Custom Blood Disorder Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in any 2-10 genes across our portfolio of curated hematology genes. This analysis 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.**

Excludes *PLAU

The custom blood disorder panel can be ordered by selecting 2-10 genes from the following curated hematology-focused genes.

ABCG5	*CDC42*	*F9*	*G6PC3*	*HOXA11*	*LAMTOR2*	*PLAU**	*SERPINA1§*	*TBXAS1*	*WAS*
ABCG8	*CSF3R*	*F10*	*GATA1*	*HPS1*	*LMAN1*	*PLG*	*SERPINC1*	*TCIRG1*	*WIPF1*
ACTB	*CXCR4*	*F11*	*GATA2*	*HPS3*	*LYST*	*PRKACG*	*SERPIND1*	*THBD*	
ACTN1	*CYCS*	*F13A1*	*GFI1*	*HPS4*	*MCFD2*	*PROC*	*SERPINE1*	*THPO*	
ADAMTS13	*DIAPH1*	*F13B*	*GFI1B*	*HPS5*	*MECOM*	*PROS1*	*SERPINF2*	*TUBB1*	
ANKRD26	*DTNBP1*	*FERMT3*	*GGCX*	*HPS6*	*MPIG6B*	*RAB27A*	*SLC37A4*	*USB1*	
ANO6	*ELANE*	*FGA*	*GNE*	*HRG*	*MPL*	*RAC2*	*SLFN14*	*VIPAS39*	
AP3B1	*ETV6*	*FGB*	*GP1BA*	*ITGA2B*	*MYH9*	*RASGRP2*	*SRC*	*VKORC1*	
AP3D1	*F2*	*FGG*	*GP1BB*	*ITGB3*	*NBEA*	*RBM8A*	*STIM1*	*VPS13B*	
ARPC1B	*F5*	*FLI1*	*GP6*	*JAGN1*	*NBEAL2*	*RNU4ATAC*	*STXBP2*	*VPS33B*	
BLOC1S3	*F7*	*FLNA*	*GP9*	*KDSR*	*P2RY12*	*RUNX1*	*TAZ*	*VPS45*	
BLOC1S6	*F8*	*FYB1*	*HAX1*	*KNG1*	*PLA2G4A*	*SBDS*	*TBXA2R*	*VWF*	

aHUS/DDD Genetic Panel includes the following genes NOT available for single gene sequencing: *C3, C4BPA, C4BPB, CD46/MCP, CFB, CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, DGKE, LMNA*

* Available via aCGH only

§ Targeted analysis of the Pittsburgh variant ONLY; NGS and aCGH of *SERPINA1* otherwise not available.

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Indications for testing:

Custom Blood Disorder Panel (NGS and/or aCGH), order code 4850:

The Custom Blood Disorder Panel should be considered:

- In patients with a suspected inherited disorder of coagulation factors, platelet function and/or number, thrombosis and/or neutrophils in which the clinical and/or laboratory phenotype is defined enough that the diagnostic possibilities are limited to 10 genes or less
- In patients with clinical and laboratory findings of an inherited disorder of coagulation factors, platelet function and/or number, thrombosis and/or neutrophils, when the patient's history and laboratory phenotype suggest multiple coexisting disorders
- In patients in whom a family history of an inherited hematologic disorder is reported but the specific gene or disorder is unknown, and an affected relative is unavailable for confirmation of diagnosis

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 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, analysis by aCGH for the involved gene may be required. Please call our laboratory to discuss prior to sending sample.

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 hemostatic disorders. Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, such as risk for syndromic manifestation, predisposition or malignancy and/or may carry individual or familial implications such 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 2-10 genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, 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: 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 Custom Blood Disorder Panel (NGS and aCGH) is highest in patients with a clearly defined and specific clinical and laboratory phenotype for the genes selected.

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 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: 4850

CPT codes: Visit Versiti.org/test-catalog for full list of recommended CPT codes for each gene in the panel.

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)

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

Additional references for inherited coagulation, platelet, neutrophil disorders and thrombosis can be found under their specific panel test descriptions

Variant interpretation references

1. 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
2. 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
3. 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
4. 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.

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