9-2-2022

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Recommended Citation

Salazar E, Long TA, Smock KJ, Wool GD, Rollins-Raval M, Chen D, Harris NS, Chan CW, Olson JD, Pham HP, Ritter J, Unold D, VanSandt AM, Goodwin Iv AJ. Analysis of College of American Pathologists von Willebrand Factor Proficiency Testing Program. Semin Thromb Hemost. 2022 Sep 2. doi: 10.1055/s-0042-1749591. Epub ahead of print. PMID: 36055272.

Analysis of College of American Pathologists von Willebrand Factor Proficiency Testing Program

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Semin Thromb Hemost

Abstract

Von Willebrand factor (VWF) level and/or function is altered in von Willebrand disease (VWD), the most common heritable bleeding disorder worldwide. Laboratory assessment of VWF is continually evolving. Historically, the primary method for the assessment of VWF platelet-binding activity was the ristocetin cofactor assay (VWF: RCo). Contemporary alternative measures of VWF platelet-binding activity include VWF:GPIbR (recombinant; using ristocetin), VWF:GPIbM (recombinant; gain-of-function mutant), and monoclonal antibody. Recently, the American Society of Hematology, International Society on Thrombosis and Haemostasis, National Hemophilia Foundation, and World Federation of Hemophilia collaboration issued guidelines recommending the use of newer assays of VWF platelet-binding activity (VWF: GPIbM, VWF: GPIbR) over VWF:RCo, given known limitations of the VWF:RCo assay. Despite this recommendation, the newer VWF:GPIbM and VWF:GPIbR assays are not United States Food and Drug Administration cleared, limiting their availability in the United States. We sought to assess assay utilization trends, agreement of VWF testing methods, and imprecision of VWF testing (based on assigned sample type) from the College of American Pathologists Proficiency Testing Surveys. The analysis confirms that, while VWF antigen testing has low imprecision, the various VWF activity assays have significant interassay variability, with VWF:RCo showing greater imprecision than the newer GPIb-binding assays. The overall trends in assay utilization reflect the barriers to complete compliance with modern VWD diagnostic guidelines in North America.

Keywords
- von Willebrand disease
- coagulation special testing
- proficiency testing

Issue Theme Laboratory Diagnostics for Thrombosis and Hemostasis Testing—Part I; Guest Editors: Robert C. Gosselin, CLS and Kristi J. Smock, MD

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0042-1749591.
ISSN 0094-6176.

Von Willebrand factor (VWF) is a multimeric, multifunctional plasma protein that plays a critical role in primary hemostasis. Circulating as a variably sized globular protein, VWF unfolds under conditions of high fluid shear stress, which can facilitate the binding between VWF and platelet surface glycoprotein Ib (GPIb)-V-IX. VWF functions include binding of subendothelial collagen at a site of vascular injury, binding and anchoring of platelets to the injured site through the platelet GPIb receptor, and serving as a carrier for factor VIII, releasing it locally near the injured site. Von Willebrand disease (VWD) can arise from a deficiency or absence of VWF and/or qualitative loss of any of the three major VWF functions. Currently, the International Society on Thrombosis and Haemostasis (ISTH) VWD classification scheme includes six different types.[1] Type 1 VWD is a partial quantitative deficiency of VWF, and type 3 is a complete absence of VWF. Type 2 VWD is a heterogeneous group of functional defects including 2A (loss of high molecular weight multimers [HMWMs] due to faulty assembly or increased proteolysis), 2B (loss of HMWMs due to aberrantly high VWF binding to platelet GPIb), 2M (loss of GPIb- or collagen binding without loss of HMWMs), and 2N (loss of FVIII binding). In addition, acquired VWD may arise in patients without inherited VWD due to multiple potential mechanisms. High fluid shear stress conditions, such as cardiac valvular disease or implantable or extracorporeal mechanical devices may lead to the consumption of HMWMs. Extreme thrombocytosis seen in myeloproliferative neoplasms can lead to the adsorption of VWF. Autoantibodies against VWF can arise in the context of autoimmune disease or plasma cell dyscrasias.[2] Lastly, patients treated with VWF concentrates, which may not contain the full complement of HMWMs, may have test results that mimic acquired VWD.

VWD is the most common heritable bleeding disorder worldwide with an epidemiologically estimated prevalence of approximately 1% of the general population.[3] The availability of treatments that mitigate and/or manage VWD-related bleeding necessitates reliable diagnostic criteria since appropriate therapies are linked to VWD types. Diagnostic criteria include the presence of bleeding symptoms, identification of similarly affected family members, and abnormal VWF test results. Owing to the multiple functions of VWF, VWD laboratory testing is increasing in complexity, with multiple different testing platforms available.[4] Historically, the primary method for the assessment of VWF platelet-binding activity was the ristocetin cofactor assay (VWF:RCo).[5,6] These assays quantitate reagent platelet agglutination in patient plasma after the addition of the VWF-activating antibiotic ristocetin. Due to high assay variability related to lyophilized reagent platelets and patient ristocetin response, as well as poor precision and limit of quantification, this assay, while long considered the gold standard for VWF platelet-binding activity, may contribute to diagnostic errors.[4] Contemporary alternative measures of VWF platelet-binding activity include VWF:GPIbR (recombinant; using ristocetin), VWF:GPIbM (gain-of-function mutant; no ristocetin), and VWF:Ab (monoclonal antibody).[7] The VWF:GPIbR assesses the ability of VWF to bind to recombinant wild-type GPIb bound to inert particles (typically latex or magnetic particles) in the presence of exogenous ristocetin. Some individuals may carry a VWF A1 domain polymorphism, such as D1472H that renders VWF insensitive to ristocetin without pathological consequences.[8] These individuals may have falsely low function in ristocetin-dependent assays but normal function in ristocetin-independent assays, which is an advantage of the assays that do not utilize ristocetin, such as the VWF:GPIbM and VWF:Ab assays. For example, the VWF: GPIbM assay employs the gain-of-function-mutated GPIb (analogous to the mutation underlying platelet-type VWD) that binds to VWF without the need for ristocetin. In contrast, the VWF:Ab assay uses a monoclonal antibody directed against the GPIb-binding region (domain A1) of VWF. Mutations in this region would disrupt platelet binding in vivo as well as antibody binding in vitro, resulting in lower measures of activity.[9]

Recently, the American Society of Hematology (ASH), ISTH, National Hemophilia Foundation (NHF), and World Federation of Hemophilia (WFH) collaboration issued guidelines on the diagnosis and management of VWD.[10] Given the known limitations of the VWF:RCo assay, the expert panel suggested the use of newer assays of VWF platelet-binding activity (VWF: GPIbM, VWF: GPIbR) over VWF:RCo. Despite this recommendation, the newer VWF:GPIbM and VWF: GPIbR assays are not United States Food and Drug Administration (U.S. FDA) cleared, significantly limiting their availability in the United States. In addition to this recommendation, the panel suggested against a platelet-dependent VWF activity/VWF antigen ratio <0.5 cutoff rather using a higher cutoff of <0.7 to identify type 2 VWD for patients with an abnormal initial VWD screen.

The College of American Pathologists (CAP) Proficiency Testing Survey program is the largest Clinical Laboratory Improvement Amendments-approved external quality assessment program in North America, providing proficiency testing worldwide, which include surveys of VWF testing. In light of the recent ASH, ISTH, NHF, WFH guidelines recommending more contemporary assays of VWF function, we sought to assess assay utilization trends, agreement of VWF testing methods, and imprecision of VWF testing (based on assigned sample type) from CAP Proficiency Testing Surveys.

Methods

The CAP proficiency testing survey program for VWF testing involved twice-yearly distribution of samples (two challenges per survey) through the CGS3 (Coagulation Special Testing VWF) surveys. The majority of CGS3 participants were from North America (157 United States and 16 Canada), with 55 other countries (broadly distributed throughout South America and Asia) based upon the 2020 CGS enrollment. The majority of samples consisted of purchased off-the-shelf products or manufactured, lyophilized plasmas prepared according to requirements set forth by the CAP Hemostasis and Thrombosis Committee (HaTC) to simulate different VWD subtypes. The Scientific and Standardization Committee (SSC)/ISTH Secondary Coagulation Standards Lot

Table 1 Sample descriptions, mean results, and ratios for samples analyzed in study from CAP CGS3 Surveys, 2015 to 2020

Mail	Intended sample type[b]	VWF:Ag mean, %	VWF:RCo mean, %	VWF:GPIb[a] mean, %	VWF:RCo/Ag ratio mean	VWF:GPIb/Ag ratio mean
2015A	Type 1	20.7	13.1	15.6	0.60	0.77
2015B	Type 2	31.6	20.0	27.1	0.64	0.79
2016A	NPP	97.5	80.0	90.8	0.86	0.89
2016B	Type 2	36.0	22.2	29.6	0.62	0.78
2017A	CPP	28.9	13.4	16.7	0.44	0.56
2017B	NPP	101.9	74.7	86.5	0.78	0.83
2018A	CPP	36.3	14.9	18.7	0.42	0.52
2018B	ISTH Lot #4	113.9	76.6	89.9	0.67	0.78
2019A	Type #2	37.7	27.2	33.7	0.75	0.85
2019B	NPP	133.1	100.3	107.6	0.78	0.80
2020A	ISTH Lot #5	115.8	86.4	94.3	0.77	0.81
2020B	Type 1	41.8	20.7	28.6	0.50	0.67

Abbreviations: CPP, cryopool plasma (type 2 like); GPIbM, recombinant; gain-of-function mutant; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; VWF:Ab, monoclonal antibody; VWF:Ag, von Willebrand factor antigen; VWF:GPIb, von Willebrand factor GPiB-based activity assays; VWF:GPIb/Ag, von Willebrand factor GPiB-based activity assay/antigen ratio; VWF:RCo, ristocetin cofactor assay; VWF:RCo/Ag, ristocetin cofactor assay/antigen ratio.

[a]Other VWF:GPIb-based assays: predominantly VWF:Ab, with some VWF:GPIbM.

[b]Results may not match intended sample type due to elements of the manufacturing process. Data are informative for method comparison but not for diagnostic performance of ratio in discriminating type 1 and type 2 samples.

#4 and Lot #5 were distributed in two challenges. Laboratories performed testing according to their test menu, regular patient testing workflow, and laboratory standard operating procedures. The results were submitted to CAP through a standardized reporting form, and the summarized data were reviewed by the HaTC.

Twelve representative proficiency testing samples (all distributed as lyophilized plasma) from surveys distributed between 2015 and 2020 were selected for analysis (see ►**Table 1** for intended sample type). These included normal pooled plasma (NPP), SSC/ISTH Lots #4 and #5 (normal coagulation standards), samples intended to represent type 1 VWD, and samples intended to be deficient in HMWMs (type 2A VWD), including cryopoor plasma (CPP). Laboratories were asked to report VWF:Ag (von Willebrand factor antigen) result, calibrator material, whether the antigen level was normal or abnormal, VWF activity, the activity method used (with the choices being VWF:RCo, collagen binding, and GPIb-binding immunobinding [other VWF:GPIb-based assays]), whether the activity was normal or abnormal, the activity to antigen ratio, and whether VWF multimer distribution was normal or abnormal (if performed).

The CAP data forms were not specifically designed to identify users of VWF:GPIbR methods, and it was presumed that there is little to no use of this method in this dataset due to the lack of availability in North America. Participants were given the option to report whether results were below the reportable range. In such cases, participants may or may not submit a numerical value indicating their lower limit of detection (analytical sensitivity).

Data from the 12 proficiency testing surveys were extracted. The number of participants per sample was defined as the number of laboratories reporting at least one VWF assay in the survey. Values reported with a "less than" value were removed from the analysis (to determine mean, standard deviation [SD], and coefficient of variation [CV]). To reduce data bias in ratio calculations, for activity results from VWF:RCo and other VWF:GPIb-based assays, the lowest assay manufacturer-recommended reportable value was assigned when laboratories indicated that the result was below their reportable range without also submitting a value for their lower limit of detection. The manufacturer provided the lowest reportable values were 19% (HemosIL VWF activity assay [VWF:Ab], Instrumentation Laboratories/Werfen, Lexington, MA) and 20% (BC Von Willebrand Reagent, Siemens Healthineers, Marburg, Germany [VWF:RCo]). The VWF activity-to-antigen ratio was calculated (regardless of whether this was submitted by the laboratory) based on the reported or imputed VWF activity and antigen results for both VWF:RCo and other VWF:GPIb-based assays. All summary statistics for ratios were assessed using these two calculated ratios. For VWF:RCo, results were additionally summarized based on whether the test was performed with automated instrumentation (Siemens BC) or manually using platelet aggregometry (all other reagent kits). For other VWF:GPIb-based assays, results were additionally summarized using the two most-represented reagent kits: HemosIL and Siemens Innovance [VWF:GPIbM]. For VWF:Ag, results were additionally summarized using the two most-represented reagent kits (both based on automated latex agglutination): HemosIL and Stago Liatest. Outliers for individual

laboratory reported or calculated VWF tests were identified by a 2-pass 3 SD scheme. The mean, median, SD, CV, and interquartile range (IQR, box on box, and whisker plots) were determined for postoutlier results from each survey for each VWF test.

All analyses were conducted with SAS (Cary, North Carolina). To test for differences in VWF assay means, an analysis of variance test was employed for each sample. Second, to test for differences in imprecision, a variance ratio test (Levene's Test) was employed for each sample. A Bonferroni correction was applied to the significance level of $\alpha = 0.05$ to control for the fact that 12 tests were conducted, one for each sample.

Results

Intended specimen type, mean assay results, and ratios are summarized in ►**Table 1**.

Von Willebrand Factor Assay and Methods Utilization

The number of laboratories reporting VWF:Ag on each specimen fluctuated between 53/62 (85%) and 55/58 (95%) participants until 2019, when analytes from other surveys were consolidated into the CGS3 survey. Subsequently, the number of laboratories reporting VWF:Ag increased to a range of 124/140 (89%) to 133/141 (95%) participants in 2019 and 2020. Most laboratories performed VWF:Ag by automated immunoassay (974/1017 results; 95%), with a minority reporting results from enzyme-linked immunoassays.

The number of laboratories reporting VWF:RCo ranged from 19/58 (33%) to 34/68 (50%) participants per sample. From 2015 to 2018, the percentage of participants reporting VWF:RCo steadily decreased from 50 to 39%. In 2019, as with VWF:Ag, the percentage of participants reporting VWF:RCo increased to 44% and this remained steady through 2020. Over half of the laboratories reporting VWF:RCo used the automated Siemens BC reagent (241/459 results; 53%), followed by manual methods reported as Bio-Data (84/459 results; 18%), Chrono-Log (49/459 results; 11%), Helena (17/459 results; 4%), laboratory-developed test (12/459 results; 3%), and "other" reagents (54/59 results; 12%).

The number of laboratories reporting other VWF:GPIb-based assays ranged from 19/68 (28%) to 63/141 (45%) participants per sample. We observed a steady increase in the percentage of participants reporting other VWF:GPIb-based assay results between 2015 and 2020 with 28% of participants reporting in 2015 and 45% of participants reporting in 2020. Laboratories reporting other VWF:GPIb-based assays indicated using either the HemosIL (presumed to be VWF:Ab) kit (331/399 results; 83%) or Siemens Innovance (VWF:GPIbM) (68/399 results; 17%) reagents. The number of laboratories reporting results for the VWF:GPIbM assay ranged from 3/68 (4%) to 12/142 (8.5%) participants per sample over the study period, and all represent international laboratories likely due to lack of availability of this method in the United States, with the potential exception of laboratory-developed methods. Although the CAP data forms were not currently designed to identify users of VWF:GPIbR methods, it is presumed that there is little to no use of this method in this dataset due to lack of availability in North America, with no evidence to the contrary. A relative minority of laboratories reported results for collagen binding, ranging from 1/68 (1.5%) participants in 2015 to 6/142 (4.2%) participants in 2020. The number of laboratories reporting a multimer interpretation was also low and ranged from 4/68 (6%) to 13/140 (9%) participants per sample.

Von Willebrand Factor Assay Imprecision

Of all VWF tests, VWF:Ag had the most consistent and best precision (►**Tables 2** and **3**). The CV per sample ranged from 6.0 to 15.1%. NPP samples consistently had a VWF antigen (CV $\leq$7%). In contrast to VWF:Ag, overall CV for VWF:RCo ranged from 14.6 to 44.8% per sample (►**Table 4**). Sample CV was inversely related to the mean activity level. When the mean activity level was

Table 2 Sample imprecision for VWF:Ag, 2015 to 2020

Mail	Sample	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	61	68	20.7	2.2	10.7
2015B	Type 2	64	72	31.6	3.9	12.3
2016A	NPP	59	65	97.5	5.2	5.3
2016B	Type 2	59	66	36.0	5.0	13.8
2017A	CPP	53	62	28.9	4.4	15.1
2017B	NPP	55	62	101.9	6.5	6.4
2018A	CPP	55	58	36.3	4.5	12.4
2018B	ISTH Lot #4	53	62	113.9	7.8	6.8
2019A	Type 2	132	141	37.3	4.6	12.2
2019B	NPP	124	140	133.1	8.0	6.0
2020A	ISTH Lot #5	133	141	115.8	8.1	7.0
2020B	Type 1	130	142	41.8	3.9	9.2

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation.
[a]*N*, all refers to the total number of participants in the survey.

Table 3 Sample imprecision for VWF:Ag by reagent method, 2015 to 2020

Mail	Sample	Reagent method	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	HemosIL	20	68	22.3	1.5	6.8
		Stago	30	68	20.5	1.7	8.2
2015B	Type 2	HemosIL	19	72	34.5	3.1	9.0
		Stago	30	72	31.1	3.2	10.2
2016A	NPP	HemosIL	21	65	100.1	4.0	4.0
		Stago	26	65	96.7	4.2	4.4
2016B	Type 2	HemosIL	21	66	40.3	2.5	6.1
		Stago	27	66	34.0	3.6	10.7
2017A	CPP	HemosIL	22	62	31.3	2.5	8.1
		Stago	24	62	27.5	4.3	15.8
2017B	NPP	HemosIL	23	62	106.0	5.2	4.9
		Stago	23	62	99.3	5.0	5.0
2018A	CPP	HemosIL	23	58	38.0	2.4	6.3
		Stago	26	58	35.8	5.1	14.3
2018B	ISTH Lot #4	HemosIL	21	62	117.2	5.0	4.2
		Stago	23	62	110.7	9.0	8.1
2019A	Type 2	HemosIL	58	141	41.1	1.8	4.3
		Stago	62	141	34.8	3.4	9.9
2019B	NPP	HemosIL	59	140	135.5	6.5	4.8
		Stago	54	140	132.0	8.5	6.4
2020A	ISTH Lot #5	HemosIL	63	141	120.7	5.5	4.5
		Stago	54	141	112.1	7.7	6.9
2020B	Type 1	HemosIL	65	142	43.3	2.7	6.2
		Stago	47	142	40.9	4.2	10.2

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation.
[a]*N*, All refers to the total number of participants in the survey.

Table 4 Sample imprecision for VWF:RCo, 2015 to 2020

Mail	Sample	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	34	68	13.1	5.9	44.8
2015B	Type 2	35	72	20.0	7.8	39.2
2016A	NPP	28	65	80.0	11.7	14.6
2016B	Type 2	24	66	22.2	6.4	28.8
2017A	CPP	25	62	13.4	4.7	35.1
2017B	NPP	22	62	74.7	17.5	23.5
2018A	CPP	19	58	14.9	6.7	44.8
2018B	ISTH Lot #4	24	62	76.6	13.1	17.1
2019A	Type 2	62	141	27.2	6.6	24.3
2019B	NPP	62	140	100.3	15.9	15.9
2020A	ISTH Lot #5	64	141	86.4	16.4	18.9
2020B	Type 1	60	142	20.7	6.6	31.9

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation; VWF:RCo, ristocetin cofactor assay.
[a]*N*, All refers to the total number of participants in the survey.

Table 5 Sample imprecision for VWF:RCo results by reagent method, 2015 to 2020

Mail	Sample	Reagent method	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	Nonautomated[b]	17	68	13.4	6.1	45.6
		Automated	17	68	13.7	5.2	38.0
2015B	Type 2	Nonautomated	15	72	25.1[c]	6.8	27.0
		Automated	20	72	16.2[c]	6.3	39.3
2016A	NPP	Nonautomated	11	65	85.5	14.0	16.4
		Automated	17	65	76.4	8.5	11.1
2016B	Type 2	Nonautomated	8	66	24.1	7.5	30.9
		Nonautomated	16	66	21.2	5.8	27.4
2017A	CPP	Nonautomated	12	62	13.1	3.1	23.8
		Automated	13	62	13.7	5.9	43.3
2017B	NPP	Nonautomated	9	62	83.3	18.2	21.9
		Automated	13	62	68.7	14.8	21.6
2018A	CPP	Nonautomated	8	58	13.0	5.9	45.4
		Automated	11	58	16.3	7.1	43.7
2018B	ISTH Lot #4	Nonautomated	9	62	81.7	14.4	17.6
		Automated	15	62	73.6	11.8	16.0
2019A	Type 2	Nonautomated	32	141	28.5	5.9	20.6
		Automated	30	141	25.7	7.1	27.6
2019B	NPP	Nonautomated	33	140	102.0	18.4	18.1
		Automated	29	140	98.3	12.5	12.7
2020A	ISTH Lot #5	Nonautomated	34	141	94.1[c]	17.5	18.6
		Automated	30	141	77.6[c]	9.2	11.8
2020B	Type 1	Nonautomated	30	142	22.8	6.8	30.0
		Automated	30	142	18.6	5.7	30.8

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation; VWF:RCo, ristocetin cofactor assay.
[a]*N*, All refers to the total number of participants in the survey.
[b]All methods apart from Siemens BC Von Willebrand Reagent and assumed to be performed on an aggregometer/nonautomated. Automated indicates Siemens BC Von Willebrand Reagent and assumed to be performed on a coagulation analyzer.
[c]Statistically significant difference in means.

<30%, CV ranged from 24.3 to 44.8%, while CV for NPP samples with a mean activity level >74% ranged from 14.6 to 23.5% (►**Table 4**). Sample CV was lower with the other VWF:GPIb-based assays (presumed to primarily comprise VWF:Ab and VWF:GPIbM) relative to the VWF:RCo assays, ranging from 8.0 to 34.8% for these assays (combined VWF:GPIb-based assay data not shown). As with VWF:RCo, other VWF:GPIb-based assay sample CVs were also inversely related to the mean activity level with other VWF:GPIb-based assays having the highest CV when the mean activity level was below 30%. NPP samples had a mean activity level >86%, with relatively low CV ranging from 8.0 to 14.4%.

Von Willebrand Factor Assay Method Agreement and Imprecision

With respect to antigen assays, while there were statistically significant differences between mean values for HemosIL and Stago VWF:Ag (most frequent reagents) for 8 of 12 samples ($p < 0.004$, multiple test correction applied), there were no statistically significant differences in variances between the two reagents for all samples. There was a trend toward higher CV with the Stago reagent relative to the HemosIL reagent (►**Table 3**).

For VWF:RCo, there were statistically significant differences between nonautomated/manual and automated means for only 2 of 12 samples ($p < 0.004$, multiple test correction applied), and yet, there were no statistically significant differences in variances between the two reagent methods for all samples as shown in ►**Table 5**.

Except for one sample (2015A), values for VWF activity assessed by HemosIL VWF:Ab assay were consistently higher than the Siemens Innovance VWF:GPIBM, including for type 2 and CPP samples (►**Table 6**). Results from the HemosIL VWF:Ab assay were also consistently higher than VWF:RCo results for the same samples (►**Tables 5** and **6**). However, it must be kept in mind that the number of laboratories reporting results from the Siemens Innovance VWF:GPIbM was low.

Table 6 Sample imprecision for other VWF:GPIb-based results by the reagent method, 2015 to 2020

Mail	Sample	Reagent method	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	HemosIL[b]	15	68	15.3	4.9	31.8
		Siemens Innovance	3	68	16.7	2.9	17.3
2015B	Type 2	HemosIL	16	72	28.8	4.9	17.0
		Siemens Innovance	3	72	18.3	1.5	8.3
2016A	NPP	HemosIL	18	65	92.3	13.6	14.7
		Siemens Innovance	3	65	86.3	8.4	9.7
2016B	Type 2	HemosIL	18	66	31.7	3.7	11.6
		Siemens Innovance	4	66	21.0	1.4	6.7
2017A	CPP	HemosIL	19	62	17.6	2.3	12.9
		Siemens Innovance	3	62	11.3	3.5	31.0
2017B	NPP	HemosIL	19	62	87.2	7.5	8.6
		Siemens Innovance	4	62	77.8	2.9	3.7
2018A	CPP	HemosIL	19	58	20.8	4.8	22.8
		Siemens Innovance	4	58	8.5	4.5	53.1
2018B	ISTH Lot #4	HemosIL	18	62	92.5	8.1	8.8
		Siemens Innovance	4	62	79.3	9.9	12.5
2019A	Type 2	HemosIL	45	141	36.2	4.4	12.0
		Siemens Innovance	9	141	22.4	3.6	16.2
2019B	NPP	HemosIL	43	140	108.6	7.8	7.2
		Siemens Innovance	8	140	100.0	6.2	6.2
2020A	ISTH Lot #5	HemosIL	50	141	96.9	7.2	7.5
		Siemens Innovance	12	141	85.6	4.1	4.8
2020B	Type 1	HemosIL	51	142	30.9	4.8	15.5
		Siemens Innovance	11	142	18.2	1.3	7.3

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation; VWF:Ab, monoclonal antibody; VWF:GPIb, von Willebrand factor GPIb-based activity assays; VWF:GPIbM, recombinant; gain-of-function mutant; VWF:GPIbR, recombinant; using ristocetin.

[a]N, All refers to the total number of participants in the survey.

[b]HemosIL assumed to be VWF:Ab, as VWF:GPIbR is not FDA cleared in the United States. Siemens Innovance indicates VWF:GPIbM assay.

Von Willebrand Factor Interpretation

With respect to interpretation of VWF:Ag level, for all samples, >85% of laboratories that provided qualitative interpretation of their numeric results correctly identified results as normal or abnormal based on the assigned sample type. Similar trends in interpretation were observed for VWF:RCo- and GPIb-based activity assays, with >95% and >89% of laboratories correctly identifying normal or abnormal for each sample, respectively.

Von Willebrand Factor Activity/Antigen Ratio

For VWF:RCo, the calculated mean VWF activity/antigen ratio ranged from 0.67 to 0.86 per sample for the five NPP samples (►**Table 7**) . The calculated mean ratio for the two samples manufactured to mimic type 1 VWD was unexpectedly reduced at 0.60 and 0.50, respectively, which may be related to the specimen manufacturing process that resulted in some loss of the highest molecular weight multimers as ratios of 0.56 and 0.45, respectively, and slightly abnormal multimers were noted at the time of sample manufacture. For the three simulated type 2 VWD samples (manufactured to simulate type 2A with missing HMWMs), the mean ratio was unexpectedly high at 0.64, 0.62, and 0.75, which could also be related to the manufacturing process. The lowest ratios were observed in the two CPP samples, 0.42 and 0.44, which were also intended to represent type 2 specimens with abnormal multimers.

Consistent with the generally higher VWF activity results with the HemosIL VWF:Ab assay, calculated VWF activity/antigen ratios were also higher relative to VWF:RCo-based ratios for the NPP samples (►**Tables 7** and **8**). For the two samples meant to represent type 1 VWD samples, the activity/antigen ratio was 0.72 ($n=15$) and 0.71 ($n=50$) using the HemosIL reagent and 0.99 ($n=3$) and 0.47 ($n=11$) using the Siemens VWF:GPIbM method (data not shown). For the three simulated type 2 VWD samples, the calculated ratios were relatively higher with the HemosIL reagent versus the VWF:GPIbM methods: 0.82 ($n=17$), 0.82 ($n=18$), and 0.90 ($n=47$) versus 0.64 ($n=3$), 0.60 ($n=4$), and 0.60 ($n=9$), respectively (data not shown).

Table 7 Sample imprecision for calculated VWF:RCo/antigen ratios, 2015 to 2020

Mail	Sample	*N*	*N*, All[a]	Mean, %	SD	CV%
2015A	Type 1	32	68	0.60	0.27	45.2
2015B	Type 2	32	72	0.64	0.25	39.6
2016A	NPP	26	65	0.86	0.12	13.6
2016B	Type 2	21	66	0.62	0.17	27.7
2017A	CPP	21	62	0.44	0.17	38.7
2017B	NPP	19	62	0.78	0.20	26.0
2018A	CPP	19	58	0.42	0.19	44.5
2018B	ISTH Lot #4	21	62	0.67	0.13	19.2
2019A	Type 2	58	141	0.75	0.18	23.5
2019B	NPP	56	140	0.78	0.12	15.3
2020A	ISTH Lot #5	61	141	0.77	0.15	20.0
2020B	Type 1	55	142	0.50	0.16	31.7

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation; VWF:RCo, ristocetin cofactor assay.
[a]*N*, All refers to the total number of participants in the survey.

Discussion

The recently issued ASH, ISTH, NHF, and WFH guidelines on the diagnosis and management of VWD suggest the use of newer assays of VWF platelet-binding activity (VWF:GPIbM, VWF:GPIbR) over VWF:RCo. Although VWF:Ab methods are also mentioned in the guideline text, the guideline recommendation chose to focus on VWF:GPIbM and VWF:GPIbR as direct measures of platelet-binding activity. Our CAP survey data show that the VWF:Ab is one of the most commonly used measures of VWF activity in the United States, and the CAP Surveys contain few participants using the new guideline's recommended assays. This is likely due to VWF:Ab being FDA cleared, as opposed to VWF:GPIbR and VWF:GPIbM, and may also be related to the selection of assay manufacturers that are aligned with laboratory instrumentation. The CAP VWF proficiency testing data confirm that VWF:RCo assays, in aggregate (automated or manual), have higher imprecision relative to GPIb-based assays. The observed higher imprecision of VWF:RCo relative to other VWF:GPIb assays is consistent with observations in several other studies,[11,12] although one study assessing a large dataset from the Royal College of Pathologists of Australasia Quality Assurance Program reported higher variability with other VWF:GPIbM assays relative to VWF:RCo.[7] It is not clear whether these differences are related to the methods used (commercial kits versus laboratory-developed tests), the specific samples that were studied, methods of analysis, or other factors. The number of laboratories reporting results for VWF:GPIbM in the CAP surveys remains relatively small at 11 results reported in the latter half of 2020.

We also sought to assess whether VWF:RCo performed on an automated platform had improved precision. There was no consistent significant difference in imprecision between automated VWF:RCo and VWF:RCo performed on nonautomated platforms, suggesting that the lack of reproducibility may be related to the use of intact platelets rather than the method used to detect platelet agglutination or aggregation.

In the United States, the VWF:RCo and the VWF:Ab are the only FDA-approved/cleared VWF activity assays.[4] Approximately, 50% of laboratories consistently reported results from VWF:RCo assays through the timeframe of this study. The lack of available FDA-approved/cleared VWF:GPIbM, VWF:GPIbR, and VWF:CB assays in the United States contributes to a relatively lower rate of usage of non-VWF:RCo functional VWF assays. Consequently, the data in this study support the notion that a barrier to complete compliance with the ASH, ISTH, NHG, and WFH guidelines exists in the United States.[4] Similarly, the number of laboratories reporting results from VWF:CB and multimer assays remains relatively low. If and when automated/semiautomated platforms become more widely available in the United States, use of these assays may increase.[13,14]

We observed a positive bias relative to VWF:RCo for samples categorized as having been run with the HemosIL reagent, presumed to be mostly, if not all VWF:Ab. This affects both the activity measurements and the activity-to-antigen ratios and has the potential to affect VWD classification that is based on these ratios. Although the reason for the bias is not entirely clear, it could be related to differences in how calibrator materials for the kits are assigned or other factors. The International Council for Standardization in Haematology has emphasized the importance of calibrator traceability to a reference method or reference material of known value, ideally an international standard.[15] Calibrator traceability to an international standard may be especially important for laboratories that employ laboratory-developed tests. For the ISTH Lot #5 international standard, we note that VWF:GPIbR and VWF:GPIbM values are now being assigned in addition to VWF:RCo, while VWF:Ab values are not assigned. The available method-specific assigned values differ. An ISTH Lot #5 assigned value for VWF:Ab could prove

Table 8 Sample Imprecision for calculated other VWF:GPIb-based[a] /antigen ratio by the reagent method, 2015 to 2020

Mail	Sample	Reagent method	*N*	*N*, All[b]	Mean, %	SD	CV%
2015A	Type 1	HemosIL	15	68	0.72	0.26	35.7
		Siemens Innovance	3	68	0.99	0.23	23.3
2015B	Type 2	HemosIL	17	72	0.82	0.14	17.5
		Siemens Innovance	3	72	0.64	0.10	14.8
2016A	NPP	HemosIL	17	65	0.89	0.12	13.5
		Siemens Innovance	3	65	0.89	0.07	7.9
2016B	Type 2	HemosIL	18	66	0.82	0.13	15.3
		Siemens Innovance	4	66	0.60	0.06	9.8
2017A	CPP	HemosIL	18	62	0.57	0.08	14.9
		Siemens Innovance	3	62	0.49	0.20	41.9
2017B	NPP	HemosIL	19	62	0.83	0.10	12.3
		Siemens Innovance	4	62	0.78	0.05	6.5
2018A	CPP	HemosIL	19	58	0.56	0.14	25.8
		Siemens Innovance	4	58	0.30	0.17	56.1
2018B	ISTH Lot #4	HemosIL	20	62	0.80	0.07	8.7
		Siemens Innovance	4	62	0.68	0.07	10.7
2019A	Type 2	HemosIL	47	141	0.90	0.10	11.6
		Siemens Innovance	9	141	0.60	0.11	17.6
2019B	NPP	HemosIL	43	140	0.80	0.07	8.2
		Siemens Innovance	8	140	0.78	0.07	9.4
2020A	ISTH Lot #5	HemosIL	50	141	0.81	0.07	8.2
		Siemens Innovance	12	141	0.79	0.05	6.8
2020B	Type 1	HemosIL	50	142	0.71	0.11	15.9
		Siemens Innovance	11	142	0.47	0.03	7.4

Abbreviations: CPP, cryopool plasma (type 2 like); CV%, coefficient of variation; ISTH, International Society on Thrombosis and Haemostasis; NPP, normal poor plasma; SD, standard deviation; VWF:Ab, monoclonal antibody; VWF:GPIb von Willebrand factor GPIb-based activity assays; VWF:GPIbM, recombinant; gain-of-function mutant; VWF:GPIbR, recombinant; using ristocetin.

[a]HemosIL assumed to be VWF:Ab, as VWF:GPIbR is not FDA cleared in the United States. Siemens Innovance indicates VWF:GPIbM assay.

[b]*N*, All refers to the total number of participants in the survey.

useful in the investigation and resolution of the positive VWF:Ab bias we and others have observed. The new VWD guideline does include recommendations for discrimination between type 1 and type 2 forms of VWD based on the ratio results. Existing literature supports the notion that the VWF:Ab assay is less sensitive to HMWM reduction relative to other assays,[4,7,16] although the trend has not been widely reported in North America.[9] The data from CAP proficiency testing support this conclusion, and yet, additional studies are required to assess the clinical significance.

We acknowledge several limitations to this analysis of proficiency testing data. First, no patient samples with confirmed and characterized VWD were distributed. Samples were, instead, commercial or manufactured products; thus, the findings may not be completely applicable to the assessment of VWD in patients. Regardless, the distribution of an aliquoted sample to many laboratories allows for reliable estimation of interlaboratory variability among the various assays. Second, the analysis was limited by the specific data elements captured in the proficiency testing reporting form. For example, we were unable to assess results from VWF:GPIbR assays. It is possible that VWF:GPIbR results were categorized by the reporting laboratory as "HemosIL" since the reporting form did not include a "HemosIL VWF:GPIbR" category. Despite this limitation, the number of laboratories reporting results from VWF:GPIbR is likely to be very small, given that no VWF:GPIbR assay is U.S. FDA-approved/cleared.

The reporting form allowed for documentation of an exception indicating that results were below the limit of detection, but laboratories did not consistently submit their lower limit of detection. To limit bias introduced by the exclusion of these data, we chose to assign the manufacturer-specific lower limit of detection and include these data in the analyses. While this approach may affect the dataset, the trends reported here were consistently seen even in the dataset where these values were excluded. The recognition of the limitations introduced by the reporting form will allow the CAP to improve the proficiency testing data capture and improve data utility.

Samples meant to represent type 1 VWD had lower VWD activity/antigen ratios than expected. This brings into question the reliability of these samples as a true representation of type 1 VWD, and future studies scrutinizing the effect of lyophilization, dilution, or other aspects of sample preparation are required. Because of this limitation, we are unable to reliably assess the proposed ASH, ISTH, NHG, and WFH VWF activity/antigen ratio of <0.7 to confirm type 2 VWD (2A, 2B, or 2M) for patients with an abnormal initial VWD screen. Finally, the data are only reflective of laboratories participating in the CAP proficiency testing program and may not be entirely applicable to settings where other assays, such as the VWF:GPIbM, VWF:GPIbR, and VWF:CB, are more widely implemented. Despite these limitations, the data presented herein represent the current state of primarily North American interlaboratory performance in the laboratory assessment of VWF level and function.

In summary, this analysis of recent CAP VWF proficiency testing program results confirms that, while VWF:Ag testing has low imprecision, the various VWF activity assays have significant interassay variability, with VWF:RCo showing greater imprecision than other GPIb-based assays. The overall trends in assay utilization reflect the barriers to complete compliance with modern VWD diagnostic guidelines in North America.

Conflict of Interest

None declared.

Acknowledgments

The authors wish to thank Vineeta Shivde, MT(ASCP) for her dedicated staffing of the Hemostasis and Thrombosis Committee of the College of American Pathologists.

References

1 Sadler JE, Budde U, Eikenboom JC, et al; Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost 2006;4(10):2103–2114
2 Langer AL, Connell NT. Acquired von Willebrand Syndrome. Hematol Oncol Clin North Am 2021;35(06):1103–1116
3 Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. Blood 1987;69(02):454–459(In Eng)
4 Favaloro EJ. Commentary on "ASH ISTH NHF WFH 2021 guidelines on the diagnosis of VWD": reflections based on recent contemporary test data. Blood Adv 2022;6(02):416–419
5 Howard MA, Firkin BG. Ristocetin—a new tool in the investigation of platelet aggregation. Thromb Diath Haemorrh 1971;26(02):362–369(In Eng)
6 Olson JD, Brockway WJ, Fass DN, Magnuson MA, Bowie EJ. Evaluation of ristocetin-Willebrand factor assay and ristocetin-induced platelet aggregation. Am J Clin Pathol 1975;63(02):210–218
7 Favaloro EJ, Dean E, Arunachalam S, Vong R, Mohammed S. Evaluating errors in the laboratory identification of von Willebrand disease using contemporary von Willebrand factor assays. Pathology 2022;54(03):308–317
8 Flood VH, Gill JC, Morateck PA, et al. Common VWF exon 28 polymorphisms in African Americans affecting the VWF activity assay by ristocetin cofactor. Blood 2010;116(02):280–286
9 Chen D, Tange JI, Meyers BJ, Pruthi RK, Nichols WL, Heit JA. Validation of an automated latex particle-enhanced immunoturbidimetric von Willebrand factor activity assay. J Thromb Haemost 2011;9(10):1993–2002
10 James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv 2021;5(01):280–300
11 Patzke J, Budde U, Huber A, et al. Performance evaluation and multicentre study of a von Willebrand factor activity assay based on GPIb binding in the absence of ristocetin. Blood Coagul Fibrinolysis 2014;25(08):860–870
12 Abdulrehman J, Ziemba YC, Hsu P, et al. Diagnosis of von Willebrand disease: an assessment of the quality of testing in North American laboratories. Haemophilia 2021;27(06):e713–e720
13 Stufano F, Baronciani L, Bucciarelli P, et al. Evaluation of a fully automated von Willebrand factor assay panel for the diagnosis of von Willebrand disease. Haemophilia 2020;26(02):298–305
14 Pikta M, Szanto T, Viigimaa M, et al. Evaluation of a new semi-automated Hydragel 11 von Willebrand factor multimers assay kit for routine use. J Med Biochem 2021;40(02):167–172
15 Gardiner C, Coleman R, de Maat MPM, et al. International Council for Standardization in Haematology (ICSH) laboratory guidance for the verification of haemostasis analyser-reagent test systems. Part 2: specialist tests and calibrated assays. Int J Lab Hematol 2021;43(05):907–916
16 Favaloro EJ, Bonar RA, Mohammed S, et al. Type 2M von Willebrand disease - more often misidentified than correctly identified. Haemophilia 2016;22(03):e145–e155

