Inherited Thrombocytopenia Panel

**BloodCenter of Wisconsin** offers a specifically designed Inherited Thrombocytopenia Panel (test code 4840) optimized for detection of germline variants in 21 genes known to cause inherited thrombocytopenia.

Inherited thrombocytopenia is a heterogeneous group of disorders characterized by low platelet counts typically less than 150,000/μL, but often can vary with age, gender 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, 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 or distinctive granulocyte inclusions, while other syndromic types present with additional non-hematologic manifestations. Certain types of inherited thrombocytopenia cause predisposition to acute myelogenous leukemia or myelodysplastic syndromes.

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 several different genes known to cause 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, including MYH9-related disorders, Bernard-Soulier syndrome, congenital amegakaryocytic thrombocytopenia, familial platelet disorder with predisposition to acute myelogenous leukemia, ANKRD26-related thrombocytopenia, WAS-related thrombocytopenia, gray platelet syndrome and others. Additional genes in this panel are associated with syndromes that have thrombocytopenia as a common finding among other non-hematologic features.

This 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 variants (CNV), are a known cause of genetic disorders, but can escape detection by next-generation sequence analysis. Further testing with the BloodCenter of Wisconsin custom designed, high density gene-focused array, aCGH Deletion/Duplication Analysis, allows for the possible detection of large deletions and duplications within a single exon of a given gene, encompassing one or more exons, or affecting an entire gene. This testing may be warranted when results of sequence analysis do not fully explain a clinical phenotype, or when a suspected disorder is known to be caused by deletions or duplications. Please refer to the aCGH Deletion/Duplication Analysis test description for more information about specific genes included in this array.

Inherited platelet disorders associated with platelet dysfunction are included in the Platelet Function Disorder Panel. For broader evaluation of unspecified platelet problems, both the Inherited Thrombocytopenia Panel and Platelet Function Disorder Panel can be ordered together as part of the Comprehensive Platelet Disorder Panel.

Refer to the table inside for further information about each gene in the Inherited Thrombocytopenia Panel, including the clinical phenotype, OMIM numbers and inheritance pattern.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Clinical Phenotype</th>
<th>Phenotype/Gene OMIM number</th>
<th>Inheritance</th>
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</thead>
<tbody>
<tr>
<td><strong>ACTN1</strong></td>
<td>Bleeding disorder, platelet-type 15 (BDPT15): macrothrombocytopenia with mild or absent bleeding tendency.</td>
<td>615193/102575</td>
<td>Autosomal Dominant</td>
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<td><strong>ANKRD26</strong></td>
<td>Thrombocytopenia-2 (THC2): mild bleeding tendency with normal platelet function and morphology with increased predisposition to hematologic myeloid malignancies.</td>
<td>188000/610855</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td><strong>CYC5</strong></td>
<td>Thrombocytopenia-4 (THC4): bleeding tendency may be mild or absent with normal platelet size and morphology.</td>
<td>612004/123970</td>
<td>Autosomal Dominant</td>
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<td><strong>ETV6</strong></td>
<td>Thrombocytopenia-5 (THC5): onset is typically in early childhood with an increased susceptibility to develop various hematologic and solid malignancies throughout life.</td>
<td>616216/600618</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td><strong>GATA1</strong></td>
<td>Inherited phenotype: thrombocytopenia and/or anemia ranging from mild to severe; may be associated with platelet dysfunction, mild β-thalassemia, neutropenia, and congenital erythropoietic porphyria (CEP). Thrombocytopenia present in infancy and anemia may range from mild to severe hydrops fetalis. Carrier females may have mild to moderate symptoms. X-linked dyserthropoietic anemia and thrombocytopenia (XLTDA): variable severity of thrombocytopenia and dyserthropoietic anemia may be present. Thrombocytopenia with beta-thalassemia, X-linked (XLTT): variable thrombocytopenia, splenomegaly, and unbalanced hemoglobin chain synthesis resembling that of beta-thalassemia minor.</td>
<td>300367/305371, 314050/305371</td>
<td>X-linked Recessive, Autosomal Dominant</td>
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<tr>
<td><strong>GP1BA</strong></td>
<td>Bernard-Soulier syndrome (BSS): mild to severe bleeding disorder due to absence or dysfunction of the platelet glycoprotein receptor Ib/V/IX complex with mild to moderate thrombocytopenia, unusually large platelets and abnormal platelet function with absent or markedly reduced aggregation response to ristocetin. Caused by homozygous or compound heterozygous pathogenic variants in GP1BA (606672), GP1BB (138720), or GP9 (173515). Bernard-Soulier syndrome (BSSA2): autosomal dominant (mono-allelic) form of BSS with mild thrombocytopenia, variable large platelets, and mild or no bleeding tendency, due to specific heterozygous variants in GP1BA or rarely in GP1BB or GP9. Platelet-type von Willebrand disease (also known as pseudo-von Willebrand disease): thrombocytopenia and mucosal bleeding due to dominant pathogenic variants in GP1BA that cause excessive binding of the GPIb-IX-V complex to von Willebrand factor.</td>
<td>231200/606672, 153670/606672, 177820/606672</td>
<td>Autosomal Recessive, Autosomal Dominant, Autosomal Dominant</td>
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<td><strong>GP1BB</strong></td>
<td></td>
<td>231200/138720, 153670/138720</td>
<td>Autosomal Recessive, Autosomal Dominant</td>
</tr>
<tr>
<td><strong>GP9</strong></td>
<td></td>
<td>231200/173515, 153670/173515</td>
<td>Autosomal Recessive, Autosomal Dominant</td>
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<tr>
<td><strong>HOXA11</strong></td>
<td>Radioulnar synostosis with amegakaryocytic thrombocytopenia 1 (RUSAT1): possible aplastic anemia and proximal fusion of the radius and ulna. Thrombocytopenia symptoms may be present at birth and require bone marrow or umbilical-cord stem-cell transplantation.</td>
<td>605432/142958</td>
<td>Autosomal Dominant</td>
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<tr>
<td><strong>ITGA2B</strong></td>
<td>Glanzmann thrombasthenia: mild to severe bleeding disorder with platelet aggregation abnormalities due to quantitative or qualitative defects of platelet glycoproteins IIb and/or IIIa. caused by homozygous or compound heterozygous recessive pathogenic variants in ITGA2B or ITGB3. Bleeding disorder, platelet-type 16 (BDPLT16): congenital macrothrombocytopenia associated with platelet anisocytosis with mild to or absent symptoms due to specific heterozygous dominant activating mutations in ITGA2B or ITGB3.</td>
<td>273800/607759, 187800/607759</td>
<td>Autosomal Recessive, Autosomal Dominant</td>
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<tr>
<td><strong>ITGB3</strong></td>
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<td>273800/173470, 187800/173470</td>
<td>Autosomal Recessive, Autosomal Dominant</td>
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<td>MPL</td>
<td><strong>Congenital amegakaryocytic thrombocytopenia (CAMT):</strong> type 1-onset in infancy with severe thrombocytopenia and progressing to pancytopenia; type II with transient increases in platelet counts with onset of bone marrow failure at age 3 or later. <strong>MYH9-related disorders:</strong> large platelets and thrombocytopenia at birth with variable later onset of non-hematologic manifestations including progressive sensorineural hearing loss, glomerulonephritis, presenile cataracts and elevation of liver enzymes. <strong>MYH9-related disorders includes previously characterized disorders:</strong> May-Hegglin anomaly: thrombocytopenia, giant platelets, and Dohle body-like inclusions. Epstein syndrome: thrombocytopenia, giant platelets, nephritis, and deafness. Fechtner syndrome: thrombocytopenia, giant platelets, and Dohle body-like inclusions in peripheral blood leukocytes, with nephritis, hearing loss, and eye abnormalities, mostly cataracts. Sebastian syndrome: thrombocytopenia, giant platelets, and leukocyte inclusions composed of highly dispersed filaments and few ribosomes. DNAF17: nonsyndromic progressive hearing loss with onset in childhood or later; hearing loss progresses from high frequency to moderate-severe deafness over time.</td>
<td>604498/159530</td>
<td>Autosomal Recessive</td>
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<tr>
<td>MYH9</td>
<td><strong>MYH9-related disorders:</strong> large platelets and thrombocytopenia at birth with variable later onset of non-hematologic manifestations including progressive sensorineural hearing loss, glomerulonephritis, presenile cataracts and elevation of liver enzymes. <strong>MYH9-related disorders includes previously characterized disorders:</strong> May-Hegglin anomaly: thrombocytopenia, giant platelets, and Dohle body-like inclusions. Epstein syndrome: thrombocytopenia, giant platelets, nephritis, and deafness. Fechtner syndrome: thrombocytopenia, giant platelets, and Dohle body-like inclusions in peripheral blood leukocytes, with nephritis, hearing loss, and eye abnormalities, mostly cataracts. Sebastian syndrome: thrombocytopenia, giant platelets, and leukocyte inclusions composed of highly dispersed filaments and few ribosomes. DNAF17: nonsyndromic progressive hearing loss with onset in childhood or later; hearing loss progresses from high frequency to moderate-severe deafness over time.</td>
<td>160775</td>
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<td>NBEAL2</td>
<td>Gray platelet syndrome (GPS): large platelets that lack α-granules leading to mild to moderate bleeding tendency and moderate thrombocytopenia with increased development of myelofibrosis and splenomegaly.</td>
<td>139090/614169</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>PRKACG</td>
<td>Bleeding disorder, platelet-type 19 (BDPLT19): severe macrothrombocytopenia and bleeding symptoms.</td>
<td>611676/176893</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>RBM8A</td>
<td>Thrombocytopenia-Absent Radius syndrome (TAR): characterized by bilateral absence of the radius with presence of thumbs; thrombocytopenia which may be congenital or develop early in life and decreases with age. Other anomalies of the skeleton (upper and lower limbs, ribs, and vertebrae), heart, and genitourinary system (renal anomalies and agenesis of uterus, cervix, and upper part of the vagina) can occur.</td>
<td>274000/605313</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Familial platelet disorder with associated myeloid malignancy (FPDMM): characterized by mild to moderate thrombocytopenia, qualitative platelet defects and a predisposition to development of myeloid malignancies.</td>
<td>601399/151385</td>
<td>Autosomal Dominant</td>
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<tr>
<td>STXB2</td>
<td>Familial hemophagocytic lymphohistiocytosis (FHL5): immune dysregulation with hypercytokinemia, defective function of natural killer cells, and macrophages that infiltrate multiple organs; characterized by variable age of onset and severity with fever, chronic diarrhea, hepatosplenomegaly, pancytopenia, coagulation abnormalities and CNS problems.</td>
<td>613101/601717</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>TUBB1</td>
<td>Congenital macrothrombocytopenia.</td>
<td>613112/612901</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td>WAS</td>
<td><strong>WAS-related disorders comprise a spectrum of disorders, not distinct entities, as clinical manifestations can vary, even within the same family.</strong> Wiskott-Aldrich syndrome (WAS): profound thrombocytopenia, small platelet size, eczema and recurrent infections; increased risk for autoimmune disorders and lymphoma; absent or decreased intracellular WAS protein (WASP) detection in hematopoietic cells by flow cytometry or western blotting. X-linked neutropenia: congenital neutropenia; variable severity, infectious history, myelodysplasia and increased risk for MDS and AML. Normal WAS protein (WASP) expression by flow cytometry or western blotting. X-linked thrombocytopenia: thrombocytopenia, possibly intermittent, with small platelet volume; variable severity of bleeding, autoimmune disease and malignancies, variable WASP expression by flow cytometry or western blotting.</td>
<td>301000/300392</td>
<td>X-linked Recessive</td>
</tr>
</tbody>
</table>
Indications for testing

**Inherited Thrombocytopenia Panel:**
- Clarification and/or confirmation of diagnosis in a patient with clinical findings of thrombocytopenia or an associated genetic syndrome when patient’s history suggests the possibility of multiple inherited thrombocytopenia disorders
- Identification of carriers with family history of an unspecified thrombocytopenia disorder to provide accurate reproductive risk assessment and genetic counseling

**Single gene sequencing or custom gene panel:**
- Analysis of genes included in the Inherited Thrombocytopenia Panel may also be ordered as a stand-alone single gene sequencing test or custom panel (2-10 genes) as dictated by the patient’s clinical and laboratory phenotype

**Targeted familial variant analysis:**
- 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 (**test code: 4970**)

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 next generation sequencing assay analyzes 21 genes, spanning the full coding regions plus a minimum 30bp of non-coding DNA including intron-exon junctions, and to approximately 200bp upstream of ANKRD26 coding region (3’ 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 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.

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. To order the Analysis of copy number variation at the exon or gene level, please refer to the aCGH Deletion/Duplication Analysis test, if available, or contact Client Services before placing your order.

Reporting of results

While this assay is designed to detect germline genetic variants associated with thrombocytopenia, 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](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](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 (2x10⁶ 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 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
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
CPT codes: 81404, 81406, 81479
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, BloodCenter Client Services: (414) 937-6396 or 800-245-3117, Option 1, or LabInfo@bcw.edu.

References