

RECOMMENDATIONS AND GUIDELINES

Platelet-dependent von Willebrand factor activity. Nomenclature and methodology: communication from the SSC of the ISTH

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von Willebrand disease (VWD) is the most common bleeding disorder in man [1]. Measuring VWF functional activity is critical for making the correct diagnosis and classification. Traditionally considered the ‘gold standard’ for evaluating platelet-dependent VWF function, the ristocetin cofactor activity (VWF:RCo) assay was developed after the discovery of platelet aggregation by ristocetin [2–6].

Imprecision and insensitivity of the VWF:RCo assay led to the development of new assays. Some of the issues related to VWF activity testing have recently been reviewed [7–11]. Because it is critical that users of these various assays recognize differences between details of functions being measured, a new nomenclature was developed by the VWF Subcommittee of the Standardization and Scientific Committee (SSC) of the International Society for Thrombosis and Haemostasis (ISTH). The guiding principles in developing a practical and accurate nomenclature included simplicity, consistency and accuracy. The result of this effort reached consensus and was formally approved by the VWF Subcommittee at the Milwaukee SSC Meeting on 23 June 2014 (Table 1).

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Ristocetin cofactor activity (VWF:RCo) assays

All VWF:RCo assays have in common the use of (i) intact (native, formalin-fixed or reconstituted lyophilized) platelets and (ii) ristocetin.

First-generation (manual) VWF:RCo assay

The original description [4] of the test used glass slides for the reaction. Although still used in some laboratories, this method is laborious, subjective and operator dependent.

Second-generation (semi-automated) VWF:RCo assay

In the second-generation assays, VWF:RCo was adapted to traditional aggregometers, and commercial reagents

Table 1 Approved nomenclature for the various activities measured by current assay systems

Abbreviation for VWF activity	Description
VWF:RCo	Ristocetin cofactor activity: all assays that use platelets and ristocetin
VWF:GPIbR	All assays that are based on the ristocetin-induced binding of VWF to a recombinant WT GPIb fragment
VWF:GPIbM	All assays that are based on the spontaneous binding of VWF to a gain-of-function mutant GPIb fragment
VWF:Ab	All assays that are based on the binding of a monoclonal antibody (mAb) to a VWF A1 domain epitope

became available. The reaction velocity (slope of the aggregation curve) is compared to the calibrator. Used in many laboratories throughout the world, this semi-automated VWF:RCo assay continues to be plagued with technical issues as it is insensitive (limit of detection, LOD: $> 10\text{--}20\text{ IU dL}^{-1}$) and imprecise (coefficient of variation [CV], up to 20–30%) [12].

Third-generation (fully automated) VWF:RCo assay

The VWF:RCo assay was successfully adapted to automated coagulometers with the advantage of improved precision and higher throughputs. Currently, most coagulation laboratories use one of the published protocols listed in Table S1.

Fourth-generation (modified fully automated) VWF:RCo assay

Recently, building on prior experience to improve assay characteristics [13], a set of modifications to the original automated assay was reported to markedly improve sensitivity (LOD, 3 IU dL^{-1}) with a CV of 6–9% (Table S1, bottom row) [14,15].

VWF:RCo flow cytometry methods

A sensitive flow cytometry analysis [16–19] has not gained wide acceptance because of the requirement for equipment and expertise not commonly available in hemostasis laboratories.

Advantages and disadvantages of the VWF:RCo assay

For decades, the VWF:RCo assay represented the gold standard for measuring VWF activity; therefore, much experience was accumulated. Furthermore, most data correlating VWF levels and treatment with desmopressin or VWF concentrates relate to VWF:RCo [5,20,21]. However, the disadvantages of the VWF:RCo assays are numerous. The tests have poor sensitivity [12], which prevents measuring VWF activity $< 10\text{ IU dL}^{-1}$, making it difficult to characterize patients with *severe VWD*. Additionally, because the VWF:RCo/VWF:Ag ratio is critical for the current classification, this poor sensitivity may lead to potential misdiagnoses. First and second-generation assays were also time consuming and poorly standardized.

The high coefficient of variation [12] may lead to false diagnoses in the *moderately reduced VWF* range. Potential sources of error include instability and batch-to-batch variability of ristocetin or of the platelet reagent (whether locally prepared or commercially lyophilized) and some intrinsic instability of the assay system.

An additional disadvantage comes from the fact that VWF:RCo actually measures two parameters, (i) binding

of ristocetin to VWF and (ii) binding of ristocetin-‘activated’ VWF to test platelets (i.e. activity is triggered by an artifact, because, although believed to induce conformational changes resembling the physiological activation of VWF brought about by immobilization on the collagen-rich subendothelial surface exposed to high shear stress [22,23], ristocetin itself is not a physiological activator of VWF). Two sequence variants, p.P1467S and the H-allele of a common polymorphism p.D1472H, in the ristocetin binding region of the A1 domain (exon 28) [19,24], cause spuriously decreased VWF:RCo levels. The H-allele of the p.D1472H polymorphism is common in the African American population and results in a decreased VWF:RCo/VWF:Ag ratio [24]. These low VWF activities do not correlate with bleeding symptoms but reflect an assay artifact. For these reasons, the time-honored notion that VWF:RCo is indispensable is rapidly changing [7–9,25].

Ristocetin-triggered GPIb binding (VWF:GPIbR) assays

Vanhoorelbeke, Deckmyn and colleagues developed a platelet-free ELISA test using a recombinant GPIb fragment captured by a monoclonal antibody coated onto ELISA plates [26] with much improved LOD and CV [27–29] (Table S2). Subsequently, the same principle was used to develop latex or magnetic particle-enhanced automated assays. Correlation with the classic VWF:RCo test is reported to be excellent, proving the validity of the concept (Table S2). However, it is important to note that these assays use different reagents for capturing the GPIb fragment as well as different recombinant or plasma-derived fragments (Table S2). The source and concentration of ristocetin are also variable. Finally, the epitope specificity of the monoclonal antibody capturing GPIb is critical [27]. Nevertheless, proper automated applications of the assay principle allow for precise and sensitive detection of VWF activity [25,30–33].

Gain-of-function mutant GPIb binding (VWF:GPIbM) assays

The newest VWF activity assays use recombinant gain-of-function mutant GPIb fragments, allowing spontaneous binding of VWF to the mutant GPIb without ristocetin (Table S3). The binding is optimized when any two of three specific mutations are introduced [34]. Recent published data support the concept that the VWF:GPIbM assays are consistently correlated with the standard VWF:RCo assay [25,35–37]. These assays are precise [35], sensitive [35,38,39] and not subject to the falsely low values seen with the p.P1467S and p.D1472H polymorphisms [34]. Additionally, with ELISA applications of these new assays it may be possible to differentiate between VWD type 2A and 2B [34,40,41]. These assays have been referred to as ‘VWF Ac’ or ‘INNOVANCE VWF Ac’.

The more descriptive 'VWF:GPIbM' is the preferred name.

Monoclonal antibody binding-based VWF activity (VWF:Ab)

The original ELISA assay used the monoclonal antibody REF-VIII:R/2, which is directed against a VWF epitope involved in VWF-GPIb binding [42–44]. The commercial latex-enhanced automated immunoturbidimetric assay (LIA) version is marketed as 'VWF activity' assay, abbreviated as *VWF:Act*. As the assay does not provide information about the function of VWF being measured, it is preferable to use the more descriptive VWF:Ab term.

The LIA (HemosIL VWF activity) performed better than the ELISA [45,46] in discriminating VWD subtypes and showed good correlation with VWF:RCO [47–51]. Advantages of the HemosIL VWF:Ab test are several, including the fact that it is user-friendly, applicable to several platforms, and thus, feasible for routine laboratories [48,49]. However, because the VWF:Ab assay reports the binding of the VWF A1 domain to a mAb and not to GPIb, it is unclear to what extent this antibody is able to accurately mimic the GPIb binding surface [50]. Some VWD type 2M mutations (e.g. p.G1324A) are not detected by the assay [51] and it is not clear to what extent the assay is sensitive to the loss of HMW multimers [47,48]. Furthermore, the VWF:Ab assay did not resolve the problem with the lower limit of detection because linearity is reported [47] to be acceptable only above 12.5 IU dL⁻¹. In addition, the current package insert indicates that the LOD is 19 IU dL⁻¹. Taken together, the good overall correlation with the VWF:RCO assay probably gives this assay a role in the routine screening of VWF patients, when combined with other tests. However, the VWF:Ab test cannot be recommended as a replacement for the VWF:RCO assay.

An assay using a similar principle uses a llama nanobody that recognizes the active conformation of VWF, allowing the detection of constitutionally active VWF in VWD type 2B and thrombotic thrombocytopenic purpura (TTP) [52], but does not measure VWF activity and is not discussed further in this review.

In summary, the past several years have seen a remarkable (r)evolution of assays measuring platelet-dependent VWF activities. The original manual and semi-automated methods have mostly been replaced by automated techniques with higher precision. In the most recent applications described here, the platelets have been substituted by recombinant GPIb fragments immobilized on ELISA wells or latex particles, allowing accurate measurement of VWF activity in the very low (< 1–10 IU dL⁻¹) range. Further innovation has led to the newest generation of accurate and sensitive ristocetin-free assays, which prevent the problems that plagued the ristocetin-based assays, including false-positive results in certain populations.

While most welcome in the battery of tests for the diagnosis of VWD, the exact behavior of the particular assays is not clear at this time. Side-by-side comparison using healthy controls as well as pathological plasma samples (including VWD type 1, type 2 and type 3 patients) is urgently needed. An ongoing comparative study [53] is expected to provide useful information for clinicians and laboratory professionals alike, and will likely lead to gradual replacement of the older VWF:RCO assay. In addition to providing the technical details of the currently available assays, this short review provides the new nomenclature approved by the ISTH VWF SSC to clearly distinguish various test principles, which is recommended for all future communications in the field.

Addendum

I. Bodó prepared the manuscript and chaired the *ad hoc* VWF Activity Nomenclature Committee. J. Eikenboom, R. Montgomery, J. Patzke, R. Schneppenheim and J. Di Paola actively participated in developing the best nomenclature and they all critically reviewed and revised the manuscript, significantly contributing to the final version. J. Eikenboom and J. Di Paola, as former and current chairmen of the ISTH SSC von Willebrand Subcommittee, were responsible for initiating and concluding the Nomenclature Committee task.

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Disclosure of Conflict of Interests

J. Patzke discloses that he is an employee of Siemens Healthcare Diagnostics GmbH. J. Patzke and R. Schneppenheim hold a patent 'Method for determining von Willebrand factor activity in the absence of ristocetin and for determining the ADAMTS-13 protease'. R. Montgomery discloses a patent licensed to GTI and that GTI-Immunocor has licensed a ristocetin-free assay from the Blood Center of Wisconsin and the Medical College of Wisconsin. All other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Third and fourth generation fully automated VWF:RCo assays.

Table S2. Ristocetin-triggered GPIb-binding (VWF: GPIbR) assays by ELISA and automated (coagulometer) systems.

Table S3. Gain-of-function mutant GPIb-binding (VWF: GPIbM) assays by ELISA and automated (coagulometer) systems.

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