Versiti offers DNA sequencing of VWF exon 28 (order code 1284) for detection of germline variants associated with type 2B or type 2M von Willebrand disease (VWD).

Von Willebrand disease (VWD) is a common inherited bleeding disorder with a reported incidence ranging from 0.01% to 1%. VWD is classified into subtypes of quantitative (types 1 and 3) and qualitative (type 2) defects, caused by pathogenic variants in VWF. Type 1 VWD, characterized by partial deficiency of von Willebrand factor (VWF), is inherited as an autosomal dominant disorder with variable penetrance. Type 1C VWD is a variant of autosomal dominant type 1 VWD characterized by decreased survival (increased clearance). The defects observed in type 2 VWD include defects in formation of multimers (types 2A), increased susceptibility of VWF to degradation by proteases (type 2A), defects in platelet binding with intact multimers (type 2M), enhanced interaction of VWF with platelets (types 2B, and GP1BA-related platelet-type VWD), decreased interaction with factor VIII (type 2N), and decreased interaction with collagen (a rare form of type 2M). Types 2B, type 2M, and the majority of type 2A cases have an autosomal dominant inheritance pattern, while type 2N is an autosomal recessive disorder. Type 3 VWD is characterized by severe quantitative deficiency with a virtual absence of VWF and is inherited as an autosomal recessive disorder. Platelet-type VWD is caused by pathogenic gain-of-function variants in platelet glycoprotein 1b encoded by the GP1BA gene, and will not be detected by VWF analysis.

Genetic testing of VWF exon 28 offers the most clinical utility for the diagnosis of types 2B and 2M VWD, in confirming the VWD type to facilitate selection of appropriate medical therapy, and accurately determine recurrence risks. Laboratory findings suggestive of type 2M VWD include evidence of abnormal interaction of platelets with VWF (as suggested by a low ratio of VWF ristocetin cofactor or GP2B10a activity to VWF antigen level) in the setting of a normal VWF multimer distribution. Alternatively, the additional laboratory findings suggestive of type 2B VWD may include absence of high molecular weight multimers, abnormal low-dose ristocetin-induced platelet aggregation result, thrombocytopenia or an abnormal platelet-VWF binding assay.

If other types of VWD are clinically suspected, alternative genetic testing strategies may be considered. VWF sequence analysis (order code 1395) is recommended for types 1 and 3 VWD as well as for other types, including 1C and 2A; although these latter types have been associated with variants in certain exons of VWF, evolving knowledge has revealed that pathogenic variants causing these phenotypes occur across the gene, and therefore testing of limited exons is no longer a recommended approach. Variants causing type 2N VWD are located in specific factor VIII-binding functional domains in exons 17-21 and 24-27; for patients with low factor VIII and suspicion of type 2N VWD or patients with functional binding assays consistent with this diagnosis, VWD Type 2N Sequence Analysis (order code 1288) is available. In families with a specific VWD diagnosis in whom prior testing has identified a pathogenic variant that fully explains the phenotype, Targeted Familial Variant Analysis (order code 4970) is appropriate for evaluation of at-risk relatives or for prenatal diagnosis.

**Indications for testing:**

- Diagnosis of von Willebrand disease, particularly when types 2B and 2M are suspected
- Evaluation of abnormal VWF activity to antigen ratio, in the context of normal multimers or enhanced VWF-platelet binding
- Facilitate selection of appropriate medical therapy
- Identification of pathogenic variant(s) to allow for familial testing or prenatal diagnosis

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services (414) 937-6396 or 800-245-3117 (ext. 6250) to be directed to genetic counselors or clinical support team.
Test method:
This next-generation sequencing assay analyzes the coding region of VWF exon 28 plus a minimum of 30bp of non-coding DNA, including intron-exon junctions, and is compared to the build GRCh37:p13 reference sequence (VWF, NM_000552.3). These 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 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.

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. This assay is not designed to detect large deletions or duplications (>20 bp). Additionally, this assay will not detect any variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology. To order the analysis of copy number variants at the exon or gene level, please refer to the Comparative Genomic Hybridization (aCGH) Deletion/Duplication Analysis (order code 4800).

The clinical sensitivity for detecting pathogenic variants in individuals with a clinical diagnosis of types 2B and 2M VWD is approximately 99%. Although pathogenic variants in exon 28 have been associated with a majority of the cases of type 2A VWD, pathogenic variants associated with this type have also been identified in other areas of the VWF gene, such that testing of VWF exon 28 alone will not detect all pathogenic variants. In order to provide the most complete analysis, we recommend testing all coding exons of the VWF gene via Sequence Analysis (order code 6128). Variants in non-coding DNA, including intron-exon junctions, can also be detected using Sequence Analysis of GP1BA (order code 1395) rather than testing of limited exons is suggested. Sequence variants are classified and reported in accordance with Human Genome Variation Society (HGVS) nomenclature (http://www.hgvs.org).

Specimen requirements:

Parental/Patient: 3-5 mL Whole Blood (EDTA tube, lavender top), 2-5 mL Bone Marrow (EDTA tube, lavender top), 3-4 Buccal Swabs, or ≥ 50ng/uL of High Quality DNA.

Fetal: 7-15 mL Amniotic fluid, 5-10 mg Chorionic villi; back up culture of amniocytes or chronic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chronic 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, place into a sturdy cardboard box, and tape securely. Shipping requirements:

Send to: Versiti Client Services Diagnostic Laboratories 638 N. 18th Street Milwaukee, WI 53233 800-245-3117 ext. 6250

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This next-generation sequencing assay analyzes the coding region of VWF exon 28 plus a minimum of 30bp of non-coding DNA, including intron-exon junctions, and is compared to the build GRCh37.p13 reference sequence (VWF_NM_000552.3). These 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 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.

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. This assay is not designed to detect large deletions or duplications (>20 bp). Additionally, this assay will not detect any variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology. To order the analysis of copy number variants at the exon or gene level, please refer to array Comparative Genomic Hybridization (aCGH) Deletion/Duplication Analysis (order code 4800).

The clinical sensitivity for detecting pathogenic variants in individuals with a clinical diagnosis of types 2B and 2M VWD is approximately 99%. Although pathogenic variants in exon 28 have been associated with a majority of the cases of type 2A VWD, pathogenic variants associated with this type have also been identified in other areas of the VWF gene, such that testing of all exons via VWF Sequence Analysis (order code 1395) rather than testing of limited exons is suggested. Only a minority of pathogenic variants causing type 1 and 3 VWD are identified in exon 28. Exon 28 Sequence Analysis will distinguish type 2B VWD from platelet-type VWD, but sequence analysis of GP1BA is required to genetically confirm a diagnosis of platelet-type VWD.

Testing of all VWF exons is suggested because of the possibility of variants of uncertain significance. Some variants may also be detected. Results are classified and reported in accordance with ACMG next-generation sequencing standards. Variants interpreted to be pathogenic, likely pathogenic, and of uncertain significance will be reported as will the benign variant VWF c.4141G>C (p.D1472H); other 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://www.hgvs.org).

Specimen requirements:

**Parental/Patient:** 3-5 mL Whole Blood (EDTA tube, lavender top), 2-5 mL Bone Marrow (EDTA tube, lavender top), 3-4 Buccal Swabs, or ≥ 50ng/uL of High Quality DNA.

**Fetal:** 7.15 mL Amniotic fluid, 5-10 mg Chorionic villi; back up culture of amnioocytes or chronic villi is highly recommended. Cultured: Two T25 flasks cultured amnioocytes or chronic 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. Sample collection, processing and shipping requirements:

- **Send to:** Versiti Client Services Diagnostic Laboratories 638 N. 18th Street Milwaukee, WI 53233 800-245-3117 ext. 6250, or LabINFO@versiti.org

**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.

**References**


