Fibrinolytic Disorder Panel

BloodCenter of Wisconsin offers a specifically designed Fibrinolytic Disorder Panel: (test code 4860 optimized for detection of germline variants in 9 genes known to cause delayed bleeding due to hyperfibrinolysis.

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 challenge. 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

This panel evaluates for single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease, in eight of the nine genes in this panel: *F13A1, F13B, FGA, FGB, FGG, SERPINA1, SERPINE1*, and *SERPINF2*. *PLAU* is evaluated by aCGH Deletion/Duplication Analysis in order to detect the heterozygous 78-kb tandem duplication responsible for the Quebec Platelet Disorder.

Only *PLAU* aCGH analysis is included in the Fibrinolytic Disorder Panel. Separate testing with aCGH Deletion/Duplication Analysis is available for some other genes on this panel to evaluate for large deletions and duplications, also referred to as copy number variation (CNV), which are a known cause of genetic disorders but can escape detection by next generation sequence analysis. Please refer to the aCGH Deletion/Duplication Analysis test description for more information about the other genes that can be evaluated with this array.

For evaluation of delayed bleeding suggestive of hyperfibrinolysis, the Fibrinolytic Disorder Panel, which includes genes associated with autosomal dominant and autosomal recessive conditions, is recommended. For broader evaluation of unspecified bleeding problems, the Comprehensive Bleeding Disorder Panel (test code 4825) may be considered.

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

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Hyperfibrinolysis / Delayed Bleeding Panel: gene, clinical phenotype, OMIM number and inheritance pattern.

Gene	Clinical Phenotype	Phenotype/ Gene OMIM number	Inheritance
F13A1	Factor XIII deficiency: rare bleeding disorder with symptoms shortly after birth. Without treatment, life-threatening intracranial hemorrhage may occur. Pathogenic variants in *F13A1* and *F13B* severely reduce the amount or activity of the factor XIII A subunit or B subunit respectively.	613225/ 134570	Autosomal Recessive
F13B		613235/ 134580	Autosomal Recessive
FGA	Pathogenic variants in *FGA*, *FGB* and *FGG* result in quantitative and/or qualitative changes in the fibrinogen alpha, beta or gamma subunit chains respectively. Congenital afibrinogenemia: rare bleeding disorder with excessive bleeding often in the newborn period. Congenital hyperfibrinogenemia results in decreased amounts of these subunit chains and may lead to varying bleeding symptoms from mild-severe. Congenital dysfibrinogenemia and congenital hypodysfibrinogenemia may result in bleeding symptoms, thromboembolic complications or both.	202400/ 134820	Autosomal Recessive
		616004/134820	Autosomal Dominant /Autosomal Recessive
FGB		202400/ 134830	Autosomal Recessive
		616004/134830	Autosomal Dominant /Autosomal Recessive
FGG		202400/ 134850	Autosomal Dominant /Autosomal Recessive
		616004/ 134850	
PLAU	Quebec Platelet Disorder (QPD): mildly reduced to normal platelet counts, delayed onset of bleeding, excessive trauma-induced bruising or hematoma, joint and muscle bleeds, spontaneous hematuria.	601709/191840	Autosomal Dominant
SERPINA1	Bleeding disorder of variable severity caused by specific variant *SERPINA1* c.1145T>G (p.Met358Arg) "Pittsburgh allele" resulting in an altered protein that inhibits thrombin, FXa and protein C. Sequencing of the full coding region of *SERPINA1* gene is not available.	613490/107400	Autosomal Dominant
SERPINE1	Plasminogen activator inhibitor-1 deficiency (PAI-1D): rare bleeding disorder associated with increased bleeding after trauma, injury or surgery due to increased fibrinolysis of fibrin blood clots.	613329/ 173360	Autosomal Recessive/ Autosomal Dominant
SERPINF2	Alpha-2-plasmin inhibitor deficiency (APLID): moderate to severe increased susceptibility to trauma-induced and spontaneous bleeding.	262850/ 613168	Autosomal Recessive

Indications for testing

Fibrinolytic Disorder Panel:

Clarification and/or confirmation of diagnosis in a patient with delayed bleeding of variable severity following trauma, surgery, venipuncture or tooth extraction, with or without other bleeding symptoms such as epistaxis or menorrhagia.

Single gene sequencing or custom gene panel:

Analysis of genes included in the Fibrinolytic Disorder Panel may also be ordered as a stand-alone single gene sequencing test or as a Custom Blood Disorder Panel (2-10 genes) as dictated by the patient's clinical and laboratory phenotype.

Targeted familial variant analysis:

Targeted variant analysis for clinical diagnosis or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant(s) is known in the family (test code: 4970). Known familial *PLAU* duplications require testing with aCGH Deletion/Duplication Analysis.

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

Test method

This assay analyzes 9 genes with next generation sequencing, the full coding regions plus a minimum 30bp of non-coding DNA including intron-exon junctions of *F13A1, F13B, FGA, FGB, FGG, SERPINE1*, and *SERPINF2* are analyzed, as well as targeted sequencing of *SERPINA1* c.1145T. 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 bi-directional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of directors, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. *PLAU* is analyzed by high density array Comparative Genomic Hybridization (aCGH) with probes approximately 60 bp in length and density of coverage in exonic regions of approximately 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; data is returned and analysis is performed.

For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

Assay sensitivity and limitations

The analytical sensitivity of this test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Our analysis does not detect large deletions or duplications (>20 bp), or deletions, duplications or variants that are outside the regions sequenced, with the exception of *PLAU*. To order the analysis of copy number variation at the exon or gene level for genes other than *PLAU*, please refer to the aCGH Deletion/Duplication Analysis test, or contact Client Services before placing your order.

Reporting of results

While this assay is designed to detect germline genetic variants associated with a bleeding phenotype, variants unrelated to the indication for testing, but with other clinical and/or reproductive implications, may also be detected. A comprehensive database of gene-phenotype relationships listed by gene name can be found at http://www.omim.org.

Results are classified and reported in accordance with ACMG next-generation sequencing standards. Variants 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).

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.

If questions, please contact the laboratory to discuss sample requirements.

Shipping requirements

SHIP

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
BloodCenter of Wisconsin
638 N. 18th St.
Milwaukee, WI 53233

Required forms

ORDER

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: 81479

Turnaround time: 21 days

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

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

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