Inherited Thrombocytopenia Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 42 genes known to cause inherited thrombocytopenia. This panel can be ordered as:

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

Inherited thrombocytopenia is a heterogeneous group of disorders characterized by low platelet counts typically less than 150,000/uL, but often can vary with age, sex and ethnic background. Symptoms of thrombocytopenia may include purpura, petechiae, prolonged bleeding from cuts, epistaxis, gum bleeding, excessive bleeding after surgery, hemoptysis, hematuria, and menorrhagia in women. Severe inherited thrombocytopenias can present in the newborn period or early childhood, while mild thrombocytopenia may remain undiagnosed until incidental detection on routine blood testing in adulthood. Some inherited types of thrombocytopenia have only hematologic manifestations, such as differences in platelet size and granularity or distinctive granulocyte inclusions, while other syndromic types present with additional non-hematologic manifestations. Certain types of inherited thrombocytopenia cause predisposition to myeloid and lymphoid malignancies.

Misdiagnosis of inherited thrombocytopenia as autoimmune thrombocytopenia (ITP) can result in inappropriate therapies and inadequate surveillance for additional medical complications, underscoring the importance of accurate diagnosis. Advances in genetic testing through next-generation sequencing allow for identification of underlying genetic defects and for distinguishing inherited cases from immune thrombocytopenia. Accurate diagnosis provides information about the 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 genes known to be associated syndromic or non-syndromic thrombocytopenia may be inherited in an autosomal recessive, autosomal dominant or X-linked recessive manner. More common and rare types of inherited thrombocytopenia 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. While thrombocytopenia may be the presenting symptom in type 2B von Willebrand disease (VWD) and congenital thrombotic thrombocytopenic purpura (TTP), these disorders are usually suspected based on specific plasma studies and genetic testing of VWF and ADAMTS13 for these conditions is offered by our laboratory separately (individually or as part of other genetic panels).

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Inherited platelet disorders associated with platelet dysfunction are included in the Platelet Function Disorder Panel. For broader evaluation of platelet problems that may include low platelet counts and/or platelet dysfunction, all genes on the Inherited Thrombocytopenia Panel and Platelet Function Disorder Panel can be analyzed together by ordering the Comprehensive Platelet Disorder Panel.

Refer to the table for further information about each gene in the Inherited Thrombocytopenia Panel, including the clinical phenotype, platelet size and inheritance pattern.

Inherited Thrombocytopenia Panel: gene, clinical phenotype, platelet size and inheritance pattern

Gene	Clinical Phenotype	Platelet Size	Inheritance
ABCG5	**Sitosterolemia with macrothrombocytopenia:** xanthomas, premature atherosclerosis, arthritis, hepatic dysfunction and hematologic abnormalities including stomatocytosis leading to hemolytic anemia and macrothrombocytopenia with mild to moderate bleeding and large platelets surrounded by vacuoles on peripheral smear, as well as splenomegaly	Large	Autosomal Recessive
ABCG8	**Sitosterolemia with macrothrombocytopenia:** xanthomas, premature atherosclerosis, arthritis, hepatic dysfunction and hematologic abnormalities including stomatocytosis leading to hemolytic anemia and macrothrombocytopenia with mild to moderate bleeding and large platelets surrounded by vacuoles on peripheral smear, as well as splenomegaly	Large	Autosomal Recessive
ACTB	***ACTB*-related thrombocytopenia:** mild developmental disability, non-specific minor facial abnormalities, microcephaly and thrombocytopenia with platelet anisocytosis	Variable (normal/large)	Autosomal Dominant
ACTN1	***ACTN1*-related thrombocytopenia** (platelet-type bleeding disorder 15): mild macrothrombocytopenia with minimal or absent bleeding tendency	Large	Autosomal Dominant
ANKRD26	***ANKRD26*-related thrombocytopenia** (thrombocytopenia 2): thrombocytopenia with normal platelet size, mild/absent bleeding and an increased predisposition to hematologic myeloid malignancies	Normal	Autosomal Dominant
ARPC1B	***ARPC1B*-related thrombocytopenia:** microthrombocytopenia, decreased platelet dense granules, allergic and inflammatory disease	Small	Autosomal Recessive
CDC42	***CDC42*-related thrombocytopenia** (Takenouchi-Kosaki syndrome): macrothrombocytopenia, variable intellectual disability, distinct facial features, lymphedema, camptodactyly, and variable involvement of other organ systems	Large	Autosomal Dominant
CYCS	***CYCS*-related thrombocytopenia** (thrombocytopenia 4): non-syndromic thrombocytopenia with normal platelet size	Normal	Autosomal Dominant
DIAPH1	***DIAPH1*-related thrombocytopenia:** macrothrombocytopenia and sensorineural hearing loss	Large	Autosomal Dominant
ETV6	***ETV6*-related thrombocytopenia** (thrombocytopenia 5), characterized by thrombocytopenia with normal platelet size, red cell macrocytosis, mild to moderate bleeding and predisposition to both myeloid and lymphoid malignancies	Normal	Autosomal Dominant
FLI1	***FLI1*-related thrombocytopenia** (platelet-type bleeding disorder-21): macrothrombocytopenia with moderate bleeding from platelet dysfunction due to alpha granule deficiency (large/fused platelet alpha granules on platelet electron microscopy), with or without delta granule deficiency	Large	Autosomal Dominant
FLNA	***FLNA*-related thrombocytopenia:** macrothrombocytopenia with or without associated periventricular heterotopia.	Large	X-linked
FYB1	***FYB1*-related thrombocytopenia** (thrombocytopenia 3): non-syndromic microthrombocytopenia and platelet dysfunction leading to increased bleeding	Small	Autosomal Recessive
GATA1	***GATA1*-related X-linked cytopenia:** characterized by macrothrombocytopenia and/or anemia with moderate bleeding due to platelet alpha granule deficiency	Large	X-linked
GFI1B	***GFI1B*-related thrombocytopenia** (platelet-type bleeding disorder-17): macrothrombocytopenia with platelet alpha granule deficiency leading to variable bleeding tendency, red cell anisopoikilocytosis, increased numbers of dysplastic megakaryocytes and increased platelet CD34 expression	Large	Autosomal Dominant
GNE	***GNE*-related thrombocytopenia:** macrothrombocytopenia with mild to moderate bleeding with or without myopathy	Large	Autosomal Recessive
GP1BA	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin	Large	Autosomal Recessive
	***GP1BA*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Large	Autosomal Dominant
	Platelet type von Willebrand disease: thrombocytopenia with mild bleeding due to loss of VWF high molecular weight multimers from increased binding of platelets and VWF	Large	Autosomal Dominant

Inherited Thrombocytopenia Panel: gene, clinical phenotype, platelet size and inheritance pattern			
GP1BB	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin.	Large	Autosomal Recessive
	***GP1BB*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Large	Autosomal Dominant
GP9	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin.	Large	Autosomal Recessive
	***GP9*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Large	Autosomal Dominant
HOXA11	**Radioulnar synostosis with amegakaryocytic thrombocytopenia** (RUSAT1): thrombocytopenia with normal platelet size and radial abnormalities	Normal	Autosomal Dominant
ITGA2B	***ITGA2B*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Large	Autosomal Dominant
ITGB3	***ITGB3*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Large	Autosomal Dominant
KDSR	***KDSR*-related thrombocytopenia** (Erythrokeratodermia variabilis et progressiva 4): thrombocytopenia with normal platelet size and platelet dysfunction with or without skin hyperkeratosis and ichthyosis	Normal	Autosomal Recessive
MECOM	***MECOM*-associated syndrome** (Radioulnar synostosis with amegakaryocytic thrombocytopenia 2): bone marrow failure with hypomegakaryocytic thrombocytopenia with normal platelet size, radioulnar synostosis, clinodactyly, cardiac and renal malformations, B-cell deficiency and hearing loss	Normal	Autosomal Dominant
MYH9	***MYH9*-related disorders** (MYH9-RD) characterized by macrothrombocytopenia with or without extra hematologic manifestations including renal dysfunction, hearing loss, cataracts and liver enzyme elevation.	Large	Autosomal Dominant
MPIG6B	***MPIG6B*-related thrombocytopenia:** macrothrombocytopenia with focal myelofibrosis	Large	Autosomal Recessive
MPL	**Congenital amegakaryocytic thrombocytopenia** (CAMT): thrombocytopenia with normal platelet size and progression to bone marrow failure	Normal	Autosomal Recessive
NBEAL2	**Gray platelet syndrome (GPS):** macrothrombocytopenia with mild to moderate bleeding due to alpha granule deficiency, splenomegaly and bone marrow fibrosis	Large	Autosomal Recessive
PRKACG	***PRKACG*-related thrombocytopenia** (platelet-type bleeding disorder 19), characterized by severe macrothrombocytopenia with associated platelet dysfunction leading to moderate to severe bleeding	Large	Autosomal Recessive
RBM8A	**Thrombocytopenia absence radius (TAR) syndrome:** bilateral absence of the radii and severe thrombocytopenia with normal platelet size that is usually transient	Normal	Autosomal Recessive
RNU4ATAC	**Roifman syndrome:** growth retardation, distinctive facial features, microcephaly, cognitive delay, retinal dystrophy, spondyloepiphyseal dysplasia, hypogammaglobulinemia and in some cases thrombocytopenia	Normal	Autosomal Recessive
RUNX1	**Familial platelet disorder with predisposition to myeloid leukemia** (FPD/AML): mild to moderate thrombocytopenia with normal platelet size, platelet delta storage pool disorder and a predisposition to development of myeloid malignancies	Normal	Autosomal Dominant
SLFN14	***SLFN14*-related thrombocytopenia** (platelet-type bleeding disorder 20): mild to moderate macrothrombocytopenia with associated platelet dysfunction from dense granule deficiency leading to variable bleeding	Variable (Normal/Large)	Autosomal Dominant
SRC	***SRC*-related thrombocytopenia** (thrombocytopenia 6): thrombocytopenia and bleeding with associated myelofibrosis and bone pathology	Variable (Normal/Large)	Autosomal Dominant
STIM1	***STIM1*-related thrombocytopenia** (Tubular aggregate myopathy and Stormorken syndrome): variable muscle weakness, miosis, thrombocytopenia with normal platelet size, hyposplenism, ichthyosis, dyslexia and short stature. Electron dense platelet inclusions and target-like organelles are characteristic	Normal	Autosomal Dominant
STXBP2	**Familial hemophagocytic lymphohistiocytosis type 5** (FLH5): prolonged fever, cytopenias and hepatosplenomegaly due to proliferation and infiltration of hyperactivated macrophages and T-lymphocytes	Normal	Autosomal Recessive

Inherited Thrombocytopenia Panel: gene, clinical phenotype, platelet size and inheritance pattern			
THPO	***THPO*-related thrombocytopenia:** characterized by severe thrombocytopenia with normal platelet size progressing to bone marrow failure	Normal	Autosomal Recessive
TUBB1	***TUBB1*-related thrombocytopenia:** mild macrothrombocytopenia and minimal/ absent bleeding	Large	Autosomal Dominant
VIPAS39	**Arthrogryposis, renal dysfunction, and cholestasis syndrome type 2** (ARCS2): macrothrombocytopenia with platelet dysfunction from alpha granule deficiency with associated arthrogryposis, renal dysfunction, and cholestasis	Large	Autosomal Recessive
VPS33B	**Arthrogryposis, renal dysfunction, and cholestasis syndrome type 1** (ARCS1): macrothrombocytopenia with platelet dysfunction from alpha granule deficiency with associated arthrogryposis, renal dysfunction, and cholestasis	Large	Autosomal Recessive
WAS	***WAS*-related disorders:** spectrum of disorders including Wiskott-Aldrich syndrome characterized by microthrombocytopenia, eczema and recurrent infections, X-linked thrombocytopenia and X-linked neutropenia	Small	X-linked
WIPF1	**Wiskott-Aldrich syndrome type 2** (WAS2): recurrent infections, eczema, thrombocytopenia with normal platelet size, defective T cell proliferation and impaired natural killer cell function	Normal	Autosomal Recessive

Indications for testing:

Inherited Thrombocytopenia Panel (NGS and/or aCGH), order code 4840:

The Inherited Thrombocytopenia Panel should be considered:

- In patients with lifelong thrombocytopenia suspected to be inherited, with or without a family history of thrombocytopenia
- In patients with thrombocytopenia that is presumed to be immune in origin but does not respond to immunosuppression

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 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 42 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 comprehensive genetic testing (NGS and aCGH) of the 42 genes in this panel is highest in patients with lifelong thrombocytopenia who have not been known to have a normal platelet count, especially when they have a positive family history of thrombocytopenia or consanguinity.

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: 4840

For suggested CPT codes, visit the 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

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Inherited platelet disorder references

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Variant interpretation references

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