MAYO CLINIC LABORATORIES

Test Definition: PRSNG

Protein S Deficiency, PROS1 Gene, Next-Generation Sequencing, Varies

Overview

Useful For

Ascertaining a pathogenic alteration in the *PROS1* gene of patient with congenital protein S deficiency

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

Genetics Test Information

This test detects pathogenic alterations in the *PROS1* gene to delineate the underlying molecular defect in a patient with a laboratory diagnosis of thrombophilia due to protein S deficiency.

The gene target for this test is:
Gene name (transcript): *PROS1* (GRCh37 [hg19] NM_000313)
Chromosomal location: 3q11.1

Testing Algorithm

At Mayo Clinic, measurement of plasma free protein S antigen is performed as the initial testing for protein S deficiency. If the free protein S antigen is abnormal, then the total plasma protein S antigen will be performed to distinguish between types I and III protein S deficiency.

Genetic testing for protein S deficiency is indicated if:
-Free protein S antigen and/or activity is abnormally reduced
-Preanalytical variables and acquired causes of protein S deficiency have been excluded (eg, acute thrombosis, surgery, disseminated intravascular coagulation [DIC], liver disease, vitamin K deficiency, therapy with vitamin K antagonists such as warfarin, pregnancy, treatment with hormonal contraceptives, HIV infection, varicella, sickle cell disease, malignancy, nephrotic syndrome)

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
For initial testing in the assessment of protein S deficiency, order PSTF / Protein S Antigen, Plasma.

For patients in whom hereditary protein S deficiency is strongly suspected and the free plasma protein S antigen level is normal, consider testing free protein S activity for detecting type II protein S deficiency. Order S_FX / Protein S Activity, Plasma.

Shipping Instructions
Ambient and refrigerated 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)/Refrigerate/Frozen

Specimen Type: Extracted DNA
Container/Tube: 1.5- to 2-mL tube with indication of volume and concentration of the DNA
Specimen Volume: Entire specimen
Collection Instructions:
1. Label specimen as extracted DNA and source of specimen.
2. Provide volume and concentration of the DNA
Specimen Stability: Frozen (preferred)/Refrigerate/Ambient

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

Gross hemolysis	OK
Gross lipemia	OK

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Protein S (PS) is a vitamin K-dependent glycoprotein that is synthesized mainly in the liver and endothelium and is part of the natural anticoagulant system. It is a cofactor in the inactivation of procoagulant factors V and VIII. Most (60%) of protein S circulates in plasma in a form bound to C4b binding protein and the rest circulates freely and is termed "free PS". The free form serves as a cofactor to anticoagulant enzyme activated protein C in the proteolytic inactivation of procoagulant factors Va and VIIIa.

A congenital deficiency of PS deficiency increases thrombotic risk by 8 to 10 fold compared to normal individuals. Nearly half of PS type I deficiency patients present with venous thromboembolism (VTE) until the age of 55; about half of these VTE events are unprovoked.(1) The use of oral contraceptives in women deficient in PS may increase the risk of VTE by 600-fold compared to individuals with normal PS levels. Women with PS deficiency also have a 3-fold increased risk for pregnancy loss and pregnancy complications.(1) The prevalence of congenital protein S deficiency in the general population is 0.16% to 0.21%. The prevalence of protein S deficiency in patients with VTE is estimated to be 2%.(2)

Congenital protein S deficiency is classified into three disease states. In type I PS deficiency, there is a decreased amount of both bound and free forms of protein S. In type II deficiency, protein function is altered but levels of PS are normal. In type III deficiency, there is a low level of free PS but a normal amount of total PS. No differences in clinical presentation and severity have been observed for the different phenotypes.

The *PROS1* gene encodes for PS. Alterations in *PROS1* can lead to sustained activation of factors V and VIII and the

continued production of thrombin, leading to an increased risk for thrombosis. Congenital PS deficiency is inherited in an autosomal dominant manner with incomplete penetrance. Heterozygotes for a pathogenic alteration in *PROS1* typically present with plasma PS levels in the 35% to 60% range.(3) Individuals who are homozygous or compound heterozygous for pathogenic alterations in *PROS1* present with massive VTE or neonatal purpura fulminans, which is life-threatening if untreated. Retinopathy has also been observed as the presenting symptoms in some cases. Genetic testing of the *PROS1* gene is indicated if free protein S antigen or activity is abnormally reduced and acquired causes of protein S deficiency have been excluded. Normal, full-term newborn infants or healthy premature infants may have decreased levels of total protein S (15%-50%); but because of low levels of C4bBP, free PS may be normal or near the normal adult level (greater than or equal to 50%). Total PS reaches adult levels by 90 to 180 days postnatal.

Acquired (nongenetic causes) of PS deficiency are much more common than hereditary PS deficiency. It is unknown if acquired deficiency of PS results in increased thrombotic risk. Vitamin K deficiency, oral anticoagulant therapy, presence of liver disease, or intravascular coagulation and fibrinolysis/disseminated intravascular coagulation, thrombotic thrombocytopenia purpura, pregnancy or estrogen therapy, and nephritic syndrome are common acquired causes of PS deficiency. As an acute-phase reactant, plasma C4bBP levels increase with acute illness and may cause acquired free PS deficiency. Additionally, many preanalytic factors may interfere with measurements of free and total PS, such as the use of heparin, having hemoglobin levels greater than 20 g/dL, bilirubin (greater than 25 mg/dL for free PS, greater than 100 mg/L for total PS), or rheumatoid factor greater than 900 IU/mL. Free PS measurements are affected by additional factors such as elevated triglycerides (greater than1,500 mg/dL), sickle cell anemia, and the presence of the factor V Leiden alteration in the *F5* gene.

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 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, and 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 protein S 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.

If there is a family history of protein S deficiency, it is often useful to test first-degree family members to help establish the clinical significance of variants of unknown significance.

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.

Contact the laboratory if additional information is required regarding the transcript and/or human genome assembly used for the analysis of this patient's results.

Clinical Reference

1. Brouwer JLP, Veeger NJGM, van der Schaaf W, Kluin-Nelemans HC, van der Meer J: Difference in absolute risk of venous and arterial thrombosis between familial protein S deficiency type I and type III. Results from a family cohort study to assess the clinical impact of a laboratory test-based classification. Br J Haematol. 2005 Mar;128(5):703-710
2. ten Kate MK, van der Meer J: Protein S deficiency: a clinical perspective. Haemophilia. 2008 Nov;14(6):1222-1228
3. Marlar RA, Gausman JN: Protein S abnormalities: a diagnostic nightmare. Am J Hematol. 2011 May;86(5):418-421
4. Duebgen S, Kauke T, Marschall C, et al: Genotype and laboratory and clinical phenotypes of protein S deficiency. Am J Clin Pathol. 2012 Feb;137(2):178-184
5. Garcia de Frutos P, Fuentes-Prior P, Hurtado B, Sala N: Molecular basis of protein S deficiency. Thromb Haemost. 2007 Sep;98(3):543-556

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

Day(s) Performed
Varies

Report Available
21 to 28 days

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

Performing Laboratory Location
Rochester

Fees & 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 US Food and Drug Administration.

CPT Code Information
81479

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PRSNG	PROS1 Gene, Full Gene NGS	92994-3

Result ID	Test Result Name	Result LOINC® Value
113100	PRSNG Result	50397-9
113094	Alterations Detected	82939-0

113093	Interpretation	69047-9
113095	Additional Information	48767-8
113096	Method	85069-3
113097	Disclaimer	62364-5
113098	Panel Gene List	48018-6
113099	Reviewed By	18771-6