Thrombosis Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 12 genes, plus two targeted variants, known to be associated with an increased risk for developing venous thromboembolism. This panel can be ordered as:

- **Next Generation Sequencing (NGS) only;**
- **NGS with reflex to Array Comparative Genomic Hybridization (aCGH) Deletion/Duplication if sequencing does not identify clinically significant variants that fully explain the patient's phenotype;**
- **NGS with concurrent aCGH Deletion/Duplication (both testing methodologies performed simultaneously); or Deletion/Duplication by aCGH only.**

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common yet complex disorder. Risk factors involved in the pathogenesis of this disorder include inherited thrombophilias that are caused by loss-of-function of anticoagulant proteins, gain-of-function of procoagulants, or defects in the fibrinolytic pathways. These inherited risk factors, together with acquired risk factors, predispose an individual to thrombosis. Not all individuals with a genetic predisposition to thrombosis will develop VTE; the relative risk for thrombosis may be influenced by the specific variant present, whether the variant(s) is heterozygous, compound heterozygous or homozygous, the concomitance of other pathogenic variants, a family history of DVT, as well as the presence of other inherited and/or acquired risk factors. Identifying individuals who have an increased genetic susceptibility for VTE may assist providers in establishing an individualized risk assessment, which in some cases may guide management decisions, assist with the identification of affected family members, and allow for accurate genetic recurrence risk assessment. In addition, the identification of women who have one or more inherited thrombophilia variants may provide important information for contraception and pregnancy management.

This panel includes analysis of the Factor V Leiden and prothrombin 20210G>A variants, as well as genes associated with coagulation factor regulatory proteins (ADAMTS13, protein C, protein S, and antithrombin) and other genes that are associated with an increased risk for thrombosis. The NGS panel evaluates for single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease. However, large deletions and duplications, also referred to as copy number variations (CNVs), are a known cause of genetic disorders, but can escape detection by next generation sequence analysis. Additional testing with aCGH Deletion/Duplication analysis is available for all genes on this panel to evaluate for large deletions and duplications encompassing one or more exons, or affecting an entire gene.

Refer to the table on the following page for further information about each gene in the Autosomal Dominant Thrombocytopenia Panel, including the clinical phenotype and platelet size.

versiti™

Thrombosis Panel: gene and clinical phenotype	
Gene	Clinical Phenotype
ADAMTS13	**Congenital ADAMTS13 deficiency** (also known as familial/inherited thrombotic thrombocytopenic purpura (TTP) and Upshaw-Schulman syndrome): pathogenic variants in *ADAMTS13* are associated with autosomal recessive ADAMTS13 deficiency, characterized by increased risk of life-threatening thrombotic microangiopathy (thrombocytopenia, microangiopathic hemolytic anemia, microvascular thrombosis and organ dysfunction) that usually presents in childhood; adult presentations, often triggered by pregnancy or acute illness, are reported
*F2**	**Prothrombin gene variant:** the pathogenic gain of function prothrombin gene variant c.*97G>A (legacy nomenclature G20210A) is associated with increased risk of venous thromboembolism due to increased prothrombin activity
*F5***	Factor V Leiden variant: the pathogenic F5 c.1691G>A (R506Q) variant is associated with increased risk of venous thromboembolism due to decreased cleavage of FV by activated protein C
FGA *FGB* *FGG*	**Dysfibrinogenemia:** pathogenic variants in *FGA, FGB* and *FGG* are associated with qualitative fibrinogen defects that can increase the risk of thrombosis
HRG	**Histidine-rich glycoprotein deficiency:** pathogenic variants in *HRG* are associated with increased risk of thrombosis due to histidine-rich glycoprotein deficiency, a modulator of the intrinsic coagulation pathway
KNG1	***KNG1*-associated increased risk of thrombosis:** pathogenic variants in *KNG1* are associated with high molecular weight kininogen (HMWK) abnormalities which increases the risk of thrombosis through a pleiotropic effect on coagulation.
PLG	**Plasminogen deficiency:** pathogenic variants in *PLG* are associated with autosomal recessive plasminogen deficiency, characterized by ligneous conjunctivitis due to formation of chronic fibrin pseudomembranous lesion
PROC	**Protein C deficiency:** pathogenic variants in *PROC* are associated with autosomal recessive quantitative or qualitative protein C deficiency, characterized by neonatal purpura fulminans, warfarin-induced skin necrosis and increased risk of thrombosis. Heterozygotes may have moderately reduced protein C levels and present with a milder thrombotic phenotype.
PROS1	**Protein S deficiency:** pathogenic variants in *PROS1* are associated with autosomal recessive quantitative or qualitative protein S deficiency, characterized by neonatal purpura fulminans, warfarin skin necrosis and increased risk of thrombosis. Heterozygotes may have moderately reduced protein S levels and present with a milder thrombotic phenotype.
SERPINC1	**Antithrombin deficiency:** pathogenic variants in *SERPINC1* are associated with congenital antithrombin deficiency, characterized by quantitative or qualitative deficiencies in antithrombin, resulting in increased risk of venous thrombosis and fetal loss
SERPIND1	**Heparin cofactor II deficiency:** pathogenic variants in *SERPIND1* are associated with increased risk of thrombosis due to heparin cofactor II deficiency
THBD	**Thrombomodulin defect:** pathogenic variants in *THBD* are associated with increased risk of thrombosis due to decreased activation of protein C, leading to increased thrombin generation

*Prothombin gene c.*97G>A variant only (legacy nomenclature G20210A)

**Factor V Leiden variant only c.1601G>A, p.Arg534Gln (legacy nomenclature G1691A, p.R506Q)

Indications for testing:

Thrombosis Panel (NGS and/or aCGH), order code 4820:

The Thrombosis Panel should be considered:

- In patients with venous thromboembolism in whom clarification or confirmation of inherited predisposition is desired
- In patients with venous thromboembolism that is recurrent, in unusual vascular territories or seemingly unprovoked and presenting at young age
- In patients with thromboembolism and a strong family history of venous thrombosis, to assist with treatment, reproductive risk and genetic counseling

Single Gene Analysis (order code 4855) or Custom Blood Disorder Panel (Order Code 4850), (NGS and/or aCGH):

Analysis of genes included in this panel may also be ordered as a standalone Single Gene Analysis or as a Custom Blood Disorder Panel (2-10 genes), by NGS and/or by aCGH, as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970):

Targeted variant analysis for clinical diagnosis, carrier identification, or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant(s) is known in the family. If the proband was not tested at Versiti, a control sample may be needed (please call the laboratory to discuss). If the familial variant is a large deletion or duplication, aCGH for the involved gene is required.

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, to be directed to our genetic counselors and clinical support team.

Informed Consent

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of and understands the indications for and implications of the genetic test. This panel is designed for clinical detection of germline genetic variants in genes with strong or definitive evidence for causality of inherited thrombocytopenia. Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, and/or may carry individual or familial implications such as risk for syndromic manifestation, predisposition to malignancy, and/or reproductive implications (such as carrier status). If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under *forms*.

Test method:

NGS: This next-generation sequencing assay analyzes the complete coding region of 12 genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence. *ANKRD26* analysis also includes approximately 200bp upstream of coding region to identify clinically significant variants in the 5'UTR. These targeted regions are captured by hybridization, amplified, and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low quality coverage are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

aCGH: The specific genes are analyzed for copy number variations due to deletion or duplication by high density gene-focused array Comparative Genomic Hybridization. Probes are approximately 60bp in length and density of coverage in exonic regions is a minimum of 4 probes per 500 bp. Genomic DNA for the samples and gender-matched references are denatured, labeled with fluorescent dye and hybridized, the array is washed and scanned, and analysis is performed for the specific genes requested.

Assay sensitivity and limitations:

NGS: The analytical sensitivity of the NGS test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology.

aCGH: Balanced chromosomal rearrangements (i.e., translocations, inversions) or point mutations that may be the cause of the clinical phenotype cannot be detected via aCGH. Any exonic deletion or duplication smaller than 500bp may not be detected. Low level of mosaicism will not be detected by aCGH. Probe performance could be affected by multiple SNPs in a given region. Breakpoints occurring outside the targeted gene(s) will not be defined.

Clinical Sensitivity

The clinical sensitivity of the Thrombosis Panel (NGS and aCGH) of the 12 genes and two targeted variants in this panel is highest in patient with a history of unprovoked or hormonally provoked thrombosis at a young age and thrombosis in unusual locations, especially if there is a family history of thrombosis in multiple family members with similar clinical characteristics.

Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards and guidelines. Sequence variants and large deletions and duplications predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

Specimen Requirements

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1ug of DNA at ≥50ng/uL of High Quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions please contact the laboratory to discuss sample requirements.

SHIP

Shipping Requirements

Ship on an ice pack at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
638 N. 18th St
Milwaukee, WI, 53233

ORDER

Required Forms

Please complete all pages of the requisition form. Clinical history (including patient's ethnicity, clinical diagnosis, family history, and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround Time

Test code: 4820

For suggested CPT codes, visit Versiti.org/test menu

Turnaround time: 21 days

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, or LabInfo@versiti.org

References

Thrombosis Panel references

1. Connors JM. Thrombophilia Testing and Venous Thrombosis. N Engl J Med. 2017;377(12):1177-1187. doi:10.1056/NEJMra1700365
2. De Stefano V, Rossi E. Testing for inherited thrombophilia and consequences for antithrombotic prophylaxis in patients with venous thromboembolism and their relatives. A review of the Guidelines from Scientific Societies and Working Groups. Thromb Haemost. 2013;110(4):697-705. doi:10.1160/TH13-01-0011
3. Lee EJ, Dykas DJ, Leavitt AD, et al. Whole-exome sequencing in evaluation of patients with venous thromboembolism. Blood Adv. 2017;1(16):1224-1237. Published 2017 Jun 29. doi:10.1182/bloodadvances.2017005249
4. Martinelli I, De Stefano V, Mannucci PM. Inherited risk factors for venous thromboembolism. Nat Rev Cardiol. 2014;11(3):140-156. doi:10.1038/nrcardio.2013.211
5. Megy K, Downes K, Simeoni I, et al. Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. J Thromb Haemost. 2019;17(8):1253-1260. doi:10.1111/jth.14479
6. Montagnana M, Lippi G, Danese E. An Overview of Thrombophilia and Associated Laboratory Testing. Methods Mol Biol. 2017;1646:113-135. doi:10.1007/978-1-4939-7196-1_9

Variant interpretation references

7. Bean LJH, Funke B, Carlston CM, et al. Diagnostic gene sequencing panels: from design to report-a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(3):453-461. doi:10.1038/s41436-019-0666-z
8. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013;15(9):733-747. doi:10.1038/gim.2013.92
9. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8.
10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424. doi:10.1038/gim.2015.30
11. Zhang S, Taylor AK, Huang X, et al. Venous thromboembolism laboratory testing (factor V Leiden and factor II c.*97G>A), 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2018;20(12):1489-1498. doi:10.1038/s41436-018-0322-z

versiti™