MAYO CLINIC LABORATORIES

Test Definition: GNVWD

von Willebrand Disease, VWF and GP1BA Genes, Next-Generation Sequencing, Varies

Overview

Useful For

Evaluating von Willebrand disease and platelet-type von Willebrand disease in patients with a personal or family history suggestive of von Willebrand disease

Confirming von Willebrand disease or platelet-type von Willebrand disease diagnoses with the identification of a known or suspected disease-causing alteration in the *VWF* or *GP1BA* genes, respectively

Determining the disease-causing alterations within the *VWF* or *GP1BA* genes to delineate the underlying molecular defect in a patient with a laboratory diagnosis of von Willebrand disease or platelet-type von Willebrand disease, respectively

Subtyping von Willebrand disease as type 1 (most common), type 2 variants (less common), or type 3 (rare), as well as distinguishing von Willebrand disease from platelet-type von Willebrand disease

Identifying the causative alteration for genetic counseling purposes

Prognosis and risk assessment based on genotype-phenotype correlations

Carrier testing for close family members of an individual with a von Willebrand disease or platelet-type von Willebrand disease diagnosis

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *VWF* and *GP1BA* genes associated with von Willebrand disease and platelet-type von Willebrand disease. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for von Willebrand disease and platelet-type von Willebrand disease.

Testing Algorithm

The laboratory workup for von Willebrand disease is complex and requires initial coagulation screening (including a complete blood cell count, platelet count, partial thromboplastin time, prothrombin time, and fibrinogen or thrombin time) should be performed prior to any consideration of genetic testing.

Genetic testing for a von Willebrand disease is indicated if:
-Coagulation tests indicate a deficiency or functional abnormality in von Willebrand factor
-There is a clinical suspicion for von Willebrand disease due to family history or atypical clinical presentation
-Acquired causes of deficiencies associated with von Willebrand disease have been excluded (eg, certain myeloproliferative disorders, plasma cell dyscrasias including monoclonal gammopathy of undetermined significance, high-shear stress-related cardiovascular conditions, and autoimmune disorders).

A clinical and laboratory testing algorithm for von Willebrand disease has been developed by the National Heart, Lung, and Blood Institute of the National Institutes of Health that is freely available at www.nhlbi.nih.gov/health-pro/guidelines/current/von-willebrand-guidelines. If von Willebrand disease is a concern, sets of clinical guidelines on testing for von Willebrand disease and platelet-type von Willebrand disease are also freely available.(1,2)

Prenatal specimens:
Prenatal genetic testing is not routinely performed without the prior identification of familial alterations. Requests for this prenatal testing without a known familial alteration are performed at the discretion of the Molecular Hematopathology Laboratory Director.

If an amniotic fluid specimen is received, an amniotic fluid culture will be performed at an additional charge.
If a chorionic villus specimen or cultured chorionic villi are received, a fibroblast culture will be performed at an additional charge.
For any prenatal specimen that is received, maternal cell contamination testing will be performed at an additional charge.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- von Willebrand Disease Patient Information

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type
Varies

Ordering Guidance
A systematic diagnosis through conventional coagulation testing is recommended before considering genetic testing for any suspected bleeding disorder. Special coagulation testing for evaluating patients suspected of having von Willebrand disease is available; order AVWPR / von Willebrand Disease Profile, Plasma.

If testing for hereditary bleeding disorders using a larger panel is desired, both a 6-gene and a 25-gene bleeding panel are available. For more information see GNBLF / Bleeding Disorders, Focused Gene Panel, Next-Generation Sequencing, Varies or GNBLC / Bleeding Disorders, Comprehensive Gene Panel, Next-Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for *VWF* and *GP1BA* genes. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements
All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the prenatal specimen and the maternal specimen as separate order numbers.

Necessary Information
von Willebrand Disease Patient Information is required. Testing may proceed without the patient information; however, the information aids in providing a more thorough interpretation. Ordering healthcare professionals are strongly encouraged to fill out the form and send with the specimen.

Specimen Required
A previous bone marrow transplant from an allogenic donor will interfere with whole blood testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.
Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Prenatal Specimens
Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

Specimen Type: Amniotic fluid
Container/Tube: Amniotic fluid container
Specimen Volume: 20 mL
Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient <24 hours
Additional information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks are required to culture amniotic fluid before genetic testing can occur.
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi
Container/Tube: 15-mL tube containing 15 mL of transport media
Specimen Volume: 20 mg
Specimen Stability Information: Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 2 to 3 weeks are required to culture amniotic fluid before genetic testing can occur.
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Confluent cultured cells
Container/Tube: T-25 flask
Specimen Volume: 2 Full flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing.

3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Forms

1. **von Willebrand Disease Patient Information (T825) is required**.
2. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
3. If not ordering electronically, complete, print, and send an Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

Blood: 1 mL; Amniotic fluid: 10 mL; Other specimen types: see Specimen Required

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

von Willebrand disease (VWD) is the most common inherited bleeding disorder, affecting approximately 1% of the population. VWD is a bleeding diathesis that usually involves mucous membranes and skin sites. It is typically of mild to moderate severity, although life-threatening bleeding in the central nervous system or gastrointestinal tract can occur. The most common presenting symptoms in individuals affected by VWD include epistaxis, menorrhagia, bleeding after dental extraction, postoperative bleeding, ecchymoses, bleeding from minor cuts or abrasions, gingival bleeding, and hemarthrosis.(1) While VWD occurs with equal frequency among men and women, symptoms in women are more obvious because of increased bleeding during menstrual periods, pregnancy, and after childbirth.

von Willebrand disease is a result of defects in the concentration, structure, or function of von Willebrand factor (VWF), leading to decreased factor VIII (FVIII) in circulation and/or impaired platelet adhesion and aggregation at the site of vascular injury. The *VWF* gene encodes for VWF, a protein that protects blood clotting factor VIII from degradation in circulation and promotes platelet adhesion and aggregation at the site of vascular injury. In circulation, VWF assembles into linear strings called multimers, the size of which is biologically important; larger multimers being more reactive than smaller multimers.

Levels of factor VIII, VWF antigen, and VWF activity may vary greatly within each individual over time and also with blood type (normal type "O" individuals may have VWF lower than normal individuals of other blood groups). VWF levels (and factor VIII) can be elevated in liver disease, pregnancy, estrogen therapy, inflammation, and after exercise (acute-phase reactant). VWF levels in hemophilia are normal.

This panel evaluates 2 genes associated with von Willebrand disease and platelet-type von Willebrand disease. Discrimination between these 2 heritable disorders, specifically concerning types 2A and 2B VWD and platelet-type VWD, using genetic analysis can help guide treatment. Genetic testing can also be used to assist in discriminating between type 2N VWD and hemophilia A. Subtyping of VWD using genetic analysis is important for prognosis and in guiding treatment, as well as determining inheritance pattern and risks for family members.(1,2)

The risk for developing bleeding associated with these disorders and subtypes varies. The *VWF* and *GP1BA* genes have established bleeding risk and expert group guidelines.(1-4)

It is recommended that genetic testing be offered to all patients where it may assist in diagnosis and management of von Willebrand disease.(1) Genetic testing is integral to the conclusive diagnosis of platelet-type von Willebrand disease.(5)

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(6) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(6) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with

caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. Laffan MA, Lester W, O'Donnell JS, et al. The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology. Brit J Haematol. 2014;167(4):453-465
2. James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021;5(1):280-300
3. International Society on Thrombosis and Haemostasis: Bleeding Thrombotic and Platelet Disorder TIER1 genes. ISTH; 2018. Updated July 2022. Accessed March 2, 2022. Available at: www.isth.org/page/GinTh_GeneLists
4. Megy K, Downes K, Simeoni I, et al. Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. J Thromb Haemost. 2019;17(8):1253-1260
5. Othman M, Gresele P. Guidance on the diagnosis and management of platelet-type von Willebrand disease: A communication from the Platelet Physiology Subcommittee of the ISTH. J Thromb Haemost. 2020;18(8):1855-1858
6. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee: 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

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the *VWF* and *GP1BA* genes, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp, and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the *VWF* and *GP1BA* genes.

There may be regions of the *VWF* and *GP1BA* genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

The reference transcript for *VWF* is NM_000552.4 and *GP1BA* is NM_000173.7. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

PDF Report

Supplemental

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Amniotic fluid, cultured amniocytes, chorionic villi, cultured chorionic villi: 1 month; Extracted DNA: 3 months

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81408
81479
81479 (if appropriate for government payers)
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)
88235-Amniotic fluid culture (if appropriate)
81265-Maternal cell contamination (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
GNVWD	VWF and GP1BA Genes, Full Gene NGS	105337-0

Result ID	Test Result Name	Result LOINC® Value
619202	Test Description	62364-5

619203	Specimen	31208-2
619204	Source	31208-2
619205	Result Summary	50397-9
619206	Result	82939-0
619207	Interpretation	59465-5
619208	Additional Results	82939-0
619209	Resources	99622-3
619210	Additional Information	48767-8
619211	Method	85069-3
619212	Genes Analyzed	82939-0
619213	Disclaimer	62364-5
619214	Released By	18771-6