INNOVANCE VWF Ac Assay

A review of guidelines and utility in diagnosis

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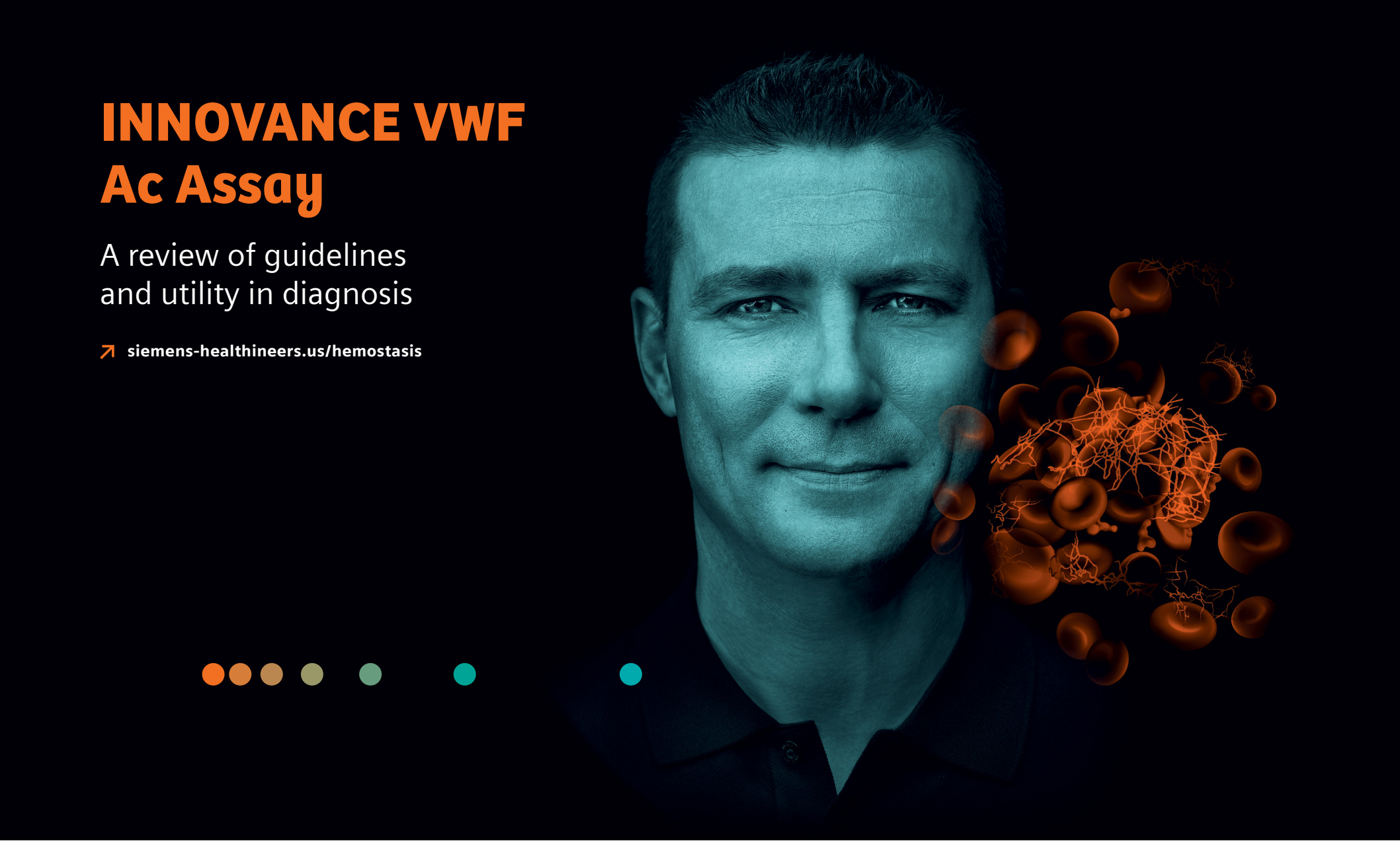

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Contents

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Recommendations and guidelines including VWF:GPIbM

1 James PD, Connell NT, Ameer B. Blood Adv. 2021 Jan 12;5(1):280-300. (free)

ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease.

First level diagnostic tests: FVIII:C, VWF:Ag and VWF-platelet GPIbα binding capacity. "New assays have been introduced to evaluate VWF-platelet GPIbα binding activity in order to over-come limitations of the VWF:RCo assay…VWF:GPIbM assays reportedly correlate with the standard VWF:RCo assay; however, the newer assays have better precision, CV, and sensitivity."

Background

von Willebrand disease (VWD) is the most common inherited bleeding disorder known in humans. Accurate and timely diagnosis presents numerous challenges.

Objective: These evidence-based guidelines of the American Society of Hematology (ASH), the International Society on Thrombosis and Haemostasis (ISTH), the National Hemophilia Foundation (NHF), and the World Federation of Hemophilia (WFH) are intended to support patients, clinicians, and other health care professionals in their decisions about VWD diagnosis.

Methods

ASH, ISTH, NHF, and WFH established a multidisciplinary guideline panel that included 4 patient representatives and was balanced to minimize potential bias from conflicts of interest. The Outcomes and Implementation Research Unit at the University of Kansas Medical Center (KUMC) supported the guideline-development process, including performing or updating systematic evidence reviews up to 8 January 2020. The panel prioritized clinical questions and outcomes according to their importance for clinicians and patients. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, including GRADE Evidence-to-Decision frameworks, to assess evidence and make recommendations, which were subsequently subject to public comment.

Results

The panel agreed on 11 recommendations.

Conclusions: Key recommendations of these guidelines include the role of bleeding-assessment tools in the assessment of patients suspected of VWD, diagnostic assays and laboratory cutoffs for type 1 and type 2 VWD, how to approach a type 1 VWD patient with normalized levels over time, and the role of genetic testing vs phenotypic assays for types 2B and 2N. Future critical research priorities are also identified.

DOI: 10.1182/bloodadvances.2020003265

2 Laffan MA, Lester W, O'Donnell JS, et al. Br J Haematol. 2014;167:453-65. (free)

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.

"The use of a recombinant GPIb with gain-of-function mutations can remove the requirement for ristocetin."

Recommendations:

"In the initial investigation for VWD, FVIII, VWF:Ag and VWF activity should be measured (1A)."

"VWF activity should be assessed by its ability to bind both GPIb and collagen (2B).

We recommend against using assays based on monoclonal antibodies directed against the VWF GPIb-binding site (1B)."

DOI: 10.1111/bjh.13064

Of special interest

3 Hubbard AR, Haberichter SL. J Thromb Haemost. 2019;17(6):1003-5. (free)

Establishment of an International Reference Reagent for standardization of von Willebrand factor binding to recombinant glycoprotein Ib (VWF:GPIbM and VWF:GPIbR): Official Communication of the SSC.

No abstract available.

DOI: 10.1111/jth.14429

4 Bodó I, Eikenboom J, Montgomery R, Patzke J, Schneppenheim R, Di Paola J. J Thromb Haemost. 2015;13:1345-50. (free)

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

"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]."

DOI: 10.1111/jth.12964

Of special interest

Reviews including INNOVANCE VWF Ac assay

5 Keesler DA, Flood VH. Res Pract Thromb Haemost. 2017;2:34-41. (free)

Current issues in diagnosis and treatment of von Willebrand disease.

"Historically, VWF binding to platelet GPIbα was measured by the ristocetin cofactor assay (VWF:RCo); a new assay using platelet GPIbα in the absence of ristocetin (VWF:GPIbM) is gradually replacing the VWF:RCo due to improved accuracy in diagnosis."

"4. RISE OF THE VWF:GPIBM ASSAY (AND DEATH OF THE VWF:RCO?)...The inconsistencies and inadequacies in these assays (RCo assays), and the potential for misdiagnosis, have led to the novel VWF:GPIbM assay."

DOI: 10.1002/rth2.12064

6 Sharma R, Flood VH. Blood. 2017;130:2386-91. (free)

Advances in the diagnosis and treatment of Von Willebrand disease.

"Fortunately, a new assay is available that avoids the use of ristocetin...The VWF:GPIbM allows greater precision, with a reported lower limit of detection of 2 IU/dL and a reported-within-laboratory coefficient of variation of 5.6%. There is reasonable correlation between VWF:RCo and VWF:GPIbM results. One study did show increased qualitative VWF defects using the VWF:GPIbM. One study did show increased qualitative VWF defects using the VWF:GPIbM. This may be due to use of ristocetin as the "gold standard," when in reality ristocetin is not the most accurate assay."

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, yet diagnosis and management remain challenging. Development and use of bleeding assessment tools allows for improved stratification of which patients may require further assessment and which patients are most likely to require treatment of their VWD. New options for laboratory assessment of von Willebrand factor (VWF) activity include a new platelet-binding assay, the VWF:GPIbM, which is subject to less variability than the ristocetin cofactor activity assay, and collagen-binding assays that provide insight into a different function of VWF. Genetic testing may be helpful in some cases where a type 2 VWD variant is suspected but is usually not helpful in type 1 VWD. Finally, treatment options for VWD are reviewed, including the use of recombinant VWF. Despite these advances, still more work is required to improve diagnosis, treatment, and quality of life for affected patients.

DOI: 10.1182/blood-2017-05-782029

7 Just S. Semin Thromb Hemost. 2017;43(1):75-91.

Laboratory testing for von Willebrand disease: the past, present, and future state of play for von Willebrand factor assays that measure platelet binding activity, with or without ristocetin.

Von Willebrand disease (VWD) was first described nearly a century ago in 1924 by Erik Adolf von Willebrand. Diagnostic testing at the time was very limited and it was not until the mid to late 1900s that more tests became available to assist with the diagnosis and classification of VWD. Two of these tests are based on ristocetin, one being ristocetin-induced platelet aggregation (RIPA) and the other the von Willebrand factor (VWF) ristocetin cofactor assay (VWF:RCo). The VWF:RCo assay provides functional assessment of in vitro VWF binding to the platelet glycoprotein (Gp) complex, GPIb-IX-V. Despite some advancements and newer technologies utilizing the principles of the original VWF:RCo assay, the original assay is still referred to as the gold standard for measurement of VWF activity. This article will review the history of VWD diagnostic assays, including RIPA and VWF:RCo over the past 40 years, as well as the newer assays that measure platelet binding with or without ristocetin, and which have been developed with the aim to potentially replace platelet-based ristocetin-dependent assays.

DOI: 10.1055/s-0036-1592164

8 Favaloro EJ, Pasalic L, Curnow J. Pathology. 2016;48(4):303-18.

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

"Testing by the INNOVANCE VWF Ac ('VWF:GPIbM') assay will provide results that closely match those of VWF:RCo...Published reports of this assay have been largely positive, with comparable diagnostic VWD performance to VWF:RCo, but improved overall lower limit of VWF sensitivity and reduced assay variability."

"Although the ELISA based method has not as yet been adapted to routine practice, or commercialised, the LIA method has been commercialized (INNOVANCE VWF Ac; Siemens, Germany) and is now adopted by many laboratories, often in place of the VWF:RCo assay. Published reports of this assay have been largely positive, with comparable diagnostic VWD performance to VWF:RCo, but improved overall lower limit of VWF sensitivity and reduced assay variability."

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.

DOI: 10.1016/j.pathol.2016.03.001

9 De Jong A, Eikenboom J. J Thromb Haemost. 2016;14:1507-16. (free)

Developments in the diagnostic procedures for von Willebrand disease.

"In our laboratory we use the VWF:GPIbM assay...The VWF:GPIbM assay is easy to use and provides swift, precise and sensitive results. Because the VWF:GPIbM assay does not require ristocetin, it is not subjective to the VWF polymorphisms that affect the VWF:RCo assay."

Von Willebrand disease (VWD) is the most common inherited bleeding disorder but its diagnosis can be challenging due to the heterogeneity of the disease. VWD is mainly associated with mild mucocutaneous bleeding, although there are more severe phenotypes with bleeding from the gastrointestinal tract or even the joints. Also, surgical interventions and trauma may lead to critical bleeding events. These bleeding episodes are all related to quantitative or qualitative defects of von Willebrand factor (VWF), a multimeric glycoprotein produced by endothelial cells and megakaryocytes, which mediates platelet adhesion and aggregation and binds factor VIII (FVIII) in the circulation. This review describes the diagnostic procedures required for correct diagnosis. Accurate diagnosis and classification is required for proper treatment and counseling. Assessment of bleeding starts with the medical history. After a positive bleeding or family history, subsequent laboratory investigations will start with a panel of standard screening tests for hemostatic defects. Patients suspected of having VWD will be tested for plasma VWF antigen levels, the ability of VWF to bind platelets and FVIII activity. When VWD is confirmed, a set of subtyping tests can classify the patients as VWD types 1, 2 (A, B, M or N) or 3. The performance of some additional assays and analyses, such as VWF pro-peptide measurement or genetic analysis, may help in identifying the pathological mechanism behind certain defects or can guide in the choice of treatment.

DOI: 10.1111/jth.13243

10 Hayward CP, Moffat KA, Graf L. Int Jnl Lab Hem. 2014;36:334-40. (free)

Technological advances in diagnostic testing for von Willebrand disease: new approaches and challenges.

"The drawback of misclassification of certain subjects by the use of ristocetin can be bypassed by platelet-dependent VWF assays using recombinant mutant gain-of-function GPIb fragments (GPIbM assay)... The VWF:RCo, VWF:GPIbR and GPIbM assays can be used to identify qualitative defects of VWF in the platelet binding."

Diagnostic tests for von Willebrand disease (VWD) are important for the assessment of VWD, which is a commonly encountered bleeding disorder worldwide. Technical innovations have been applied to improve the precision and lower limit of detection of von Willebrand factor (VWF) assays, including the ristocetin cofactor activity assay (VWF:RCo) that uses the antibiotic ristocetin to induce plasma VWF binding to glycoprotein (GP) IbIXV on target platelets. VWF-collagen-binding assays, depending on the type of collagen used, can improve the detection of forms of VWD with high molecular weight VWF multimer loss, although the best method is debatable. A number of innovations have been applied to VWF:RCo (which is commonly performed on an aggregometer), including replacing the target platelets with immobilized GPIbα, and quantification by an enzyme-linked immunosorbent assay (ELISA), immunoturbidimetric, or chemiluminescent end-point. Some common polymorphisms in the VWF gene that do not cause bleeding are associated with falsely low VWF activity by ristocetin-dependent methods. To overcome the need for ristocetin, some new VWF activity assays use gain-of-function GPIbα mutants that bind VWF without the need for ristocetin, with an improved precision and lower limit of detection than measuring VWF:RCo by aggregometry. ELISA of VWF binding to mutated GPIbα shows promise as a method to identify gain-of-function defects from type 2B VWD. The performance characteristics of many new VWF activity assays suggest that the detection of VWD, and monitoring of VWD therapy, by clinical laboratories could be improved through adopting newer generation VWF assays.

DOI: 10.1111/ijlh.12220

11 Boender J, Kruip MJ, Leebeek FW. Int J Lab Hematol. 2014;36(3):334-40. (free)

A diagnostic approach to mild bleeding disorders

"The INNOVANCE VWF Ac method has a better lower limit of detection, and better precision, than VWF:RCo estimated by aggregometry....Furthermore, use of the INNOVANCE VWF Ac instead of VWF:RCo increased the number of cases considered to have qualitative defects of VWF, possibly from an increased sensitivity to qualitative defects in VWF-GPIba binding, including the loss of HMWM."

Mild inherited bleeding disorders are relatively common in the general population. Despite recent advances in diagnostic approaches, mild inherited bleeding disorders still pose a significant diagnostic challenge. Hemorrhagic diathesis can be caused by disorders in primary hemostasis (von Willebrand disease, inherited platelet function disorders), secondary hemostasis (hemophilia A and B, other (rare) coagulant factor deficiencies) and fibrinolysis, and in connective tissue or vascular formation. This review summarizes the currently available diagnostic methods for mild bleeding disorders and their pitfalls, from structured patient history to highly specialized laboratory diagnosis. A comprehensive framework for a diagnostic approach to mild inherited bleeding disorders is proposed.

DOI: 10.1111/jth.13368

12 Bolton-Maggs PH, Favaloro EJ, Hillarp A, Jennings I, Kohler HP. Haemophilia. 2012 Jul;18 Suppl 4:66-72. (free)

Difficulties and pitfalls in the laboratory diagnosis of bleeding disorders.

von Willebrand disease (VWD) is the most common inherited bleeding disorder, but variable severity and several classification types mean that diagnosis is often not straightforward. In many countries, the assays are not readily available and/or are not well standardized. The latest methods and the basis of VWD are discussed here, together with information from the international quality assessment programme (IEQAS). Factor XIII deficiency is a rare, but important bleeding disorder, which may be missed or diagnosed late. A discussion and update on this diagnosis is considered in the final section of our review.

DOI: 10.1111/j.1365-2516.2012.02830.x

13 Lillicrap D. Blood. 2013;122(23):3735-40. (free)

von Willebrand disease: advances in pathogenetic understanding, diagnosis, and therapy.

"Central to the confirmation of VWD diagnosis are appropriately standardized measurements of VWF:Ag and VWF platelet-dependent function. This latter analysis currently involves testing for VWF:RCo, but this test is notoriously difficult to standardize and is relatively insensitive at VWF levels <10%. Therefore, the recent development of assays to quantify direct binding of VWF to platelet GPIbα appears to offer significant advantages, although a more comprehensive evaluation of different qualitative VWD variants must first be completed to ensure that the assays are sensitive to all appropriate structural changes."

"Another complicating issue that has arisen in the interpretation of the platelet-dependent functional assay for VWF is the interference in the VWF:RCo assay with a polymorphism at codon 1472 (D1472H)."

von Willebrand disease (VWD) is the most common autosomally inherited bleeding disorder. The disease represents a range of quantitative and qualitative pathologies of the adhesive glycoprotein von Willebrand factor (VWF). The pathogenic mechanisms responsible for the type 2 qualitative variants of VWF are now well characterized, with most mutations representing missense substitutions influencing VWF multimer structure and interactions with platelet GPIbα and collagen and with factor VIII. The molecular pathology of type 3 VWD has been similarly well characterized, with an array of different mutation types producing either a null phenotype or the production of VWF that is not secreted. In contrast, the pathogenetic mechanisms responsible for type 1 VWD remain only partially resolved. In the hemostasis laboratory, the measurement of VWF:Ag and VWF:RCo are key components in the diagnostic algorithm for VWD, although the introduction of direct GPIbα-binding assays may become the functional assay of choice. Molecular genetic testing can provide additional benefit, but its utility is currently limited to type 2 and 3 VWD. The treatment of bleeding in VWD involves the use of desmopressin and plasma-derived VWF concentrates and a variety of adjunctive agents. Finally, a new recombinant VWF concentrate has just completed clinical trial evaluation and has demonstrated excellent hemostatic efficacy and safety.

DOI: 10.1182/blood-2013-06-498303

14 Castaman G, Linari S. Expert Opinion on Orphan Drugs. 2019;7(4):147-55.

Advances in the diagnosis of von Willebrand disease.

"Finally, the novel VWF:GPIbM assay, which uses mutated GPIb fragment able to bind VWF without ristocetin, introduces a non-physiological binding to a mutated receptor. However, the assay is reported to be precise, sensitive, and relatively easy to perform. A recent Dutch study has compared the different VWF activity tests in a large population of patients with VWD, showing that all assays correlated excellently, but discrepant results led to a different classification for up to 20% of the patients."

Introduction: The diagnosis of von Willebrand disease (VWD) may be difficult and is based on the assessment of bleeding history and several diagnostic assays, which evaluate the pleiotropic function of von Willebrand factor (VWF). Laboratory diagnosis requires a series of assays to determine VWF concentration and function, and factor VIII activity, but no single test is available to explore all VWF activities to confirm or exclude diagnosis.

Areas covered: This review describes the advances in diagnosing VWD, starting from how to define and quantify the bleeding history to the new assays exploring VWF activities.

Expert opinion: VWD is the most common inherited bleeding disorder, is highly heterogeneous, and its appropriate diagnosis may represent a complex laboratory task, especially for type 2 variants. Until recently, the ristocetin cofactor activity assay has represented the standard method for measuring VWF activity, as its ability to bind to platelets in presence of ristocetin, but it has low sensitivity and high variability of results. Novel assays are increasingly used, are often automated and correlate excellently with the standard assay but sometimes discrepant results may lead to a different classification of VWD. The VWF-collagen binding assay is a useful complementary assay to better categorize type 2 variants.

DOI: 10.1080/21678707.2019.1609352

15 Favaloro EJ. Hamostaseologie. 2020b;10.1055/s-0040-1713735. (free)

Navigating the myriad of von Willebrand factor assays

"...essentially a GPIb binding assay that does not use platelets, and which currently comprises the Siemens Healthineers INNOVANCE VWF Ac assay (by LIA), as well as non-commercialized ELISA-based assays. These assays essentially generate test results that are very similar to those generated using VWF:RCo assays, but do not use ristocetin in the assay."

von Willebrand factor (VWF) represents a large and complex adhesive plasma protein whose main function is to provide a bridge between blood platelets and damaged endothelium, and thus facilitate primary hemostasis. VWF also binds to FVIII, preventing early proteolysis, and delivers this cargo to sites of vascular injury, thereby promoting clot formation and secondary hemostasis. An absence, deficiency, or defect in VWF can lead to a bleeding diathesis called von Willebrand disease (VWD), considered the most common inherited bleeding disorder. Contemporary laboratory assays used in VWD diagnosis/exclusion comprise a myriad of assays that identify the quantity (level) of VWF, as well as the multitude of VWF activities. These may use the following test abbreviations: VWF:Ag, VWF:RCo, VWF:CB, VWF:GPIbR, VWF:GPIbM, VWF:FVIIB, VWF:Ab. The current review explains what these assays are, as well as their place in VWD diagnostics.

DOI: 10.1055/s-0040-1713735

16 Fogarty H, Doherty D, O'Donnell JS. Br J Haematol. 2020 Nov;191(3):329-39.

New developments in von Willebrand disease.

"Consequently, this Gp1b variant binds to the A1 domain of VWF even in the absence of ristocetin. In addition to not being affected by the common VWF P1467S and D1472H polymorphisms, accumulating data suggests that the new VWF activity assays have reduced CVs and lower limits of detection compared to the original VWF:RCo assays."

Von Willebrand disease (VWD) constitutes the most common inherited human bleeding disorder. It is associated with a mucocutaneous bleeding phenotype that can significantly impact upon quality of life. Despite its prevalence and associated morbidity, the diagnosis and subclassification of VWD continue to pose significant clinical challenges. This is in part attributable to the fact that plasma von Willebrand factor (VWF) levels vary over a wide range in the normal population, together with the multiple different physiological functions played by VWF in vivo. Over recent years, substantial progress has been achieved in elucidating the biological roles of VWF. Significant advances have also been made into defining the pathophysiological mechanisms underpinning both quantitative and qualitative VWD. In particular, several new laboratory assays have been developed that enable more precise assessment of specific aspects of VWF activity. In the present review, we discuss these recent developments in the field of VWD diagnosis, and consider how these advances can impact upon clinical diagnostic algorithms for use in routine clinical practice. In addition, we review some important recent advances pertaining to the various treatment options available for managing patients with VWD.

DOI: 10.1111/bjh.16681

17 Higgins RA, Goodwin AJ. Am J Hematol. 2019;94(4):496-503. (free)

Automated assays for von Willebrand factor activity.

"Good correlation was demonstrated between VWF:GPIbM and VWF:RCo, and the mean difference between methods was 6 IU/dL to 7 IU/dL lower by VWF Ac INNOVANCE. Analytical performance characteristics were most extensively evaluated for Sysmex coagulation instruments."

von Willebrand factor (VWF) ristocetin cofactor activity (VWF:RCo) by platelet aggregometry has been considered the gold standard for evaluating the ability of VWF to bind platelets for over 40 years. Many automated systems no longer require platelets and rather rely on agglutination of latex particles. Automated methods of measuring VWF activity have improved performance characteristics and are performed on the same coagulation instruments used for routine testing via immunoturbidimetric methodology. Alternatively, a newer chemiluminescence assay system for measuring VWF activity demonstrates excellent performance characteristics. As these methods are becoming widely used, it is important to assess their performance in diagnosing and monitoring different types of von Willebrand disease. We review the automated methodologies and the published performance of these VWF assays. Advantages and limitations of these automated methods are discussed.

DOI: 10.1002/ajh.25393

18 Wasef E. N Z J Med Lab Sci. 2016;70:30-40.

Is it time for ristocetin to step down? Comparison study between a new automated von Willebrand factor activity assay and the von Willebrand factor ristocetin activity assay.

Background: von Willebrand disease (VWD) is one of the most frequent bleeding disorders; arising from absence or dysfunction of von Willebrand factor (VWF), a multimer plasma protein. Laboratory diagnosis of VWD requires an accurate detection and measurement of VWF in the patient's plasma. These measurements are required for classification and are a guide for best patient management. To date there is no one functional test available to detect VWD; rather a panel of testing is used to classify the disease, including VWF: RCo (ristocetin cofactor), VWF: CB (collagen binding) and VWF: Ag (antigen). Currently the test of choice for functional screening is VWF activity using ristocetin as a cofactor (VWF: RCo). Although this test is widely used, it lacks sensitivity and precision with a very high coefficient of variation. Moreover, no other available screening test has been established to replace VWF: RCo.

Aim: To evaluate the diagnostic efficiency and accuracy of the INNOVANCE VWF Ac assay for measurement of VWF activity based on binding of VWF to platelet GPIb receptors using polystyrene particles coated with an antibody against GPIb.

Methods: 30 normal, healthy donor samples and 30 patients previously diagnosed with VWD were assessed for VWF: RCo, INNOVANCE VWF Ac and collagen binding assays. Results were analysed for correlation between these three assays and sensitivity and specificity for INNOVANCE VWF Ac.

Results: There was a good correlation between the VWF:Ac and VWF:RCo assays, with the VWF:Ac being superior in sensitivity and limit of detection over the VWF:RCo assay.

Conclusions: Our results showed that the INNOVANCE VWF Ac assay is a suitable activity assay that fulfils laboratory diagnosis of VWD and, after assessment of all VWD types and response to therapy, can replace the VWF:RCo assay in the screening panel for VWD.

DOI: 10.3316/informit.260151988932585

19 Leebeek FW, Eikenboom JC. N Engl J Med. 2016;375(21):2067-80.

Von Willebrand's disease

"The VWF:RCo assay may be replaced by newer assays that measure the binding of VWF to a recombinant wild-type GPIb fragment with the use of ristocetin or the spontaneous binding of VWF to a gain-of-function recombinant mutant GPIb fragment."

DOI: 10.1056/NEJMra1601561

20 Patzke J, Favoloro EJ. Methods Mol Biol. 2017;1646:453-60.

Laboratory testing for von Willebrand factor activity by glycoprotein Ib binding assays (VWF:GPIb).

"However, the available studies indicate a very good comparability of VWF:GPIb results to VWF:RCo results."

"A sample with an implausible VWF:GPIb result for a specific method should be investigated with the platelet based VWF:RCo assay or other VWF:GPIb methods."

In addition to assessment of von Willebrand factor (VWF) antigen (VWF:Ag), the first-line laboratory investigation of possible von Willebrand disease (VWD) often includes an assay to measure GPIb (glycoprotein Ib) binding activity of VWF. A decreased GPIb binding activity is characteristic for most of the VWD types. For many years, the most frequently used assay for measuring GPIb binding activity was the ristocetin cofactor assay (VWF:RCo), which measures the agglutination of fixed human platelets by VWF in the presence of ristocetin. Because of performance issues, including high assay variability and a lack of VWF sensitivity, this assay is currently being replaced or supplemented by assays based on the binding of VWF to recombinant GPIb. One published method (now abbreviated VWF:GPIbR) uses wild-type GPIb for triggering the binding reaction in the presence of ristocetin. Another more widely used method (now abbreviated VWF:GPIbM) uses gain-of-function GPIb without ristocetin; this permits spontaneous binding of VWF to GPIb and avoids problems associated with the nonphysiological substance ristocetin. The binding of VWF to GPIb can be quantified by using different principles, e.g., ELISA, particle agglutination, or chemiluminescence. The following chapter describes a ristocetin-free method based on particle agglutination in more detail.

DOI: 10.1007/978-1-4939-7196-1_33

Studies involving the INNOVANCE VWF Ac assay

21 Favaloro EJ, Bonar R, Hollestelle MJ, et al. Thromb Res. 2018;166:96-105.

Differential sensitivity of von Willebrand factor activity assays to reduced VWF molecular weight forms: a large international cross-laboratory study.

"Activity assays have variable utility, in part due to differential sensitivity to high molecular weight (HMW) VWF.

"This study identifies HMW sensitivity in the order VWF:CB, VWF:GPIbM, VWF:RCo, VWF:GPIbR, VWF:Ab. This study identifies HMW sensitivity in the order VWF:CB, VWF:GPIbM, VWF:RCo, VWF:GPIbR, VWF:Ab."

"The lowest CVs (below 12.5%) were observed for VWF:Ag (LIA), VWF:GPIbM and VWF:GPIbR methods."

Introduction: von Willebrand disease (VWD), the most common inherited bleeding disorder, is due to deficiencies/defects in von Willebrand factor (VWF). Effective diagnosis requires testing for FVIII, VWF antigen and one or more VWF 'activity' assays. Classically, 'activity' is assessed using ristocetin cofactor (VWF:RCo), but collagen binding (VWF:CB) and/or other assays are used by many laboratories. This extensive international cross-laboratory study has specifically evaluated contemporary VWF activity assays for comparative sensitivity to reduction in high molecular weight (HMW) VWF, and their ability to differentiate type 1 vs 2A VWD-like samples.

Materials and methods: A set of four samples representing step wise reduction in HMW VWF were tested by over 400 laboratories worldwide using various assays. A second set of two samples representing type 1 or type 2A VWD-like plasma was tested by a subset of 251 laboratories.

Results: Combined data identified some differences between VWF activity assays, with sensitivity for reduction of HMW being highest for VWF:CB and VWF:GPIbM, intermediate for VWF:RCo and VWF:GPIbR, and lowest for VWF:Ab. 'Within' method analysis identified the Stago method as the most sensitive VWF:CB assay. A large variation in inter-laboratory CV (e.g., 7-24% for the normal sample) was also demonstrated for various methods. Although performance of various methods differed significantly, most laboratories correctly differentiated between type 1 and 2 samples, irrespective of VWF activity assay employed.

Conclusions: These results hold significant clinical implications for diagnosis and therapy monitoring of VWD, as well as potential future diagnosis and therapy monitoring of thrombotic thrombocytopenic purpura (TTP).

DOI: 10.1016/j.thromres.2018.04.015

22 Szederjesi A, Baronciani L, Budde U, et al. J Thromb Haemost. 16:1604-13. (free)

An international collaborative study to compare different von Willebrand factor glycoprotein Ib binding activity assays: the COMPASS-VWF study.

Method comparison study of 5 assays (including INNOVANCE VWF Ac) versus VWF:RCo, including 53 healthy controls and 42 well-characterized VWD patients (type 1, 2 and 3):

"All VWF activity assays correlated well with each other and the VWF:RCo assay."

Essentials

New VWF activity assays are increasingly used but information on their comparability is limited.

This is an ISTH SSC-organized study (expert labs, 5 countries) to compare all available assays. VWF activity by six assays correlated well with each other.

The new assays show improved characteristics - minor differences are noted.

Summary

Background: Several new assays have become available to measure von Willebrand factor (VWF) activity. The new assays appear to have improved performance characteristics compared with the old reference standard, ristocetin cofactor activity (VWF:RCo), but information is limited about how they compare with VWF:RCo and each other.

Of special interest

Methods: The von Willebrand factor Subcommittee of the International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) designed a collaborative study involving expert laboratories from several countries to compare available tests with each other and with VWF:RCo. Eight laboratories from five countries were provided with blinded samples from normal healthy individuals and well-characterized clinical cases. Laboratories measured VWF activity using all tests available to them; data from six laboratories, not affected by thawing during transportation, are included in this study.

Results: All tests correlated well with VWF:RCo activity (r-values ranged from 0.963 to 0.989). Slightly steeper regression lines for VWF:Ab and VWF:GPIbM were clinically insignificant. The new assays showed improved performance characteristics. Of the commercially available assays, the VWF:GPIbR using the AcuStar system was the most sensitive and could reliably detect VWF activity below 1 IU dL-1 . The lower limit of the measuring interval for the VWF:GPIbM and the VWF:GPIbR assays was in the 3-4 and 3-6 IU dL-1 range, respectively. Inter-laboratory variation was also improved for most new assays.

Conclusion: All VWF activity assays correlated well with each other and the VWF:RCo assay. The slight differences in characteristics found in the COMPASS-VWF study will assist the VWF community in interpreting and comparing activity results.

DOI: 10.1111/jth.14206

23 Boender J, Eikenboom J, van der Bom JG, et al. J Thromb Haemost. 2018;16(12):2413-24. (free)

Clinically relevant differences between assays for von Willebrand factor activity.

VWF:RCo was not sensitive enough to classify 18% of patients and misclassified half of genotypic 2B VWD patients, especially those with p.Arg1306Trp.

VWF:GPIbM was the most precise assay but misclassified over a quarter of genotypic 2A, 2B and 3 patients. Unexpected high levels >5% in 8 of 21 genotype 3 samples, which is in contrast to previous studies.

Essentials

It is unclear whether there are differences between von Willebrand factor (VWF) activity assays.

We compared the four most used VWF activity assays in 661 von Willebrand disease (VWD) patients.

All assays correlated excellently, but a discrepant classification was seen in 20% of patients.

Differences between VWF activity assays have a large impact on the classification of VWD.

Summary

Background: Measuring the ability of von Willebrand factor (VWF) to bind to platelets is crucial for the diagnosis and classification of von Willebrand disease (VWD). Several assays that measure this VWF activity using different principles are available, but the clinical relevance of different assay principles is unclear.

Objective: To compare the four most widely used VWF activity assays in a large VWD patient population.

Methods: We measured VWF:RCo (ristocetin to activate VWF + whole platelets), VWF:GPIbR (ristocetin + platelet glycoprotein Ib receptor [GPIb] fragments), VWF:GPIbM (gain-of-function GPIb fragments that bind VWF spontaneously without ristocetin) and VWF:Ab (monoclonal antibody directed against the GPIb binding epitope of VWF to mimic platelets) in 661 VWD patients from the nationwide 'Willebrand in the Netherlands' (WiN) Study.

Results: All assays correlated excellently (Pearson $r > 0.9$), but discrepant results led to a different classification for up to one-fifth of VWD patients. VWF:RCo was not sensitive enough to classify 18% of patients and misclassified half of genotypic 2B VWD patients, especially those with p.Arg1306Trp. VWF:GPIbR was more sensitive, accurately classified the vast majority of patients, and was unaffected by the p.Asp1472His variant that causes artificially low VWF:RCo. VWF:GPIbM was the most precise assay but misclassified over a quarter of genotypic 2A, 2B and 3 patients. VWF:Ab, often not considered an actual VWF activity assay, performed at least equally to the other assays with regard to accurate VWD classification.

Conclusion: Although the different VWF activity assays are often considered similar, differences between assays have a large impact on the classification of VWD.

DOI: 10.1111/jth.14319

24 Vangenechten I, Mayger K, Smejkal P, et al. J Thromb Haemost. 2018;16(7):1268-77. (free)

A comparative analysis of different automated von Willebrand factor glycoprotein Ib-binding activity assays in well typed von Willebrand disease patients.

Essentials

Von Willebrand ristocetin cofactor activity (VWF:RCo) is not a completely reliable assay.

Three automated VWF activity assays were compared within a von Willebrand disease (VWD) cohort.

Raw values for all three assays were virtually the same.

An overall problem within type 2A/IIE VWD using VWF:GPIb-binding activity/VWF:Ag was observed.

"INNOVANCE seems to be the best choice as a first-line VWF:GPIb-binding activity assay, providing the best balance between sensitivity and specificity for type 2 VWD."

"Although no significant difference between those three assays was seen...the INNOVANCE has the advantage of no longer having to rely upon ristocetin, and as such is not influenced by VWF polymorphisms (e.g. p.D/H1472 and p.P/S1467) [5] affecting the capacity of ristocetin to close the VWF A1 domain loop in vitro, but there could conceivably be circumstances in which the presence of the gain-of-function mutations could potentially misrepresent or even result in increased values for VWF:GPIb-binding activity."

Summary

Background: von Willebrand disease (VWD) is an inherited bleeding disorder caused by quantitative (type 1 and 3) or qualitative (type 2) von Willebrand factor (VWF) defect. VWD diagnosis and classification require numerous laboratory tests. VWF: glycoprotein Ib (GPIb)-binding activity assays are used to distinguish type 1 from type 2 VWD.

Objectives: Three different automated VWF:GPIb-binding activity assays were compared. Patients and methods BC-VWF:RCo (Siemens Healthcare Diagnostics), HemosIL® VWF:RCo (Instrumentation Laboratory) and INNOVANCE VWF:Ac (Siemens Healthcare Diagnostics) were performed in a well typed VWD cohort (n = 142).

Results: Based on the three most used VWD parameters (FVIII:C, VWF:Ag and VWF:GPIb-binding activity) and using a cut-off of <0.70 for type 2 VWD revealed sensitivity and specificity of, respectively, 92% and 72.4% for VWF:RCo/VWF:Ag, 84% and 89.7% for VWF:GPIbR/VWF:Ag, and 92% and 85.1% for VWF:GPIbM/VWF:Ag, whereas a lowered cut-off of < 0.60 resulted in reduced sensitivity with increased specificity for all assays.

Conclusion: VWD classification based on FVIII:C, VWF:Ag and VWF:GPIb-binding activity revealed an overall problem with normal VWF:GPIb-binding activity/VWF:Ag within type 2, especially type 2A/IIE. Although all assays were practically identical, BC-VWF:RCo had higher %CV compared with both new assays but comparable lower limit of quantification (LLOQ) ~4 IU dL-1 . No clear improved distinction between type 1 and 2 VWD with new assays was seen.

BC-VWF: RCo and HemosIL® are ristocetin dependent, whereas INNOVANCE does not rely upon ristocetin and is not influenced by VWF polymorphisms increasing VWF:GPIb-binding activity levels. INNOVANCE seems to be the best choice as a first-line VWF:GPIb-binding activity assay, providing the best balance between sensitivity and specificity for type 2 VWD.

DOI: 10.1111/jth.14145

25 de Maistre E, Volot F, Mourey G, et al. Thromb Haemost. 2014;112:825-30

Performance of two new automated assays for measuring von Willebrand activity: HemosIL AcuStar and INNOVANCE

"Our results showed that both new tests could replace the "gold standard" VWF:RCo in aggregometry with several benefits: they are fully automated, easier and faster to perform, better adapted to emergency situations if necessary."

"However, the activity/antigen ratio obtained with VWF:Ac INNOVANCE seemed to be better than that obtained by the two other methods at predicting type 2B VWD."

The ristocetin cofactor activity assay (VWF:RCo) is the reference method for assessing von Willebrand factor (VWF) activity but remains difficult to perform, and the coefficient of variation of the method is high (about 20-30%). This study evaluated and compared the performance for measuring the VWF activity of two newly commercialised assays [VWF:Ac INNOVANCE (VWF:Ac) and VWF:RCo Acustar

(VWF:RCo Acu)] with the reference VWF:RCo aggregation in 123 pathological plasma samples. The correlation and concordance between both new tests (VWF:RCo-Acu and VWF:Ac) and the reference VWF:RCo were good. The results of the VWF activity to VWF antigen ratio were also comparable whatever the method for the classification of VWF deficiency in all patients. Our results showed that both new tests could replace the "gold standard" VWF:RCo in aggregometry with several benefits: they are fully automated, easier and faster to perform, better adapted to emergency situations if necessary.

DOI: 10.1160/TH14-02-0108

26 Graf L, Moffat KA, Carlino SA, et al. Int J Lab Hematol. 2014;36(3):341-51.

Evaluation of an automated method for measuring von Willebrand factor activity in clinical samples without ristocetin.

"We conclude that INNOVANCE VWF Ac is suitable for the diagnosis, classification, and monitoring of VWD, and that it has a number of advantages over VWF:RCo method."

"Our study, and the recent report... provide considerable evidence that replacing VWF:RCo with VWF:Ac in VWD screens is acceptable to evaluate patients for bleeding disorders with quantitative or qualitative defects in VWF binding to GPIba. More patients were diagnosed as having qualitative VWF abnormalities using VWF:Ac than VWF:RCo, and we suspect that this reflects better sensitivity and precision along with an improved detection of some functional abnormalities."

"These observations indirectly suggest that replacing VWF:RCo with VWF:Ac might reduce the number of false-negative VWD screens, for both congenital and acquired defects."

Introduction: The development of an automated, von Willebrand factor (VWF) activity assay, INNOVANCE VWF Ac (VWF:Ac), which measures VWF binding to the platelet receptor glycoprotein Ibα without ristocetin, led us to evaluate the assay for diagnosing von Willebrand disease (VWD) and monitoring therapy.

Methods: After validating that the assay could be performed on an instrument from a different manufacturer, we compared VWF:Ac to VWF ristocetin cofactor activity (VWF:RCo) findings, including ratios of activity/antigen, for 100 healthy controls and 262 consecutive clinical samples from 217 patients (197 adults, 64 children, n = 1 age unknown) referred for VWF testing.

Results: There was excellent correlation (R(2) = 0.96) between VWF:Ac results run at two different sites on two different instruments. VWF:Ac had greater precision and sensitivity to low levels of VWF than the VWF:RCo method. Although there was good correlation between VWF:Ac and VWF:RCo results among healthy controls and patient subjects, VWF:Ac results were undetectable and/or significantly lower than VWF:RCo among patients who had types 2A, 2B, or 2M VWD. Additionally, a higher proportion of patient samples were classified as showing qualitative defects using the VWF:Ac compared with VWF:RCo method. While most samples drawn on VWD therapy had similar VWF levels by VWF:Ac and VWF:RCo, a type 2B VWD subject on replacement had much lower activity estimated by VWF:Ac.

Conclusion: We conclude that INNOVANCE VWF Ac is suitable for the diagnosis, classification, and monitoring of VWD, and that it has a number of advantages over VWF:RCo method.

DOI: 10.1111/ijlh.12218

27 Favaloro EJ, Mohammed S. Thromb Res. 2014;134(6):1292-300.

Towards improved diagnosis of von Willebrand disease: comparative evaluations of several automated von Willebrand factor antigen and activity assays.

"VWF:RCo and VWF:Ac are largely interchangeable."

"Importantly, the VWF:Ac assay seemed equally sensitive to HMW deficiency as compared to both VWF:RCo and VWF:CB."

"Again, there was greater concordance between VWF:RCo/VWF:Ag and VWF:Ac/VWF:Ag ratios. "

"Information provided by other EQA organizations similarly informs on the increasing usage of this assay in normal test practice."

Introduction: von Willebrand disease (VWD) is reportedly the most common bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF). Laboratory diagnosis and typing has important management implications and requires a wide range of tests, including VWF activity and antigen, and involves differential identification of qualitative vs quantitative defects.

Methods: We have assessed several VWF antigen and activity assays (collagen binding [VWF:CB], ristocetin cofactor [VWF:RCo] and the new Siemens Healthineers INNOVANCE assay [VWF:Ac], employing latex particles and gain of function recombinant glycoprotein Ib to facilitate VWF binding and agglutination without need for ristocetin) using different instrumentation, including the new Sysmex CS-5100, with a large sample test set (n=600). We included retrospective plus prospective study designs, and also evaluated desmopressin responsiveness plus differential sensitivity to high molecular weight VWF.

Results: VWF:Ag and VWF:RCo results from different methods were respectively largely comparable, although some notable differences were evident, including one high false normal VWF:Ag value (105 U/dL) on a type 3 VWD sample, possibly due to heterophile antibody interference in the latex-based CS-5100 methodology. VWF:Ac was largely comparable to VWF:RCo, but VWF:CB showed discrepant findings to both VWF:RCo and VWF:Ac with some patients, most notably patients with type 2M VWD.

Conclusions: (a) VWF:Ag on different platforms are largely interchangeable, as are VWF:RCo on different platforms, except for occasional (some potentially important) differences, and manufacturer recommended methods may otherwise require some assay optimization; (b) VWF:RCo and VWF:Ac are largely interchangeable, except for occasional differences that may also relate to assay design (differing optimizations); (c) VWF:CB provides an additional activity to supplement VWF:RCo or VWF:Ac activity assays, and is not interchangeable with either.

DOI: 10.1016/j.thromres.2014.09.024

28 Patzke J, Budde U, Huber A, et al. Blood Coagul Fibrinolysis. 2014;25:860-70.

Performance evaluation and multicentre study of a von Willebrand factor activity assay based on GPIb binding in the absence of ristocetin.

"Because of the excellent performance, the new assay is perfectly suited for decision-making in the range between 4 and 70% of norm. Very strong deficiencies can be detected and a reliable activity/antigen ratio can be calculated even in the very low range of VWF levels."

The functional activity of von Willebrand factor (VWF) is most frequently measured by using the ristocetin cofactor assay (VWF:RCo). However, the method's drawbacks include unsatisfactory precision, sensitivity and availability of automated system applications. We have developed an alternative assay (INNOVANCE VWF Ac) that is based on the binding of VWF to recombinant glycoprotein Ib (GPIb). Two gain-of-function mutations were introduced into a GPIb fragment, allowing an assay format without ristocetin. Fully automated assay applications are available for the BCS/BCS XP systems and the Sysmex CS-2000i, Sysmex CA-7000, Sysmex CA-1500 and Sysmex CA-560 systems. The INNOVANCE VWF Ac assay measuring range extends from 4 to 600% VWF for all systems except the Sysmex CA-560 system. Within-device precision values were found to be between 2 and 7%. The limit of detection was below 2.2% VWF. In a study on the BCS XP system, a total number of 580 sample results yielded a correlation to the VWF:RCo assay of r equal to 0.99 (slope = 0.96). Very similar results were observed when von Willebrand disease samples type 1, 2A, 2B, 2M, 2N and 3 were investigated with the new assay and the VWF:RCo assay. The excellent performance data and comparability to VWF:RCo, together with the ease of use, led us to the conclusion that the ristocetin cofactor assay can be replaced by the new GPIb-binding assay to reliably diagnosing patients with von Willebrand disease.

DOI: 10.1097/MBC.0000000000000169

29 Lawrie AS, Stufano F, Canciani MT, et al. Haemophilia. 2013;19(2):338-42. (free)

A comparative evaluation of a new automated assay for von Willebrand factor activity.

"The INNOVANCE VWF Ac assay was shown to be reliable and precise."

The ristocetin cofactor assay (VWF:RCo) is the reference method for assessing von Willebrand factor (VWF) activity in the diagnosis of von Willebrand's Disease (VWD). However, the assay suffers from poor reproducibility and sensitivity at low levels of VWF and is labour intensive. We have undertaken an evaluation of a new immunoturbidimetric VWF activity (VWF:Ac) assay (INNOVANCE VWF Ac. Siemens Healthcare Diagnostics, Marburg, Germany) relative to an established platelet-based VWF:RCo method. Samples from 50 healthy normal subjects, 80 patients with VWD and 50 samples that exhibited 'HIL' (i.e. Haemolysis, Icterus or Lipaemia) were studied. VWF:Ac, VWF:RCo and VWF:Ag were performed on a CS-analyser (Sysmex UK Ltd, Milton Keynes, UK), all reagents were from Siemens

Of special interest

Healthcare Diagnostics. The VWF:Ac assay, gave low intra- and inter-assay imprecision (over a 31-day period, n = 200 replicate readings) using commercial normal (Mean 96.2 IU dL(-1), CV < 3.0%) and pathological (Mean 36.1 IU dL(-1), CV < 3.5%) control plasmas. The normal and clinical samples exhibited good correlation between VWF:RCo (range 3-753 IU dL(-1)) and VWF:Ac (rs = 0.97, P < 0.0001), with a mean bias of 5.6 IU dL(-1). Ratios of VWF:Ac and VWF:RCo to VWF:Ag in the VWD samples were comparable, although VWF:Ac had a superior lower level of detection to that of VWF:RCo (3% and 5% respectively). A subset (n = 97) of VWD and HIL samples were analysed for VWF:Ac at two different dilutions to assess the effect on relative potency, no significant difference was observed (P = 0.111). The INNOVANCE VWF Ac assay was shown to be reliable and precise.

DOI: 10.1111/hae.12064

30 Szederjesi A, Baronciani L, Budde U. J Thromb Haemost. 2020;18(10):2513-23. (free)

Comparison of von Willebrand factor platelet-binding activity assays: ELISA overreads type 2B with loss of HMW multimers

Background: A number of new assays with different measuring principles are available to measure von Willebrand factor (VWF) glycoprotein Ib (GPIb)-binding activity, but little is known about how these assays might behave differently for subtypes of von Willebrand disease (VWD).

Objectives: The Comparison of Assays to Measure VWF Activity (COMPASS-VWF) study was designed to compare all available VWF GPIb-binding activity assays for VWF. We specifically searched for particular assay behavior differences.

Patients/methods: To sort out random differences from systematic assay behavior deviations, all assays were performed in different laboratories on the same samples in a blinded fashion. Samples from 53 normal controls and 42 well-characterized VWD patients were reanalyzed in this study to dissect assay-specific discrepancies.

Results: No assay behavior differences were found for 53 normal controls. For VWD patients, we found the following systematic assay behavior patterns: (a) All ELISA assays for VWF:GPIbR as well as VWF:GPIbM are insensitive to detect the low VWF activity of VWD type 2B patients with loss of high molecular weight multimers; (b) VWF:Ab assay reports higher activity for the p.V1665E mutation than all other assays; and (c) all ristocetin-based assays (including VWF:RCo using fixed platelets) but the AcuStar assay report discrepantly low VWF activity for the p.P1467S polymorphism. No systematic assay-specific difference was observed for either the particle agglutination VWF:GPIbM assay or the AcuStar assay using magnetic beads.

Conclusions: Different assay principles may lead to discrepant results for certain VWD types or mutations. Therefore, a more extensive study for a large number of patients is needed to better characterize the incidence and relevance of such assay-specific differences.

DOI: 10.1111/jth.14971

31 Bowman M, Rimmer E, Houston DS, Israels SJ, James P. Haemophilia. 2018;24(2):e57-e59.

Discordant von Willebrand factor (VWF) activity in patients with VWF p.Gly1324Ser confirmed in vitro.

No abstract available.

"The results of our cellular studies are consistent with the patients' laboratory tests showing abnormal VWF activity levels using VWF:RCo and VWF:GPIbM assays but normal results when testing with the VWF:Ab assay."

DOI: 10.1111/hae.13401

32 Bowyer AE, Guy S, Shepherd MF, Sampson BM, Kitchen S, Makris M. Haemophilia. 2016;22(1):e74-e76

Von Willebrand factor activity assay errors.

No abstract available.

"The results presented in this report clearly demonstrate that some automated VWF activity assays may misdiagnose patients with AVWS and VWD. When using these VWF activity assays, which do not include ristocetin and platelets, in the initial diagnosis of acquired haemophilia and VWS, it is imperative that a VWF antigen should be tested at the same time."

PMID: 26635234 DOI: 10.1111/hae.12862

33 Favaloro EJ, Mohammed S. Thromb Res. 2016b;141:202-211. (free)

Evaluation of a von Willebrand factor three test panel and chemiluminescent-based assay system for identification of, and therapy monitoring in, von Willebrand disease.

"VWF:RCo by agglutination yielded good comparability to HemosIL AcuStar VWF:RCo (and INNOVANCE VWF Ac)."

Von Willebrand disease (VWD) is reportedly the most common bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF). Laboratory diagnosis and typing of VWD has important management implications and requires a wide range of tests, including VWF antigen (VWF:Ag) and various activities, involving differential identification of qualitative vs quantitative VWF defects. We have assessed a new hemostasis instrument, the chemiluminescent assay based ACL AcuStar™, and an associated HemosIL AcuStar three test panel comprising VWF:Ag, VWF ristocetin cofactor (VWF:RCo) and VWF collagen binding (VWF:CB) (Instrumentation Laboratory, Bedford, MA, USA) for ability to identify VWD, to help provisionally type VWD, and for potential use in therapy monitoring. This test system was compared to previously evaluated and validated test systems including VWF:RCo on CS-5100 and BCS analyzers, the new Siemens Healthineers INNOVANCE assay (VWF Ac) on CS-5100, and VWF:Ag and VWF:CB assays performed by automated ELISA. We employed a large total sample test set (n=535) comprising plasma and platelet-lysate samples from individuals with and without VWD, some on treatment, normal plasmas, and normal and pathological controls. We also evaluated desmopressin (DDAVP) responsiveness, plus differential sensitivity to reduction in high molecular weight (HMW) VWF. The chemiluminescent test panel (VWF:Ag, VWF:RCo, VWF:CB) showed good comparability to similar assays performed by alternate methods, and broadly similar data for identification of VWD, provisional VWD type identification, DDAVP and VWD therapy, and HMW VWF sensitivity, although some notable differences were evident. The chemiluminescent system showed best low level VWF sensitivity, and lowest inter-assay variability, compared to all other systems. In conclusion, we have validated the ACL AcuStar and the chemiluminescent HemosIL AcuStar VWF test panel for use in VWD diagnostics, and have identified some favorable characteristics that may improve the future diagnosis of VWD.

DOI: 10.1016/j.thromres.2015.12.010

34 Favaloro EJ, Oliver S, Mohammed S, Vong R. Haemophilia. 2020a;26(3):503-512

Comparative assessment of von Willebrand factor multimers vs activity for von Willebrand disease using modern contemporary methodologies.

Introduction: Diagnosis of von Willebrand disease (VWD) is challenging due to heterogeneity of VWD and test limitations. Many von Willebrand factor (VWF) assays are utilized, including antigen (Ag), activity and multimer analysis. Activity assays include ristocetin cofactor using platelets (VWF:RCo) or other particles incorporating recombinant glycoprotein I ('VWF:GPIbR'), or other GPI binding assays using gain-of-function mutations ('VWF:GPIbM'), or collagen binding (VWF:CB).

Aim: To comparatively evaluate modern contemporary VWF activity assays vs VWF multimer analysis using modern contemporary methods.

Materials and methods: Several VWF activity assays (VWF:RCo, VWF:GPIbR, VWF:GPIbM, VWF:CB) assessed (typically as a ratio against VWF:Ag) against a new semi-automated procedure for different types of VWD (1, 3, 2A, 2B, 2M), plus control material (n = 580). The evaluation also focussed on relative loss of high and very high molecular weight multimers (HMWM and VHMWM) by densitometric scanning.

Results: All evaluated VWF activity/Ag ratios showed high correlation to the presence/absence of HMWM and VHMWM, although VWF:CB/Ag and VWF:GPIbR/Ag ratios using an automated chemiluminescence method yielded highest correlation coefficients

(r = .909 and .874, respectively, for HMWM). Use of the investigative procedure (VHMWM) identified fewer false positives for 'loss' in type 1 VWD.

Conclusions: This comparative investigation identified that new automated chemiluminescence VWF activity assays best identified relative loss or presence of HMWM and VHMWM according to activity to Ag ratios and an alternative investigative method for identifying VHMWM in multimer testing for a new commercial multimer method may lead to fewer false identifications of HMW loss in type 1 VWD.

DOI: 10.1111/hae.13957

35 Gardiner C, Lane P, Tailor H, Mackie IJ. Int J Lab Hematol. 2020 Apr;42(2):140-4. (free)

A practical method for reducing the interference due to lipaemia in coagulation tests.

Introduction: Plasma samples with gross lipaemia present a challenge for coagulation laboratories using optical analysers. High-speed centrifugation may be used to remove excess lipids but it has not established whether this affects haemostasis tests. The aims were to determine whether the removal of lipid by centrifugation affects PT, APTT, fibrinogen, D-dimer and von Willebrand factor activity measurements.

Methods: Twenty-six lipaemic samples (median [range]): triglyceride 4.6 mmol/L [0.5–17.0]; cholesterol: 4.06 mmol/L [2.20–9.41] and 20 plasmas spiked with Intralipid 20 or lipid isolated from patient plasmas (median triglyceride of 11.95 mmol/L [5.0–17.0] and cholesterol 4.33 [3.22–7.06]), were tested before and after the removal of the lipid layer by centrifugation (10000 g for 10 minutes). Tests were performed using the CS-5100 (Sysmex) coagulation analyser.

Results: Thirteen, 9, 3 and 1 of the lipaemic or spiked samples failed to give PT, APTT, fibrinogen and D-dimer results, respectively. Centrifugation significantly reduced triglyceride (median 2.7, [0–6.1 mmol/L]) and cholesterol (median 0.52 [0–3.5]), allowing clot detection in all tests. There were no statistically significant differences in fibrinogen, D-dimer or VWF levels in samples before and after lipid removal. A small but clinically insignificant change in PT and APTT was observed after lipid removal.

Conclusion: High-speed centrifugation reduces lipaemia sufficiently to allow testing on an optical coagulation analyser without introducing clinically significant differences PT, APTT, fibrinogen, D-dimer or VWF activity values.

DOI: 10.1111/ijlh.13129

36 Florin C, Garraud O, Molliex S, Tardy B, Campos L, Scherrer C. Ann Biol Clin (Paris). 2016 Jun 1;74(3):355-64. (free)

Biological diagnosis of von Willebrand disease: analytical characteristics of INNOVANCE vWF:Ac assay kit on STA-R Evolution Expert series analyzer (Stago).

The INNOVANCE VWF:Ac test (Siemens Healthineers) has the particularity to assess the binding capacity of von Willebrand factor (VWF) to recombinant platelet GPIb mutated in the absence of ristocetin. Our study aimed to evaluate and validate according to standard NF EN ISO 15189 the original protocol adaptation on STA-R Evolution series analyser (Diagnostica Stago). We evaluated the performance in terms of imprecision and we validate additional parameters necessary in range B as recommended by the SH GTA 04 (Cofrac). We compared the new assay with the reference assay: ristocetin cofactor activity (VWF:RCo) performed on the BCS-XP analyser by testing retrospectively samples from 82 healthy normal subjects and 61 patients with von Willebrand disease (VWD). This new assay is consistent with objectives set in terms of imprecision with CV around 4%. Excepted limit of quantification higher, additional parameters evaluated in range B have been validated. The INNOVANCE VWF: Ac assay allowed the detection of all deficits of VWF already detected by the VWF:RCo test on the BCS-XP. This adjustment on STA-R analyser therefore has satisfactory analytical performance criteria. Apart from the limit of quantification, this reagent can be used according to the recommendations specified in the original protocol adaptation. Its performance and compatibility with the spot measurement allow the diagnosis and therapeutic monitoring of VWD according to current requirements and guidelines.

DOI: 10.1684/abc.2016.1145

37 Geisen U, Zieger B, Nakamura L, Weis A, Heinz J, Michiels JJ, Heilmann C. Thromb Res. 2014 Aug;134(2):246-50.

Comparison of Von Willebrand factor (VWF) activity VWF:Ac with VWF ristocetin cofactor activity VWF:RCo.

Introduction: Ristocetin cofactor activity of Von Willebrand factor (VWF:RCo) and the ratio VWF:RCo to its antigen VWF:Ag are used as routine screening to estimate VWF function and to detect types of Von Willebrand disease (VWD) caused by loss of high molecular weight multimers. However, the VWF:RCo test is prone to analytic imprecisions due to various reasons. We compared an assay for VWF activity (VWF:Ac) with VWF:RCo putting emphasis on the ratios to VWF:Ag.

Materials and methods: We evaluated 942 samples from 432 patients and evaluated three groups in detail: normal patients (normal multimers, VWF:Ag and VWF:RCo >0.5 U/ml, VWD type 1 excluded; n=258), VWD type 1 (n=76) and acquired Von Willebrand syndrome (AVWS, n=326). In addition, 30 healthy subjects were analysed.

Results: VWF:Ac and VWF:RCo correlated well (Pearson´s r=0.96, p<0.01), so did their ratios to VWF:Ag (Pearson´s r=0.82, p<0.01). We calculated the normal range of VWF:Ac/VWF:Ag for healthy subjects as 0.8-1.16. In comparison, the reference range (mean±2std) derived from normal patient samples was 0.73-1.14. The corresponding ranges for VWF:RCo/VWF:Ag came to 0.74-1.23 (healthy) and 0.62-1.25 (normal patients). The ratios showed similar results regarding VWD type 1. The sensitivity for AVWS was higher with VWF:Ac/VWF:Ag than with VWF:RCo/VWF:Ag (97.5% versus 84.7%).

Conclusions: The data suggest that the results obtained with the VWF:Ac assay are comparable to that of the VWF:RCo assay. An AVWS was more reliably detected by VWF:Ac/VWF:Ag. We assume that the VWF:Ac assay could replace VWF:RCo for routine screening for AVWS, especially when prompt evaluation is required.

DOI: 10.1016/j.thromres.2014.04.033

38 Timm A, Hillarp A, Philips M, Goetze JP. Thromb Res. 2015 Apr;135(4):684-91.

Comparison of automated von Willebrand factor activity assays.

Introduction: Von Willebrand Disease (VWD) is the most common inherited bleeding disorder. Measurement of von Willebrand factor (VWF) activity in plasma is often based on platelet agglutination stimulated by the ristocetin cofactor activity. Novel assays, based on latex beads with recombinant glycoprotein Ib instead of platelets, have recently been developed but it is unclear whether these can improve the diagnostic capability for VWD.

Aim: To compare four automated VWF activity methods in a mixed population of patients referred for evaluation of bleeding tendency.

Methods: The analytical performances of three ristocetin and one non-ristocetin cofactor activity assays were compared in 170 consecutive plasma samples from patients referred for VWD evaluation.

Results: All methods correlated well with concordance correlation coefficients ranging from 0.90–0.95. However, when comparing the VWF activity/antigen ratios in samples classified as having VWD (activity <0.4 IU/mL) the number of samples below a ratio of 0.7 differed between 16 and 8%.

Conclusion: Despite overall correlation between assays we found that differences in classification power might interfere with the interpretation of individual samples.

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39 Lassalle F, Jeanpierre E, Zawadsky C, Boisseau P, Veyradier A, Rauch A, Goudemand J, Susen S. Poster presented at ISTH congress. 2020 Jul 12–14.

The VWF variant D1472H affects binding to ristocetin in vitro in the platelet agglutination assay but not with latex particels (HemosIL® Acustar VWF:GpIbR): the usefulness to change practices to avoid VWF genotyping

Background: The diagnosis of von Willebrand disease (VWD) relies on measurements of von Willebrand factor antigen (VWF:Ag) and functional activity. Until now, the gold standard assay for measuring VWF functional activity is the ristocetin cofactor activity (VWF:RCo),that quantifies the binding of VWF to platelets through GpIb, induced by ristocetin. However, some polymorphisms as the variant D1472H can falsely decrease VWF:RCo by interacting in vitro with ristocetin. Other tests are available to measure VWF activity, both using latex particles, as the HemosIL®Acustar VWF:GpIbR assay by chemiluminescence (with ristocetin) or the INNOVANCE VWF:GpIbM assay (no ristocetin).

Aims: Verify the sensitivity of VWF:GpIbR assay to the D1472H and reevaluate the practices for the biological diagnosis of VWD to avoid useless explorations and especially genotyping.

Methods: By studying the database of the French Reference Center for VWD, we noticed that 137 patients had a VWF:Ag>30% and a VWF:RCo/VWF:Ag ratio < 0.7 with local methods but were finally excluded from congenital VWD as no pathogenic variant was identified in VWF after genotyping. Among them, 79 presented the only variant D1472H. We were able to retest VWF:Ag and VWF activity with the VWF:GpIbM and the VWF:GpIbR assays for 30 patients.

Results: The VWF activity was significantly higher in 23/30 patients with the VWF:GpIbM and VWF:GpIbR methods (p< 0,0001), compared to VWF:RCo leading to a normalized ratio (p< 0,0001). Two patients still had a low ratio and 5 were discordant between the 2 methods but the results were very close to the 0.7 threshold.

Conclusions: The HemosIL VWF:GpIbR assay seems not to be sensitive to the D1472H variant, nor the INNOVANCE VWF:GpIbM assay. Genotyping could have been avoided for these patients that don't have VWD. We suggest that in case of low ratio with VWF:Ag>30%, the activity should be checked using VWF:GpIbR or VWF:GpIbM.

The VWF Variant D1472H Affects VWF Binding to Ristocetin in vitro [...] ISTH Academy. Lassalle F. Jul 11 2020; 296705

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COMPASS-VWF: an international multicenter study to compare VWF activity assays. Report on assay performance.

Background: Several new assays have become available to measure VWF activity. The new assays appear to have improved performance characteristics compared to the (g)old standard ristocetin cofactor activity (VWF:RCo), but information is limited about how they compare to VWF:RCo and each other.

Methods: The von Willebrand factor Subcommittee of the International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) designed a collaborative study involving expert laboratories from several countries to compare available tests with each other and with VWF:RCo. Eight laboratories from 5 countries were provided with blinded samples from normal healthy individuals and well characterized clinical cases. Laboratories measured VWF activity using all tests available to them; data from 6 laboratories, not affected by thawing during transportation, are included in this study.

Results: All tests correlated well with the VWF:RCo activity (r-values ranged from 0.963 to 0.989). Slightly steeper regression lines for VWF:Ab and VWF:GPIbM were clinically insignificant. The new assays showed improved performance characteristics. Of the commercially available assays, the VWF:GPIbR using the AcuStar system was the most sensitive, and could reliably detect VWF activity below 1 IU/dL. The lower limit of the measuring interval for the VWF:GPIbM and the VWF:GPIbR assays was in the 3–4 and 3-6 IU/dL range, respectively. Inter-laboratory variation was also improved for most new assays.

Conclusion: All VWF activity assays correlated well with each other and the VWF:RCo assay. The slight differences in characteristics found in the COMPASS-VWF study will assist the VWF community in interpreting and comparing activity results.

Doi: 10.1111/jth.14206

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Analytical performance of a new immunoturbidimetric assay for von Willebrand factor (VWF) activity testing.

Background: Von Willebrand disease requires laboratory confirmation with quantitative and qualitative measurements of von Willebrand factor (VWF). Qualitative VWF-activity (VWF-Ac) tests have poor inter- and intra-laboratory reproducibility with coefficients of variation (CVs) as high as 64%, often lacking accuracy at low VWF-Ac levels.

Methods: This study evaluated the recently launched immunoturbidometric STAGO® STA-VWF:RCo® reagent for VWF-Ac. Accuracy was evaluated on 32 samples by comparing results using the Siemens Healthineers INNOVANCE reagent. An intra-run reproducibility study was performed on controls. Linearity and lower limit of detection was studied on external-quality-assurance (EQA) material with a known VWF-Ac level.

Results: STA-VWF:RCo® reagent results were within clinical interpretation agreement with Siemens Healthineers INNOVANCE. The reproducibility study yielded % CVs of 8.41 for normal and 11.46 for abnormal controls and the assay was linear between 73 and 14.6% and remained linear to 2% with extrapolation.

Conclusions: The STAGO® STA-VWF:RCo® reagent showed clinically meaningful accuracy and acceptable precision.

DOI: 10.7754/Clin.Lab.2018.180919

42 Rao ES, Ng CJ. Transfusion and Apheresis Science. 2018;57:463-5.

Current approaches to diagnostic testing in von Willebrand disease.

Von Willebrand Disease (VWD) is considered the most common inherited bleeding disorder. It has multiple subtypes and a primary symptom of mucocutaneous bleeding. Some researchers in this field speculate that inherited disorders of platelet function may be as common but underdiagnosed due to the difficulty of accessing testing. The diagnostic approach for this disease has evolved as new instruments and diagnostic testing have become available. The ISTH-Bleeding Assessment Tool is a validated instrument that is used to screen patients referred for bleeding symptoms for further laboratory testing. The three main screening tests used in the diagnosis of VWD include von Willebrand Factor (VWF) antigen, platelet-dependent VWF activity, and factor VIII activity. Improvements in laboratory assays discussed include changes in how traditional assays are performed as well as the addition of new laboratory assays. The role of genetic testing and management of patients with borderline low von Willebrand factor are also discussed.

DOI: 10.1016/j.transci.2018.07.005

43 Reilly-Stitt C, Coppell J, Mumford AD. Haemophilia. 2014 Jul;20(4):e341-4.

Discrepancy in von Willebrand factor activity determined by ristocetin cofactor and immunotubidometric assays.

No abstract available.

"For 36 of the 37 test samples, there was a good correlation between the VWF:Ac and VWF:RCo measurements."

"Our findings highlight that assay artefact should be considered with the VWF:Ac assay as well as other immunoturbidometric assays, and that caution should be exercised in interpreting assay results unless concordance with a VWF:RCo reference method is confirmed and the test is performed in parallel with a VWF:Ag assay. This phenomenon is a particular hazard if the VWF:Ac assay is used alone as a screening test for VWD."

DOI: 10.1111/hae.12443

44 Michiels JJ, Smejkal P, Mayger K, Moore G, Blatny J, Penka M, Budde U, Berneman Z, Vangenechten I, Gadisseur A. International Journal of Clinical and Experimental Medical Sciences. 2019a;5(5):80-91. (free)

Superiority of the rapid von Willebrand factor (VWF) VWF:GPIbR and VWF:GPIbM assays in type 2A, 2B and 2M von Willebrand disease.

"The present Brno VWF VWD study demonstrates the superiority of the novel rapid VWF assays in detecting VWD 2A, 2B and 2M similar as has been documented by Michiels et al. in another recent report on the performance of rapid and classical assays in VWD 1, 2N and 2E."

A complete set of rapid activity and classical von Willebrand factor (VWF) assays for Willebrand disease (VWD) diagnosis was used in the present study to characterize VWD type 1, 2A, 2B and 2M patients due to mutations in the A1, A2 and A3 domains. The VWF:RCo/VWF:Ag, VWF:GPIbM/VWF:Ag and VWF:GPIbR/VWF:Ag ratios at cutoff value of 0.7 separated VWD type 1 and LowVWF from VWD type 2. The results from the Brno cohort of VWD 2A patients with the G1579R mutation in the A2 domain in sixteen affected member from five families and in one case with the G1609R in the A2 domain revealed that the VWF:GPIbM/VWF:Ag and VWF:GPIbR/VWF:Ag ratios are marked decreased (range 0.03-0.27) to a similar degree as compared to VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios (range 0.03-0.27) due to the proteolytic loss of large and intermediate VWF multimers. The results in VWD 2B patients due to gain of ristocetin induced platelet agglutination

(RIPA) function mutations R1306W, R1308C and R1341 in the A1 domain demonstrated that the ratios for VWF:GPIbM/VWF:Ag and VWF:GPIbR/VWF:Ag as compared to VWF:RCo/VWF:Ag ratio were markedly decreased in VWD 2B, whereas the VWF:GPIbM/VWF:Ag ratio was somewhat higher (range 0.32 to 0.36) in VWD 2M. VWD 2M patients due to loss of RIPA function mutation R1359K in the A1 domain are featured by decreased VWF ratios for WVF:RCo/Ag and VWF:GPIbR/Ag, but less decreased for the VWF:GPIbM/Ag ratio and normal VWF ratio for VWF:CB/Ag ratio the need to retain the VWF:CB assay to make a correct diagnosis of VWD 2M for its differentiation from VWD type 1. The G1415D mutation in the A1 domain is featured by decreased RIPA and decreased VWF:RCo/VWF:Ag ratio (VWD 2M) but normal values for VWF:CB/VWF:Ag, VWF:GPIbM/VWF:Ag and VWFGPIbR/VWF:Ag ratios consistent with VWD 2M. Double heterozygous P1266L/V1278I mutation in two patients and heterozygous E1292D/WT mutation in three patients in the A1 domain were diagnosed as VWD 2M or 1M associated with a secretion defect (SD). The Platelet Function Analyzer Closure Times (PFA-CT) are strongly prolonged in VWD 2A, 2B and 2M. and moderately prolonged between the upper limit of normal to 300 seconds in heterozygous mutated VWD type 1 patients.

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45 Moonla C, Akkawat B, Kittikalayawong Y, Sukperm A, Meesanun M, Uaprasert N, Sosothikul D, Rojnuckarin P. Clinical and Applied Thrombosis/Hemostasis. 25:1-8. (free)

Bleeding symptoms and von Willebrand factor levels: 30-year experience in a tertiary care center.

Correlations between bleeding symptoms and von Willebrand factor (VWF) levels may help to predict hemorrhagic severity in the Westerners with von Willebrand disease (VWD), but data in Asians are lacking. In this study, Thai patients with VWF levels <50 IU/dL without any secondary causes were enrolled from 1988 to 2018 to determine the relationship between VWF levels and hemorrhagic manifestations. According to the current concept, we reclassified VWD and low VWF by VWF levels ≤30 and 30 to 50 IU/dL, respectively. Type 2 VWD was diagnosed if VWF activity to antigen ratio was ≤0.6. Bleeding severity was determined by the condensed MCMDM-1VWD bleeding score (BS). Among 83 patients, VWF activities showed negative correlations with BS (P = .001), which were higher in type 2 (median: 7, interquartile range [IQR]: 5-11) compared with type 1 VWD (median: 3, IQR: 2-4) and low VWF (median: 4, IQR: 2-8). Bleeding symptoms were indistinguishable between type 1 VWD and low VWF using the 30 IU/dL cutoff point. However, VWF ristocetin cofactor activity or gain-of-function mutant glycoprotein Ib binding activity <36.5 IU/dL and VWF collagen binding activity <34.5 IU/dL could predict increased bleeding risk (BS ≥3) by 92.3% specificity and 70.0% sensitivity (P < .0001).

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