Coagulation Disorder Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 19 genes, plus one targeted variant, known to cause coagulation disorders. 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.**

Coagulation disorders, including hemophilia A and B, von Willebrand disease (VWD) and rare bleeding disorders (RBDs), are a heterogeneous group of inherited bleeding disorders with overlapping clinical phenotypes. Bleeding symptoms can include epistaxis, easy bruising, gingival bleeding, prolonged bleeding following an injury, surgery or dental extractions, gastrointestinal or urinary bleeding, hematomas, hemoptysis, intracranial bleeding, muscle bleeding, hemarthrosis, and menorrhagia or postpartum bleeding in women. Symptoms can present at any age and range in severity: in mild cases, individuals may remain asymptomatic until the event of a trauma or surgery, and in severe cases, patients may present with spontaneous life-threatening hemorrhage or bleeding symptoms in the newborn period. Heterozygous carriers of autosomal recessive clotting factor deficiencies and female carriers of X-linked disorders can present with moderate decreased factor activity and a milder or absent bleeding phenotype.

Although results of functional hemostasis testing often guide genetic testing for a specific inherited coagulation disorder, there are situations where functional tests are not definitive, cannot be obtained, or may suggest two or more factor deficiencies in a patient. For cases in which the laboratory phenotype is not fully consistent with clinical symptoms, combined factor deficiencies are suspected, or the specific coagulation disorder is unclear, the Coagulation Disorder Panel offers an efficient and cost-effective means of diagnostic genetic evaluation. Accurate diagnosis provides information about phenotype and prognosis, guides medical management decisions, assists with the identification of affected family members, and allows for accurate genetic recurrence risk assessment.

Variants in several different genes known to cause bleeding disorders may be inherited in an autosomal recessive, autosomal dominant or X-linked manner. More common and rare types of inherited coagulation disorders will be identified with this panel.

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.

Analysis of genes included in the Coagulation Disorder Panel may also be ordered as a stand-alone single gene test as dictated by the patient's laboratory phenotype. Alternatively, custom panels (2 to 10 genes) may be ordered if a patient's history suggests a specific bleeding disorder with multiple causative genes, or if functional testing results narrow the diagnosis to specific phenotypes that can be due to different underlying genetic conditions. Targeted familial variant testing can also be performed on any gene in the panel when the specific genetic variant is known in a family.

Additional types of inherited bleeding disorders associated with platelet dysfunction are included in the Platelet Function Disorder Panel (test code 4835). Both the Coagulation Disorder Panel and Platelet Function Disorder Panel can be ordered together as part of the Comprehensive Bleeding Disorder Panel (test code 4825).

versiti™

Refer to the table for further information about each gene in the Coagulation Disorder Panel, including the clinical phenotype and inheritance pattern.

Platelet Function Disorder Panel: gene, clinical phenotype and inheritance pattern		
Gene	Clinical Phenotype	Inheritance
F2	**Factor II deficiency** (prothrombin deficiency): severe bleeding including post procedure bleeding, umbilical stump bleeding, hemarthrosis, muscle hematomas and mucosal bleeding	Autosomal Recessive
F5	**Factor V deficiency:** moderate to severe bleeding, including mucocutaneous bleeding, postoperative bleeding, menorrhagia and gastrointestinal bleeding	Autosomal Recessive
F7	**Factor VII deficiency:** bleeding diathesis of variable severity	Autosomal Recessive
F8	**Hemophilia A:** mild to severe bleeding due to a quantitative or qualitative deficiency in factor VIII	X-linked
F9	**Hemophilia B:** mild to severe bleeding due to a quantitative or qualitative deficiency in factor VIII	X-linked
F10	**Factor X deficiency:** bleeding of variable severity and a weak association between coagulation factor activity and severity of bleeding phenotype	Autosomal Recessive
F11	**Factor XI deficiency:** quantitative or qualitative deficiency of coagulation factor XI leading to variable degrees of decreased factor activity which are weakly correlated with the severity of bleeding manifestations	Autosomal Dominant / Autosomal Recessive (most common)
F13A1 F13B	**Factor XIII deficiency:** umbilical cord bleeding, spontaneous intracranial bleeding, delayed bleeding after surgery, menorrhagia, impaired wound healing and infertility.	Autosomal Recessive
FGA *FGB* *FGG*	**Afibrinogenemia:** severe/delayed bleeding from markedly decreased or absent fibrinogen.	Autosomal Recessive
	Hypofibrinogenemia: mild to moderate delayed bleeding due to decreased fibrinogen levels	Autosomal Dominant (most common)/ Autosomal Recessive
	Hypodysfibrinogenemia: mild to moderate delayed bleeding with or without thrombosis due to deficient and dysfunctional fibrinogen	Autosomal Dominant (most common)/ Autosomal Recessive
	Dysfibrinogenemia: absent or mild/moderate delayed bleeding with or without thrombosis due to dysfunctional fibrinogen	Autosomal Dominant (most common)/ Autosomal Recessive
GGCX	**Combined deficiency of vitamin K-dependent clotting factors type 1** (VKCFD1): bleeding tendency of variable severity due to deficiency of factors II, VII, IX and X	Autosomal Recessive
LMAN1	**Combined factor V and VIII deficiency:** decreased factor levels (between 5% and 30%) leading to mild to moderate bleeding	Autosomal Recessive
MCFD2	**Combined factor V and VIII deficiency:** decreased factor levels (between 5% and 30%) leading to mild to moderate bleeding	Autosomal Recessive
SERPINA1§	**Antithrombin Pittsburgh:** the pathogenic variant *SERPINA1* c.1145T>G (p.Met358Arg) is associated with variable bleeding due to enhanced inhibition of thrombin. Targeted analysis of the Pittsburgh variant ONLY; NGS and aCGH of *SERPINA1* otherwise not available.	Autosomal Dominant
SERPINE1	**Plasminogen activator Inhibitor 1 (PAI-1) deficiency:** variable bleeding due to increased fibrinolysis	Autosomal Recessive
SERPINF2	**Alpha 2-antiplasmin deficiency:** variable bleeding tendency due to increased fibrinolysis	Autosomal Recessive
VKORC1	**Combined deficiency of vitamin K-dependent clotting factors type 2 (VKCFD2):** bleeding tendency of variable severity due to deficiency of factors II, VII, IX and X	Autosomal Recessive
VWF	**von Willebrand Disease (VWD):** mild to severe bleeding due to quantitative (types 1 and 3) or qualitative defects (type 2) in *VWF*	Autosomal Dominant (most common) / Autosomal Recessive (type 2N and 3)

§ Targeted variant of the Pittsburg allele in exon 5 only

Indications for testing:

Coagulation Disorder Panel (NGS and/or aCGH), order code 4815:

The Coagulation Disorder Panel should be considered:

- In patients with clinical and laboratory findings of a congenital bleeding disorder from a coagulation factor deficiency or dysfunction, when the patient's history suggests multiple coagulation disorders
- In patients with a suspected congenital bleeding disorder from a coagulation factor deficiency or dysfunction that have inconclusive functional hemostatic testing, or in situations where functional hemostatic testing cannot be obtained
- In patients in whom a family history of a bleeding disorder is reported but unspecified, without an affected relative available for confirmation

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 is preferred and may be required (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 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 of causality of coagulation disorders, including hemophilia A and B, von Willebrand disease, and rare bleeding disorders. 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 19 genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, as well as one targeted variant, and is compared to the build GRCh37.p13 reference sequence. In addition to the complete coding regions, *F7* analysis includes 59 bp upstream of exon 1 to cover HNF-4 and Sp1 binding sites in the promoter region, 67bp upstream of *F9* exon 1 is analyzed to cover *F9* Leyden variants, and VWF includes5' 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 comprehensive genetic testing (NGS and aCGH) of the 19 genes and one targeted variant is highest in patients with a history of lifelong clinically significant bleeding with a phenotype compatible with coagulation factor deficiency or dysfunction, who have a family history of bleeding and present with persistent abnormalities on functional hemostatic testing.

Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards. 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: 4815

CPT codes: For suggested CPT codes, visit the versiti.org/test-catalog.

Turnaround time: NGS only, aCGH only, or NGS and aCGH concurrently: 21 days

NGS reflex to aCGH: 21 days (if NGS only, aCGH not needed) or 42 days (with reflex to aCGH)

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

Coagulation Disorder references

1. Bastida JM, Benito R, Lozano ML, et al. Molecular Diagnosis of Inherited Coagulation and Bleeding Disorders. Semin Thromb Hemost. 2019;45(7):695-707. doi:10.1055/s-0039-1687889
2. Casini A, Blondon M, Tintillier V, et al. Mutational Epidemiology of Congenital Fibrinogen Disorders. Thromb Haemost. 2018;118(11):1867-1874. doi:10.1055/s-0038-1673685
3. de Moerloose P, Schved JF, Nugent D. Rare coagulation disorders: fibrinogen, factor VII and factor XIII. Haemophilia. 2016;22 Suppl 5:61-65. doi:10.1111/hae.12965
4. Flood VH, Christopherson PA, Gill JC, et al. Clinical and laboratory variability in a cohort of patients diagnosed with type 1 VWD in the United States. Blood. 2016;127(20):2481-2488. doi:10.1182/blood-2015-10-673681
5. Franchini M, Marano G, Pupella S, et al. Rare congenital bleeding disorders. Ann Transl Med. 2018;6(17):331. doi:10.21037/atm.2018.08.34
6. Johnsen JM, Fletcher SN, Huston H, et al. Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative. Blood Adv. 2017;1(13):824-834. Published 2017 May 18. doi:10.1182/bloodadvances.2016002923
7. Lillicrap D. Molecular testing for disorders of hemostasis. Int J Lab Hematol. 2013;35(3):290-296. doi:10.1111/ijlh.12078
8. 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
9. Menegatti M, Palla R. Clinical and laboratory diagnosis of rare coagulation disorders (RCDs) [published online ahead of print, 2019 Sep 7]. Thromb Res. 2019;S0049-3848(19)30402-5. doi:10.1016/j.thromres.2019.09.006
10. Veyradier A, Boisseau P, Fressinaud E, et al. A Laboratory Phenotype/Genotype Correlation of 1167 French Patients From 670 Families With von Willebrand Disease: A New Epidemiologic Picture. Medicine (Baltimore). 2016;95(11):e3038. doi:10.1097/MD.0000000000003038

Variant interpretation references

11. 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
12. 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
13. 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
14. 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.

versiti™