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REVIEW

# Laboratory tests used to help diagnose von Willebrand disease: an update

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## Summary

von Willebrand disease (VWD) is due to quantitative deficiencies and/or qualitative defects in von Willebrand factor (VWF), and is reportedly the most common inherited bleeding disorder. However, diagnosis of VWD is problematic, and is subject to over-, under-, and misdiagnosis. This is due to many factors, including limitations in current test procedures and an over-reliance on these imperfect test systems for clinical diagnosis. VWF is a complex plasma protein with multiple functions, but essentially acts to assist in the formation of a platelet thrombus to stop blood loss from sites of injury. VWF achieves this by several activities, including binding to platelets [primarily through the glycoprotein Ib (GPIb) receptor], binding to subendothelial matrix components (primarily collagen), and binding to factor VIII (FVIII), thus protecting FVIII from degradation and enabling its delivery to sites of vascular injury. Laboratory assessment of VWD entails performance of a battery of tests, some of which aim to mimic *in vivo* VWF activity. VWD is classified into six separate types, based on quantitative deficiencies [types 1 (partial deficiency) and 3 (total deficiency)] of VWF, or qualitative defects (type 2 VWD), which comprise four 'subtypes'. The current report briefly overviews the diagnosis of VWD, describing the currently available armamentarium of laboratory tests, as well as emerging options for laboratory-assisted diagnostics. Although some methodologies suffer from significant limitations that challenge the accurate diagnosis of VWD, newer methodologies and specific approaches can improve detection of this common bleeding disorder, and the appropriate characterisation and typing of patients.

*Key words:* von Willebrand disease; VWD; diagnosis; von Willebrand factor; VWF; laboratory testing.

*Abbreviations:* ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CB/Ag, collagen binding to antigen ratio; CLIA, chemiluminescent immunoassay; DDAVP, desmopressin; FVIII, factor VIII; ELISA, enzyme linked immunosorbent assay; FVIII:C, factor VIII coagulant; GPIb, (platelet) glycoprotein Ib; HMW, high molecular weight; ITP, immune or idiopathic thrombocytopenia; LIA, latex immunoassay; PT, platelet type; RCo/Ag, ristocetin cofactor to antigen ratio; RIA, radio-immunoassay; RIPA, ristocetin induced platelet aggregation; VWD, von Willebrand disease; VWF Ac, (Siemens Innovance) von Willebrand factor–activity (platelet GPIb binding assay); VWF:Act, von Willebrand factor activity [assay (generic)]; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding; VWF:FVIIIB, von Willebrand factor – Factor VIII binding; VWF:Multimers, multimeric assessment or structural profile for VWF; VWF:RCo, von Willebrand factor ristocetin cofactor.

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## INTRODUCTION

von Willebrand disease (VWD) is reportedly the most common inherited bleeding disorder. The prevalence of VWD is described as being up to 1% of the population based on epidemiological data; however, a more realistic prevalence, based on numbers of symptomatic patients presenting to medical facilities, is around 0.05% (or 1 in 20,000) people.[1] VWD is due to quantitative deficiencies and/or qualitative defects in von Willebrand factor (VWF), a complex plasma protein with multiple functions, which overall contribute to the formation of a platelet thrombus at sites of injury to help prevent blood loss.[2] VWF accomplishes this major haemostasis function by anchoring platelets to sites of vascular injury (primary haemostasis function), as well binding to factor VIII (FVIII), thus protecting FVIII from degradation, and delivering it to sites of vascular injury (thereby facilitating secondary haemostasis). VWF binds to platelets via several receptors, most notably glycoprotein Ib (GPIb), but also GPIIb/IIIa (now often termed integrin $\alpha_{IIb}\beta_3$). VWF also binds to subendothelial matrix components, most notably collagen, and in this way facilitates anchoring of platelets to damaged endothelium by forming an adhesive bridge. The peculiar structure of VWF, in terms of its size and formation of multimers has particular relevance here (see below), as the larger VWF molecules facilitate better anchoring of platelets and thus thrombus formation and prevention of bleeds.

*In vivo* biosynthesis of VWF is limited to endothelial cells and megakaryocytes,[3,4] where it is constructed as a series of oligomers containing a variable numbers of subunits, ranging from a minimum of two to a maximum of 40, with the largest multimers having molecular weights in excess of 20,000 kDa. The VWF is stored as large multimeric species ('ultra-large VWF'; UL-VWF) in internal organelles known as Weibel–Palade bodies, and released into the plasma as required upon endothelial stimulation. During release, VWF temporarily unfolds into ultra-long strings, and is then cleaved by ADAMTS-13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13) at the A2 domain (Fig. 1). The A1 domain represents the platelet GPIb binding site, as well as binding sites for heparin, sulphated glycolipids, the snake venom botrocetin, and some

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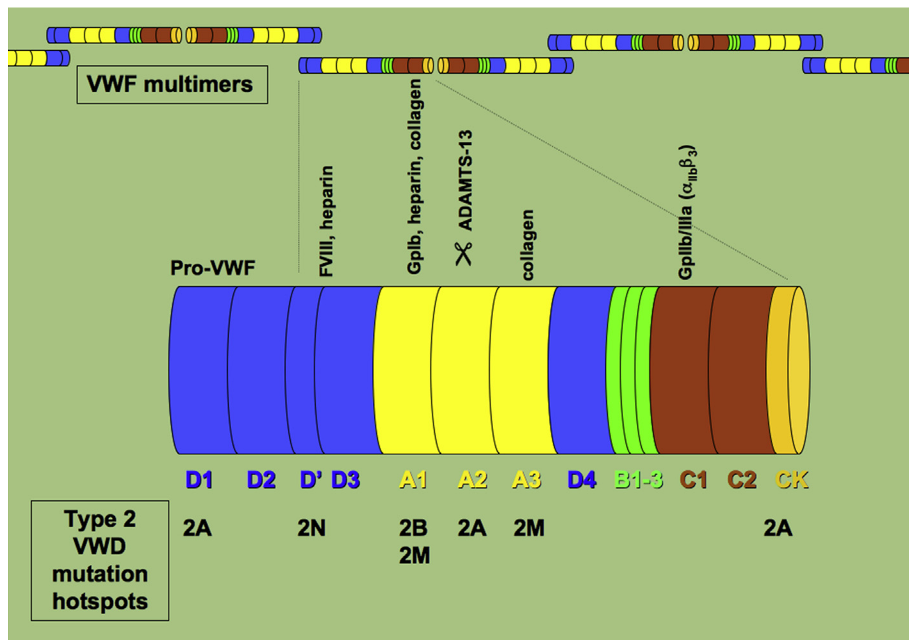


**Fig. 1** The basic structure of VWF. Figure includes the 'functional' position of various interactions, assembly into dimers and multimers, and 'hotspot' positions for type 2 *VWF* mutations. Mutations leading to types 1 and 3 VWD can occur anywhere on the *VWF* gene.

forms of collagen, notably type VI. The A3 domain represents the binding site for fibrillar collagens types I and III, and the C1 domain comprises the RGD (Arg-Gly-Asp) tripeptide sequence, or the binding site for the platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb/IIIa). Mutations in the A and C1 domains therefore can lead to forms of VWD that express failures in such binding (i.e., qualitative defects within type 2 VWD). The D'-D3 domains of VWF represent the binding site for FVIII, and mutations here can lead to type 2N VWD.

## CLASSIFICATION (TYPING) OF VWD

The current classification scheme assigns VWD to six types (Table 1),[5] characterised on the basis of quantitative deficiencies of VWF (VWD types 1 and 3), or qualitative defects in VWF (type 2 VWD), which may or may not be also associated with a quantitative deficiency of VWF.

Type 1 VWD represents the most common form of VWD in developed countries (40–70% of all cases[1]) and defines a partial deficiency of VWF (which is otherwise functionally normal). The severity of bleeding is related to the extent of VWF deficiency, so that the lower the VWF level, the more severe the bleeding risk. FVIII levels fall in parallel with VWF, but are normally present at ~1.5× the level of VWF.

Type 3 VWD represents the rarest form of VWD in developed countries (<5% of all VWD cases[1]), is essentially characterised by an absence of VWF, and thus defines the most severe VWF defect. As VWF is absent and unavailable to bind and protect FVIII, these levels are also very low (typically <10 U/dL).

Type 2 VWD patients express qualitative defects of VWF. The level of VWF protein itself may be normal or decreased, and FVIII levels may also be decreased or normal.

Type 2A VWD is classically considered the most common form of type 2 VWD[1] and describes an absence or deficiency in high molecular weight (HMW) VWF,[5] being those forms of VWF that are most biologically active. This HMW deficiency is due to either faulty VWF assembly or enhanced *in vivo* proteolysis of VWF.

Type 2B VWD is a relatively rare form of type 2 VWD, affecting <5% of all VWD patients,[1] and reflects VWF that is hyper-adhesive,[5] 'spontaneously' binding to platelets in the absence of typical haemostatic triggers. This VWF is then cleared from the circulation together with the bound platelets, typically leading to loss of HMW VWF (similar to type 2A VWD), and also sometimes (mild) thrombocytopenia. FVIII levels are often normal, although they can be low in some patients.

Type 2N VWD is another relatively rare form of type 2 VWD, affecting <5% of all VWD cases,[1] and identifying defects in VWF that do not allow proper binding to FVIII.[5] Like type 3 VWD, this leads to early proteolysis and loss of plasma FVIII, with bleeding symptoms that mimic those of haemophilia A.

Type 2M VWD describes qualitative VWF defects not associated with a loss of HMW VWF,[5] and has classically been considered a rare form of VWD. Many clinicians and scientists have conceptual difficulty with this type of VWD, which actually reflects a broadly heterogenous group of VWF defects. A simpler way to think about type 2M VWD is by exclusion of other VWD types; thus, if a patient's VWD type cannot be easily characterised as type 1, 2A, 2B, 2N or 3, then it will likely fulfil the criteria of type 2M VWD.[6] Moreover, although classically considered rare, type 2M VWD is probably as common as type 2A VWD, since most cases of type 2M are inappropriately identified as type 1 or 2A VWD.[1]

Although not considered a true VWD, since the defect lies in the platelet GPIb molecule, the laboratory phenotype of platelet type (PT)-VWD essentially mimics 2B VWD.[7,8] PT-VWD is a rare disorder that occurs at the rate of ~10% that of type 2B VWD,[1] although it is often misdiagnosed as type 2B VWD or idiopathic thrombocytopenia (ITP).

The correct diagnosis and typing of VWD is important, and this will influence patient management.[9] Expressed another

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