Fibrinolytic Disorder Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 8 genes and one targeted variant known to cause delayed bleeding due to hyperfibrinolysis. 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 analysis with concurrent aCGH Deletion/Duplication (both testing methodologies performed simultaneously); or**
- **Deletion/Duplication by aCGH only.**

***Includes *PLAU* performed by aCGH**

Delayed bleeding due to hyperfibrinolysis is characterized by excessive or delayed bleeding of variable severity following trauma, surgery, venipuncture or tooth extraction, but may also include bleeding symptoms such as epistaxis or menorrhagia. In the newborn period, patients can present with delayed umbilical bleeding, though mild cases may remain undiagnosed until adulthood, presenting after significant hemostatic challenges. While some inherited types of delayed bleeding due to hyperfibrinolysis can have indicative laboratory abnormalities on hemostasis testing, many have no distinguishing findings outside of the bleeding phenotype.

Establishing a specific diagnosis underlying a delayed bleeding phenotype enables the provision of appropriate therapies and adequate surveillance during bleeding challenges. Advances in genetic testing through next generation sequencing and molecular deletion/duplication analysis allow for identification of underlying genetic defects contributing to hyperfibrinolysis.

Variants in several different genes known to cause fibrinolytic disorders may be inherited in an autosomal recessive or autosomal dominant manner. Heterozygous carriers of autosomal recessive clotting factor deficiencies (such as FXIII deficiency) can present with moderate decreased factor activity and a milder or absent bleeding phenotype. More common and rare types of inherited fibrinolytic 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. Note that the Quebec Platelet Disorder (QPD) is associated with a heterozygous 77.9-kb tandem duplication of the *PLAU* gene which will be detected by aCGH and not by NGS; analysis of *PLAU* by aCGH is included in the otherwise NGS-only version of this panel.

For evaluation of delayed bleeding suggestive specifically of hyperfibrinolysis, the Fibrinolytic Disorder Panel is recommended. For broader evaluation of unspecified bleeding problems, the Comprehensive Bleeding Disorder Panel (test code 4825) may be considered. For specific evaluation of fibrinogen abnormalities, the Fibrinogen Disorders Panel (test code 4885) offers analysis of *FGA, FGB,* and *FGG*.

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Analysis of genes included in the Fibrinolytic Disorder Panel may also be ordered as a stand-alone single gene test as dictated by the patient's laboratory phenotype. 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.

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

Fibrinolytic Disorder Panel: gene, clinical phenotype and inheritance pattern

Gene	Clinical Phenotype	Inheritance
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
*PLAU**	**Quebec Platelet Disorder (QPD):** delayed onset bleeding, large trauma induced hematomas, hemarthrosis, muscle bleeds and hematuria from hyperfibrinolysis due to increased platelet urokinase plasminogen activator from a tandem 77.9kb duplication encompassing the *PLAU* gene	Autosomal Dominant
SERPINA1§	**α1-Antitrypsin (α1-AT) 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

*Available by aCGH only

§ Targeted variant of the Pittsburg allele in exon 5 only

Indications for testing:

Fibrinolytic Disorder Panel (NGS and/or aCGH), order code 4860:

The Fibrinolytic Disorder Panel should be considered in:

- Patients with delayed bleeding of variable severity following trauma, surgery, venipuncture or tooth extraction, with or without other bleeding symptoms, for clarification and/or confirmation of diagnosis
- In patients with a suspected fibrinolytic disorder 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 fibrinolytic 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 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 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 fibrinolytic 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 reproductive implications (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 7genes (excluding *PLAU*) 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. 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: 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 8 genes and one targeted variant known to be associated with fibrinolytic disorders is highest in patients presenting with lifelong delayed bleeding.

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 or 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: 4860

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

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Variant interpretation references

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