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 (F2D).

The gene target for this test is:

Gene name (transcript): F2 (GRCh37 [hg19] NM_000506)
Chromosomal location: 11p11.2

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

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

Advisory Information
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.

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 (168 hours of draw), and frozen specimens must arrive within 14 days (336 hours of draw).

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

Necessary Information
Rare Coagulation Disorder Patient Information is required, see Special Instructions. 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: Peripheral blood

Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD) or green top (sodium citrate)

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.

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, see Special Instructions. Fax the completed form to 507-284-1759.

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 in Special Instructions:
   - [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.

**Specimen Minimum Volume**

Blood: 1 mL
Extracted DNA: 100 mcL at 50 ng/mcL concentration

**Reject Due To**

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**Specimen Stability Information**

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**Clinical and Interpreive**

**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 isnâ€™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 (PT), prolonged activated partial thromboplastin time (aPTT), normal thrombin time (TT), 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 mutation that is not identified by the methods performed. The absence of a mutation, therefore, does not eliminate the possibility of factor II deficiency (F2D). This assay does not distinguish between
germline and somatic alterations, particularly with variant allele frequencies (VAF) 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 polymorphisms may be present that could lead to false negative or 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 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**


**Performance**

**Method Description**

Next-generation sequencing 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 next-generation sequencing in these regions are confirmed by an independent reference method. However, this does not rule out the possibility of a false-negative result in
Test Definition: F2NGS
F2 Gene, Full Gene NGS

these regions.

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

PDF Report
No

Day(s) and Time(s) Test Performed
Performed weekly; Varies

Analytic Time
21 days

Maximum Laboratory Time
28 days

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

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

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 U.S. Food and Drug Administration.

CPT Code Information
81479

LOINC® Information

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