VWF Sequence Analysis (all exons)

Versiti offers *VWF* sequence analysis of exons 1-52 (order code 1395) for detection of germline variants associated with von Willebrand disease (VWD). *VWF* analysis is appropriate for all types of 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 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); in addition to VWF antigen and proportionately low VWF ristocetin cofactor activity, patients with this type might have an elevated VWF propeptide to VWF antigen ratio and occasionally a persistence of larger than normal size multimers.

The defects observed in type 2 VWD include defects in formation of multimers (type 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, 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. Complex and sometimes severe phenotypes resulting from compound heterozygosity for qualitative and quantitative defects, such as 2N/1 and 2A/1, have been observed and can be misdiagnosed based on clinical and laboratory phenotype alone.

Genetic testing of the *VWF* gene offers clinical utility in the diagnosis of VWD, in confirming the VWD type to aid in management, and accurately determining recurrence risks. Types 1 and 3 VWD variants have been identified throughout the *VWF* gene. Although other types, including 1C and 2A, 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. All type 2B variants and the vast majority of type 2M variants are found in exon 28; for patients with suspected 2B or 2M VWD by specific plasma assays, *VWF* Exon 28 Sequence Analysis (order code 1284) can be considered. Variants causing type 2N 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.

Among patients without pathogenic variants detected by sequence analysis, large deletions in *VWF* may be detected by array Comparative Genomic Hybridization (aCGH) in approximately 30% of type 1 VWD (VWF antigen less than 30 IU/dL) and 50% of type 1C, as well as 40% of patients with type 3 in whom sequencing did not identify two pathogenic variants (Christopherson et al, 2016). Large deletions or duplications have been reported rarely in type 2 VWD (Goodeve et al, 2009; updated 2017). aCGH for *VWF* (order code 4800) is available for analysis of large deletions and duplications.

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Indications for testing:

- Diagnosis of von Willebrand disease
- Confirmation of VWD type, including distinction between severe quantitative defects (severe type 1 and type 3) or other clinically similar phenotypes
- 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 to be directed to our genetic counselors and clinical support team.

Test method:

This next-generation sequencing assay analyzes the complete coding region of *VWF* plus a minimum of 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence (*VWF*, NM_000552.3). *VWF* analysis also includes the 5' UTR. 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, clinical hematologists, 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).

Clinical Sensitivity

The clinical sensitivity for detecting pathogenic variants in individuals with a clinical diagnosis of type 1 von Willebrand disease is approximately 65%; however, in patients with type 1 VWD with VWF antigen levels of less than 30 IU/dL, pathogenic variants are identified in over 80% of cases (Sharma and Flood, 2017). The clinical sensitivity for detecting type 2 pathogenic variants is greater than 99%; for type 3 VWD, pathogenic variants are detected in more than 90% of cases (Veyradier, 2016). *VWF* Sequence Analysis is generally not recommended in individuals with "low VWF" as decreased VWF values may be attributable to both abnormalities in the *VWF* gene and other genes, including ABO; thus the clinical sensitivity for detecting pathogenic variant in this subgroup of patients would be expected to be low.

Reporting of results:

This assay targets the gene regions listed above but may nonetheless yield genetic findings unrelated to the clinical presentation for which the patient is undergoing testing. Additionally, while this assay is designed to detect germline genetic variants, variants with reproductive implications (such as carrier status), 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.4414G>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/Pediatric: 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 ($2x10^6$ 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.

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; place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines.

Label with the following address:

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

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:

Order code: 1395

CPT code: 81408

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 (ext. 6250), or Labinfo@versiti.org

References

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