013; Sep39(6):596-606

4. Pozzi N, Chen Z, Gohara DW, et al: Crystal structure of prothrombin reveals conformational flexibility and mechanism of action. J Biol Chem. 2013 Aug;288(31):22734-22744

5. Lane DA, Philippou H, Huntington JA: Directing Thrombin. Blood.Oct 2005;106(8):2605-2612

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
F2NGS	F2 Gene, Full Gene NGS	94237-5

Result ID	Reporting Name	LOINC®
113019	F2NGS Result	50397-9
113013	Alterations Detected	82939-0
113012	Interpretation	69047-9
113014	Additional Information	48767-8
113015	Method	85069-3
113016	Disclaimer	62364-5
113017	Panel Gene List	24477-2
113018	Reviewed By	18771-6