MAYO CLINIC LABORATORIES

Test Definition: AVWPR

von Willebrand Disease Profile, Plasma

Overview

Useful For

Detection of deficiency or abnormality of von Willebrand factor (VWF) and related deficiency of factor VIII coagulant activity

Subtyping von Willebrand disease (VWD) as type 1 (most common), type 2 variants (less common), or type 3 (rare)

This test is **not useful for** detection of hemophilia carriers.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
AVWPI	von Willebrand Disease Tech Interp	No	Yes
F8A	Coag Factor VIII Activity Assay, P	Yes	Yes
VWAG	von Willebrand Factor Ag, P	Yes	Yes
VWACT	von Willebrand Factor Activity, P	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
F8IS	Coag Factor VIII Assay Inhib Scrn,P	No	No
AVWPQ	von Willebrand Disease Interp	No	No
VWFMP	von Willebrand Factor Multimer, P	Yes, (order VWFMS)	No
RIST	Ristocetin Cofactor, P	No	No
8BETH	FVIII Bethesda Units, P	No	No

Testing Algorithm

Initial testing includes coagulation factor VIII activity assay, von Willebrand factor antigen (VWF Ag), VWF activity, and interpretation.

If the factor VIII, VWF Ag, VWF activity, and VWF activity:VWF Ag ratio results are normal, then a computer-generated interpretive comment indicating no evidence of von Willebrand disease will be provided.

If the VWF activity result is less than 55% or the VWF activity:VWF Ag ratio is abnormally increased, then the VWF

ristocetin cofactor activity assay will be performed at an additional charge.

If the VWF Ag, VWF activity, or ristocetin cofactor activity result is less than 40% or the VWF activity:VWF Ag/ristocetin activity:VWF Ag ratio is abnormal, then VWF multimer analysis will be performed at an additional charge.

If any test results are abnormal, all results will be reviewed by a coagulation consultant and an interpretation will be provided at an additional charge.

For more information see von Willebrand Disease Profile.

Special Instructions

- Coagulation Guidelines for Specimen Handling and Processing
- von Willebrand Disease Profile
- Coagulation Patient Information
- Coagulation Profile Comparison

Method Name

AVWPI: Technical Interpretation
AVWPQ: Medical Interpretation
F8A, F8A, 8BETH, F8IS: Optical Clot-Based
RIST: Ristocetin-Induced Agglutination
VWAG, VWACT: Latex Immunoassay (LIA)
VWFMP: Agarose Gel Electrophoresis

NY State Available

Yes

Specimen

Specimen Type

Plasma Na Cit

Ordering Guidance

Multiple coagulation profile tests are available. See Coagulation Profile Comparison for testing that is performed with each profile.

Shipping Instructions

Send all 3 aliquots in the same shipping container.

Necessary Information

1. If priority specimen, mark request form, give reason, and request a call-back.
2. Note if patient is currently receiving anticoagulant treatment (eg, heparin, warfarin).

Specimen Required

Specimen Type: Platelet-poor plasma
Patient Preparation:
1. Patient **should not** be receiving anticoagulant treatment (eg, warfarin, heparin). If not possible for medical reasons, note on request.
a. If medically feasible, for 4 to 6 hours before specimen collection, **do not** administer intravenous heparin.
b. If medically feasible, for 10 to 14 days before specimen collection, **do not** administer subcutaneous heparin or warfarin**.**
2. Patient **should not** be receiving fibrinolytic agents (streptokinase, urokinase, tissue plasminogen activator [tPA]).
3. It is recommended that specimens be collected pretransfusion. If patient has been transfused, **a specimen should not be collected for 48 hours.**
Collection Container/Tube: Light-blue top (3.2% sodium citrate)
Submission Container/Tube: 13-mm x 75-mm, 5-mL, plastic vials (polypropylene preferred)
Specimen Volume: 3 mL Platelet-poor plasma in three (3) 13-mm x 75-mm, 5-mL, plastic vials (polypropylene preferred), each containing 1 mL
Collection Instructions:
1. Specimen must be collected prior to factor replacement therapy.
2. For complete instructions, see Coagulation Guidelines for Specimen Handling and Processing.
3. Within 4 hours of collection, centrifuge and transfer all plasma into a plastic vial, and centrifuge plasma again.
4. Aliquot plasma (1-2 mL per aliquot) into 3 separate 13-mm x 75-mm, 5-mL, polypropylene vials , leaving 0.25 mL in the bottom of centrifuged vial.
5. Immediately (within 2 hours of processing) freeze plasma at -20 degrees C or, ideally, -40 degrees C or below.

Forms

1. Coagulation Patient Information (T675)
2. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

Platelet-poor plasma: 2 13-mm x 75-mm, 5-mL, Polypropylene vials , each containing 1 mL platelet-poor plasma

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Plasma Na Cit	Frozen	14 days	

Clinical & Interpretive

Clinical Information

von Willebrand factor (VWF) is synthesized by the endothelial cell and megakaryocyte and is present in these cells, as well as in platelets, subendothelial tissue, and plasma.

von Willebrand factor serves as an adhesive protein important in adhering platelets to subendothelial tissue at the site of vascular injury and for adhering platelets to each other (aggregation). Platelet adhesion and aggregation are essential to form a mechanical hemostatic "plug" and as the focus for interaction of clotting factors and phospholipid required for the formation of the fibrin platelet clot. VWF also stabilizes plasma factor VIII by binding it and protecting it from proteolysis and serves as a carrier protein for that clotting factor.

von Willebrand factor circulates in the blood in two distinct compartments. Plasma VWF mainly reflects VWF synthesis and release from vascular endothelial cells. Platelet VWF (about 10% of the blood VWF) reflects VWF synthesis by bone marrow megakaryocytes with storage primarily in the alpha granules of circulating platelets.

Plasma VWF circulates normally in multimeric forms with molecular weights ranging from 500,000 to as much as 20,000,000. The high-molecular-weight (HMW) forms of VWF are the most effective components for interaction with platelets. This primary activity of plasma VWF is measured in the laboratory with the VWF activity assay, whereas VWF antigen testing measures the amount of VWF protein, and factor VIII coagulant activity indirectly reflects VWF interaction with factor VIII. VWF multimer analysis visualizes the distribution of VWF multimers and is useful as a reflexive test for subtyping von Willebrand disease (VWD).

Levels of factor VIII, VWF antigen, and VWF activity may vary greatly within an individual over time and also with blood type (normal blood 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.

von Willebrand factor antigen measurement assesses the mass of plasma VWF protein but does not reflect VWF functions or platelet VWF. The function of VWF (mediating platelet-platelet or platelet-vessel interaction) is most commonly assessed by measurement of plasma VWF activity.

von Willebrand disease is the most common inherited bleeding disorder, affecting up to 1% of the population. It can also occur as an acquired bleeding disorder. Bleeding symptoms in all types of VWD are primarily mucosal, including epistaxis, menorrhagia, gastrointestinal bleeding, and ease of bruising, but surgical or posttraumatic bleeding can also occur.

Subtypes of inherited VWD are:
Type 1 VWD:
von Willebrand factor plasma levels (antigen and activity) typically are concordantly reduced in type 1 VWD. Because of this reduction, the level of coagulation factor VIII is often secondarily reduced. Type 1 VWD is the most common VWD variation, representing 70% to 80% of clinical VWD. It is typically inherited in autosomal dominance fashion, although recessively inherited VWD also occurs (eg, type 3 VWD). Clinical severity ranges from mild or minimal to a moderately severe bleeding diathesis and tends to correlate most closely with VWF activity. Severe type 1 disease is also called type 3 VWD, but the distinction between the two may sometimes be difficult.

Type 2 VWD:
Type 2 VWD variants represent 20% to 30% of clinical VWD, typically autosomal dominant in inheritance. There are 4

subtypes of type 2 VWD: 2A, 2B, 2M, and 2N. Abnormal plasma HMW VWF function and multimeric structure with decreased or absent HMW multimers are characteristic of types 2A and 2B but are normal in type 2M or 2N.

von Willebrand factor activity is decreased in types 2A, 2B, and 2M and typically is discordantly lower than VWF antigen. Type 2N (Normandy) has substantially decreased factor VIII coagulant activity (usually 5%-30% of mean normal), with normal VWF antigen and activity and normal VWF multimers with clinical manifestation as autosomally inherited mild hemophilia (in contrast to classical X chromosome-linked hemophilia A).

Type 2A is the most common of the 4. Type 2B manifests thrombocytopenia, either persistent or transient, and is distinguished from type 2A by abnormally heightened aggregation response of patient platelets and plasma to low dose ristocetin stimulation. Type 2M typically demonstrates hypofunctional VWF with decreased VWF activity discordantly lower than VWF antigen not due to loss of HMW multimers. One variant of type 2M, Vicenza variant VWD, has ultralarge VWF multimers in plasma.

Type 3 VWD:
von Willebrand factor is absent or markedly decreased in type 3 VWD (VWF antigen and activity either undetectably low or below 5% to 10% of mean normal, with secondary decrease of factor VIII coagulant activity (5%-30%). VWF multimers may be undetectable or, if present, have a normal distribution. Platelet VWF may also be absent.

Acquired VWD:
von Willebrand disease can also occur on an acquired basis by a variety of mechanisms not well understood. Disorders associated with acquired VWD include certain myeloproliferative or lymphoproliferative disorders, plasma cell dyscrasias including monoclonal gammopathy of undetermined significance, autoimmune disorders (eg, rheumatoid arthritis, systemic lupus erythematosus), and a variety of other diseases. In some cases, no associated disorder is detected. Laboratory testing currently cannot distinguish between congenital and acquired VWD; clinical correlation is required.

Reference Values

An interpretive report will be provided.

COAGULATION FACTOR VIII ACTIVITY
Adults: 55-200%
Normal, full-term infants or healthy premature infants typically have levels greater than or equal to 40%.*

*See Pediatric Hemostasis References in Coagulation Guidelines for Specimen Handling and Processing.

VON WILLIEBRAND FACTOR ANTIGEN
55-200%
Note: Individuals of blood group "O" may have lower plasma von Willebrand factor (VWF) antigen than those of other ABO blood groups, such that apparently normal individuals of blood group "O" may have plasma VWF antigen as low as 40% to 50%, whereas the lower limit of the reference range for individuals of other blood groups may be 60% to 70%.
Children: Neonates, infants, and children have normal or mildly increased plasma VWF antigen, with respect to the adult reference range.

VON WILLEBRAND FACTOR ACTIVITY
55-200%

Normal, full-term infants may have mildly increased levels which reach adult levels by 90 days postnatal. Healthy, premature infants (30-36 weeks gestation) may have increased levels that reach adult levels by 180 days.

Note: Individuals of blood group "O" may have lower plasma von Willebrand factor (VWF) activity than those of other ABO blood groups, such that apparently normal individuals of blood group "O" may have plasma VWF activity as low as 40% to 50%, whereas the lower limit of the reference range for individuals of other blood groups may be 60% to 70%.

COAGULATION FACTOR VIII INHIBITOR SCREEN
Negative

RISTOCETIN COFACTOR
Adults: 55-200%
Note: Individuals of blood group "O" may have lower plasma von Willebrand factor (VWF) ristocetin cofactor activity than those of other ABO blood groups, such that apparently normal individuals of blood group "O" may have plasma VWF ristocetin cofactor activity as low as 40% to 50% whereas the lower limit of the reference range for individuals of other blood groups may be 60% to 70%.

Normal, full-term infants or healthy premature infants usually have levels in the adult range.

FACTOR VIII BETHESDA UNITS
< or =0.5 Bethesda Units

Interpretation
An interpretive report will be provided when testing is complete.

Cautions
Treatment with heparin causes false-positive results of in vitro coagulation testing for lupus anticoagulant.

Warfarin treatment may impair ability to detect the more subtle varieties of lupus-like anticoagulants.

Testing should be performed prior to and in the absence of recent transfusion or von Willebrand factor (VWF) replacement therapy (eg, Humate P or DDAVP: desmopressin). If the patient has received any such therapy, this information should be provided. von Willebrand disease (VWD) patients receiving Humate P therapy may have a VWF activity level 10% to 20% lower than the VWF ristocetin cofactor activity level. Low normal levels of VWF antigen or activity do not exclude possible diagnosis of VWD (repeat testing may be indicated). Use of estrogens may result in a mild increase in VWF levels, thus masking a diagnosis of mild VWD.

Borderline low or slightly decreased levels of VWF antigen or activity may be observed in clinically normal individuals of blood group O.

This test is not useful for differentiating type 2A versus 2B VWD or platelet-type VWD (pseudo-VWD). This differentiation requires ristocetin-induced platelet aggregation testing, which must be performed using freshly obtained patient platelets and plasma.

Clinical correlation is required for differentiating acquired from congenital (hereditary) forms of VWD. Repeat testing may be helpful for confirming or evaluating low or borderline low levels of VWF (antigen and activity), especially when

there is strong suspicion of VWD.

The milder forms of the disease, especially type 1 VWD, can be difficult to diagnose or exclude, reflecting the variability of baseline VWF levels. In addition to demonstration of persistently decreased levels of VWF, clinical correlation is required for diagnosis of all VWD subtypes, especially mild type 1 VWD.

Clinical Reference

1. Federici AB, Mannucci PM. Advances in the genetics and treatment of von Willebrand disease. Curr Opin Pediatr. 2002;14(1):23-33
2. Budde U, Schneppenheim R. von Willebrand factor and von Willebrand disease. Rev Clin Exp Hematol. 2001;5(4):335-368
3. Kumar S, Pruthi RK, Nichols WL. Acquired von Willebrand disease. Mayo Clin Proc. 2002;77(2):181-187
4. Favaloro EJ, Gosselin RC. eds. Hemostasis and Thrombosis Methods and Protocols. 2nd ed. Humana Press; 2023

Performance

Method Description

Factor VIII Activity
The factor VIII assay is performed on the Instrumentation Laboratory ACL TOP using the activated partial thromboplastin time (APTT) method and a factor-deficient substrate. Patient plasma is combined and incubated with a factor VIII-deficient substrate (normal plasma depleted of factor VIII by immunoadsorption) and an APTT reagent. After a specified incubation time, calcium is added to trigger the coagulation process in the mixture. Then the time to clot formation is measured optically at a wavelength of 671 nm.(Owen CA Jr, Bowie EJW, Thompson JH Jr. Diagnosis of Bleeding Disorders. 2nd ed. Little, Brown and Company; 1975; Cielsa B. Defects of plasma clotting factors. In: Hematology in Practice. 3rd ed. FA Davis; 2019:chap 17)

von Willebrand Factor Antigen
This assay is performed using the HemosIL von Willebrand Factor Antigen kit on the Instrumentation Laboratory ACL TOP. This is a latex immunoassay method using microlatex particles coated with specific rabbit-polyclonal antibody directed against von Willebrand factor (VWF). In the presence of VWF antigen, antibody-coated latex particles agglutinate to form aggregates of diameters greater than the wavelength of the light passing through the sample and more light is absorbed as aggregation increases. The increase in absorption is proportional to the concentration of VWF antigen present in the sample.(Package insert: HemosIL von Willebrand Factor Antigen, Instrumentation Laboratory, R11 05/2018)

von Willebrand Factor Activity
This is a latex particle-enhanced immunoassay to quantify von Willebrand factor (VWF) activity in plasma. The activity of VWF is determined by measuring the increase of turbidity produced by the agglutination of the latex reagent. A specific anti-VWF monoclonal antibody adsorbed onto the latex reagent, directed against the platelet-binding site of VWF (glycoprotein Ib receptor), reacts with the VWF of patient plasma. The degree of agglutination is directly proportional to the activity of VWF in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.(Package insert: HemosIL von Willebrand Factor Activity. Instrumentation Laboratory Company; 12/2020)

Factor VIII Inhibitor Screen
The factor VIII inhibitor screen is performed on the Instrumentation Laboratory ACL TOP. The assay consists of measuring the factor VIII activity (activated partial thromboplastin time based assay) before and after incubation of a mixture of normal plasma and patient's plasma for 1 hour at 37 degrees C. Interpretation of the presence or absence of the indication of a factor VIII inhibitor is determined by comparing the factor VIII activity results and the calculated expected values.(Owen CA Jr, Bowie EJW, Thompson JH Jr. The Diagnosis of Bleeding Disorders. 7nd ed. Little Brown and Company; 1975:143-145; Meijer P, Verbruggen HW, Spannagi M. Chapter 33: Clotting factors and inhibitors: Assays and Interpretation. In: Kottke-Marchant K, ed. Laboratory Hematology Practice. Wiley Blackwell Publishing; 2012:435-446)

Ristocetin Cofactor
The von Willebrand factor (VWF) ristocetin cofactor activity is a manual assay that measures the ability of plasma VWF to bind platelet glycoprotein (GP) Ib in the presence of the antibiotic ristocetin. The method uses washed normal donor platelets and patient plasma. Ristocetin-induced, patient VWF-mediated agglutination of normal donor platelets is measured by light transmission aggregometry in the Platelet Aggregation Profiler (PAP; model PAP-8E) instrument. The slope of the agglutination response is proportional to the concentration of VWF ristocetin cofactor activity present in patient plasma.(Olson JD, Brockway WJ, Fass DN, et al. Evaluation of ristocetin-Willebrand factor assay and ristocetin-induced platelet aggregation. Am J Clin Pathol 1975;63[2]:210-218; Favaloro EJ and Lippi G. eds. Hemostasis and Thrombosis, Methods and Protocols. Humana Press 2017)

Factor VIII Bethesda Units
Heat inactivated patient plasma, undiluted and serially diluted, is mixed with an equal volume of normal pooled plasma (NPP). The NPP supplies the factor against which the inhibitor is directed in a known concentration. The patient plasma mixtures, along with a control (Bethesda pool) of diluted NPP are incubated at 37 degrees C for 2 hours, after which factor activity is measured. The factor activity in the undiluted patient and its serial dilutions are compared to the factor activity recovered in the Bethesda pool. These values are then used to calculate Bethesda units. One Bethesda unit is defined as the amount of antibody that will destroy 50% of the coagulation factor activity in 2 hours.(Owen CA Jr, Bowie EJW, Thompson JH Jr. Diagnosis of Bleeding Disorders. 2nd ed. Little, Brown and Company; 1975; Meijer P, Verbruggen HW, Spannagi M. Clotting factors and inhibitors: Assays and interpretation. In: Kottke-Marchant K, ed. Laboratory Hematology Practice. Wiley Blackwell Publishing; 2012:435-446)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
2 to 12 days

Specimen Retention Time
7 days

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

See Individual Test IDs

CPT Code Information

85240-Coagulation factor VIII assay
85246-von Willebrand factor antigen
85397-von Willebrand factor activity
85390-Technical interpretation
85245-von Willebrand factor ristocetin cofactor activity (if appropriate)
85247-von Willebrand factor multimer (if appropriate)
85335-Bethesda titer (if appropriate)
85335-Coagulation factor VIII inhibitor screen (if appropriate)
85390-26-Special coagulation interpretation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AVWPR	von Willebrand Disease Prof	48593-8

Result ID	Test Result Name	Result LOINC® Value
F8A	Coag Factor VIII Activity Assay, P	3209-4
VWAG	von Willebrand Factor Ag, P	27816-8
AVWPI	von Willebrand Disease Tech Interp	48595-3
VWACT	von Willebrand Factor Activity, P	68324-3