Laboratory Diagnosis of von Willebrand Disorder

Current Practice in the Southern Hemisphere

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Abstract

A survey of 44 laboratories was conducted to evaluate current testing proficiency in the diagnosis of von Willebrand disorder (vWD) and to assess recent changes in test practices. Laboratories performed their usual panel of tests for vWD and interpreted results for the likelihood of vWD and potential subtype. Samples were as follows: normal plasma; borderline normal or abnormal levels of von Willebrand factor (vWF) and factor VIII; type 3 vWD; type 2A vWD; and 2 samples from a healthy person, processed after handling at 22°C and 4°C, respectively. Interassay and within-method coefficients of variation were similar for all assays (approximately 15%-25%). Most laboratories reported test values consistent with expected findings and made correct interpretations, although discrepant results for 5% to 10% of responses are of concern. For the sample stored at 4°C, all laboratories detected low or borderline levels of vWF and factor VIII coagulant, and no laboratory identified this sample as from a healthy person. In contrast, for the sample stored at 22°C, most laboratories reported normal results. Compared with previous results, performance of some assays has declined while that of others has increased. Laboratories generally are proficient in tests for vWD, and transport of samples at 4°C before processing may lead to false identification of vWD, suggesting that NCCLS guidelines should be reviewed.

von Willebrand disorder (disease; vWD) is recognized as the most common inherited bleeding ailment. People with vWD have defects in or reduced levels of von Willebrand factor (vWF), an adhesive plasma protein essential for primary hemostasis. vWD is a heterogeneous disorder, which is subtyped using clinical and laboratory criteria. Typically, the diagnostic process requires performance of a panel of tests. This is because of the heterogeneity of vWD and the limitations of assays, such that no single laboratory procedure is sufficiently robust to detect all forms of vWD. Complicating matters, there is considerable interlaboratory variation in assay methods and the panel of assays used, as well as considerable variation in the diagnostic power of particular assays or test panels. Much change in the laboratory test process has occurred during the past 10 or so years.

The test panel might include any of the following: (1) a routine coagulation screening assay such as the activated partial thromboplastin time (aPTT); (2) skin bleeding time; (3) factor VIII coagulant (FVIII:C) activity; (4) vWF antigen (vWF:Ag) levels; (5) functional vWF activity using the ristocetin cofactor (vWF:RCo) assay or the vWF:collagen binding activity (vWF:CB) assay; (6) assessment of the composite vWF structure or molecular weight profile (vWF:multimer analysis); (7) platelet function analysis (eg, ristocetin-induced platelet aggregation [RIPA] procedure or using automated systems such as the PFA-100 [Dade Behring, Newark, NJ]); and (8) platelet counts. Furthermore, test methods encompass a multitude of different assay procedures, including clot-based assays, enzyme-linked immunosorbent assay (ELISA), electroimmunodiffusion (EID; Laurell gel), latex immunoassay (LIA), platelet aggregometry, and flow cytometry.

Since the composite panel of tests performed can affect the accuracy of the diagnosis and the vWD subtype determination, assessments of testing practices by evaluation of laboratory-
based test proficiency using multilaboratory surveys have been undertaken. \(^\text{10-12}\) In the first reported plasma test-based survey, which was conducted on behalf of the RCPA Quality Assurance Program (QAP) in Haematology in 1998, 25 laboratories participated by testing a set of 10 plasma samples,\(^\text{11}\) and in the second survey, conducted on behalf of the Australasian Society for Thrombosis and Haemostasis in 1999, 19 laboratories participated by testing 7 plasma samples (different from those used in the 1998 survey).\(^\text{12}\) Given the ongoing changes to test practice, partly resulting from the findings of such surveys and initiation of altered laboratory processes and partly driven by automation and advances in testing procedures, we reevaluated current test practices.

Major objectives of the study were as follows: (1) assess each laboratory’s overall performance (or proficiency) in the vWF/vWD testing process; (2) identify current laboratory practice in Australasia as it relates to the vWD diagnostic process and as potentially changed from previous surveys; (3) reassess the effectiveness of various tests, test procedures, and composite test panels for detecting or excluding vWD; and (4) reassess the suitability of the current National Committee for Clinical Laboratory Standards (NCCLS) guidelines for handling and transport of coagulation specimens. These guidelines recommend that specimens for hemostasis assays, in addition to specimens for prothrombin time and aPTT, be kept at 2°C to 4°C or 18°C to 24°C until they are centrifuged and that they be tested within 4 hours after collection, ie, both temperature ranges are acceptable.\(^\text{13}\) However, it has been noted that storage of whole blood at 4°C may lead to artifactually low vWF results in a substantial proportion of tested samples.\(^\text{14}\) In this pilot study, a substantial fall in vWF:CB (with a resultant increase in the vWF:Ag/vWF:CB ratio) was observed in 3 of 10 healthy subjects and in 2 of 2 people with type 1 vWD.\(^\text{14}\)

As in the previous multilaboratory surveys, there were 2 major components of analysis in the present survey: (1) analysis and assessment of laboratory numeric data for blindly tested samples and (2) analysis and assessment of the laboratories’ interpretations of their results. In addition to evaluation of test-based efficacy in the diagnosis of vWD, we highlight the major changes to test practice during the past 5 years, highlight potential diagnostic errors that might arise from test-based limitations, and outline some strategies to assist and improve the future laboratory testing and diagnostic processes for vWD.

**Materials and Methods**

This report focuses on an assessment of present diagnostic laboratory practices for people undergoing evaluation for vWD, and conducted on behalf of the RCPA QAP in Haematology Haemostasis Committee. The RCPA QAP in Haematology conducts ongoing testing proficiency for a large number of laboratories (approximately 640) on behalf of the Royal College of Pathologists of Australasia. Participants in this QAP derive from a wide geographic area, including Australia, New Zealand, parts of Southeast Asia, and Europe. The present survey was conducted in mid 2002. Participants were identified from expressions of interest and details provided in a questionnaire sent by the RCPA QAP. As a result, 47 laboratories were invited to participate; 44 laboratories (94%) returned test results. The laboratories were from Australia (n = 25), New Zealand (n = 8), Malaysia (n = 4), South Africa (n = 2), Singapore (n = 2), India (n = 1), Oman (n = 1), and Hong Kong (n = 1). Each participant was provided with a sample set that comprised up to 8 plasma samples, in part depending on the test panel performed by that laboratory.

**Sample Set Preparation and Study Rationale**

A plasma sample set comprising vials of lyophilized plasma was provided to each participant, containing all or part of the panel described in the following sections.

**QAP-1a and QAP-1b: Samples to Mimic Normal Plasma**

These samples were from separate single lots of a commercially available lyophilized normal control plasma (SAC-1, Helena Laboratories, Melbourne, Australia). Stated values for vWF and factor VIII were similar to each other and were as follows: 1a, vWF:Ag, 148%; vWF:RCo, 99%; and FVIII:C, 105%; and 1b, 139%, 106%, and 89%, respectively. Presurvey testing in the host laboratory provided similar results (1a, 111%, 111%, and 87%; and 1b, 122%, 99%, and 90%, respectively).

**QAP-2a and QAP-2b: Samples With Borderline Normal or Abnormal Findings**

The samples were from separate single lots of a commercially available lyophilized level 2 control plasma (SAC-2, Helena Laboratories). Stated values for vWF and factor VIII were similar and were as follows: 2a, vWF:Ag, 57%; vWF:RCo, 38%; FVIII:C, 40%; and 2b, 62%, 49%, and 47%, respectively. Presurvey testing in the host laboratory provided similar results (2a, 46%, 55%, and 38%; and 2b, 47%, 47%, and 44%, respectively).

**QAP-3: vWF-Deficient Sample**

This sample was from a single lot of a commercially available lyophilized vWF-deficient plasma (Biopool, Abacus Diagnostics, Yeerongpilly, Australia) described by the manufacturer as having “less than 30%” ristocetin cofactor activity and that in presurvey testing consistently provided levels of less than 2% vWF:Ag, vWF:CB, vWF:RCo, and FVIII:C.

**QAP-4: Sample to Mimic Type 2 vWD**

The sample was prepared from normal cryosupernatant that initially provided test results around 8% for vWF:Ag,
This sample was intended to mimic that of a normal whole blood sample handled according to the NCCLS guidelines previously stated. This sample was divided into 1.0-mL aliquots, lyophilized. For this exercise, the reconstitution volume also was 1.0 mL. The plasma was from a single donor (female, 32 years old; Caringbah, Australia). The plasma was from a single donor (female, 32 years old; Caringbah, Australia).

Table 1

Summarized Multilaboratory Test Sample Results

<table>
<thead>
<tr>
<th>QAP Sample/Plasma Type</th>
<th>Factor VIII</th>
<th>vWF:Ag</th>
<th>vWF:RCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 44 [100])</td>
<td>All (n = 43 [98])</td>
<td>ELISA (n = 21 [48])</td>
<td>LIA (n = 20 [45])</td>
</tr>
<tr>
<td>1a/Normal</td>
<td>92.5 (12.5)</td>
<td>110.7 (16.5)</td>
<td>100.9 (14.5)</td>
</tr>
<tr>
<td>1b/Normal*</td>
<td>86.2 (19.8)</td>
<td>130.4 (14.9)</td>
<td>126.1 (14.9)</td>
</tr>
<tr>
<td>2a/Equivocal</td>
<td>34.5 (13.8)</td>
<td>48.0 (15.1)</td>
<td>44.8 (15.0)</td>
</tr>
<tr>
<td>2b/Equivocal*</td>
<td>42.3 (14.3)</td>
<td>53.4 (16.3)</td>
<td>55.9 (11.5)</td>
</tr>
<tr>
<td>3/vWF deficient</td>
<td>1.7 [78]</td>
<td>2.2 [136]</td>
<td>2.9 [124]</td>
</tr>
<tr>
<td>(type 3 vWD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(type 2 vWD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/Normal (22°C)</td>
<td>82.9 (15.5)</td>
<td>72.0 (20.9)</td>
<td>676 (18.9)</td>
</tr>
<tr>
<td>6/Normal (4°C)</td>
<td>52.6 (18.4)</td>
<td>43.1 (19.5)</td>
<td>42.8 (20.9)</td>
</tr>
</tbody>
</table>

Ag, antigen; Agg, aggregometry; CB, collagen binding activity; Com, commercial; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; I-H, in-house procedure; LIA, latex immunoassay; QAP, quality assurance program; RCo, ristocetin cofactor; vWF, von Willebrand factor.

1 Values with column headings are the number [percentage] of laboratories performing a particular test method or procedure (of 47 laboratories sent a set of samples for testing, 44 returned results). Values in the body of the table are multilaboratory mean values given as percentages or ratios followed by CVs given as percentages in parentheses, except for CVs that should be viewed with caution, which are given in brackets. CVs to be considered with caution are those for sample sets in which the absolute test result values fall below approximately 15% or the number of laboratories reporting the value was low (eg, samples 3 and 4). For further details about the samples, test methods, and procedures, see the “Materials and Methods” section.

2 When the vWF antigen assay results fall below approximately 15% (ie, sample 3), calculation of test ratios such as Ag/CB and Ag/RCo should be treated with caution because assay sensitivity limitations at low vWF levels may create artificially high ratios owing to falsely low denominator values; generally, it is not advisable that ratios be calculated in these cases (see also the text and Favaloro5,6).

3 No data are given for some sample test cases because samples were not tested by the laboratories.

1% to 2% for vWF:CB, and 8% for FVIII:C. For the purpose of the survey, the sample was divided into 2-mL aliquots, lyophilized by a standard commercial process, and used as a 0.5-mL reconstitution vial (ie, to produce a 4× cryosupernatant concentrate that would mimic a type 2 vWD sample with derived levels around 4× prelyophilization values, ie, vWF:Ag of approximately 32%, vWF:CB of approximately 6%, and FVIII:C of approximately 32%).

QAP-5 and QAP-6: Normal Donor Plasma Stored at 22°C and 4°C

All blood was collected into standard buffered sodium citrate tubes (Greiner Vacuette, Interpath Services, Caringbah, Australia). The plasma was from a single donor with group O Rh-positive blood.

For the QAP-5 sample, after collection, the whole blood sample was left at ambient temperature (~22°C) for 3.5 hours with intermittent mixing and then processed normally by high-speed centrifugation. Plasma was separated from the cell pellet and snap frozen at ~80°C. The sample later was thawed, divided into 1.0-mL aliquots, and lyophilized. For this exercise, the reconstitution volume also was 1.0 mL. This sample was intended to mimic that of a normal whole blood sample transported at ambient temperature to the laboratory within the 4-hour time frame generally accepted for hemostasis samples and otherwise handled according to current NCCLS guidelines.13 Prelyophilization testing provided the following results: vWF:Ag, 76%; vWF:CB, 75%; and FVIII:C, 82%. Sample QAP-5 was derived from the same person tested in a previous survey,11 in which the sample was identified as sample 7. In the previous survey, similar mean results were obtained: vWF:Ag, 75%; vWF:CB, 72%; vWF:RCo, 68%; and FVIII:C, 82%.

The QAP-6 sample was derived from the same collection of normal donor sample as the QAP-5 sample. After collection, the whole blood sample was left at approximately 4°C for 3.5 hours with intermittent mixing and processed in the same manner as sample QAP-5. This sample was intended to mimic a sample obtained from a healthy person and transported as whole blood at approximately 4°C to the laboratory within the same time frame and otherwise handled according to the NCCLS guidelines previously noted.13 Thus, the sole difference between samples QAP-5 and QAP-6 was the temperature of whole blood storage before centrifugation and processing. In prelyophilization testing, sample QAP-6 provided the following results at the host laboratory: vWF:Ag, 52%; vWF:CB, 42%; and FVIII:C, 55%. (All assay values are mean values unless otherwise stated.)

The person providing samples QAP-5 and QAP-6 also was tested in a previous study in which 4°C treatment was noted to provide loss of vWF that can lead to artifactual vWD results in a substantial proportion of tested samples.14 It therefore was of interest to us to determine how such low temperature storage might differentially affect test results and interpretation in a multilaboratory test situation.
Sample Testing

All testing was performed blindly (ie, samples were identified only by their codes). To conserve plasma and concentrate efforts on the most relevant assays, participants were advised not to perform routine coagulation testing. Sample processing instructions were provided, and laboratories were asked to perform all other plasma-based tests available to them to establish a laboratory diagnosis of vWD (ie, FVIII:C and specific vWF tests such as vWF:Ag, vWF:CB, vWF:RCo, and vWF:multimers). Because of sample limitations of QAP-1 and QAP-2, some laboratories received only one or the other of QAP-1a and QAP-2a or QAP-1b and QAP-2b. In particular, laboratories performing vWF:RCo by ELISA and vWF:CB generally tested QAP-1a and QAP-2a, but not QAP-1b and QAP-2b.

Evaluation of Numeric Data

All returned numeric data were assessed on an individual laboratory basis and as group method data. This permitted appraisal of individual laboratory performance (ie, relative proficiency, including identification of potential assay bias) and assessment of methods. Numeric data returned in a semi-quantitative form (eg, <10%) were converted to a numeric value midway between the upper and lower limit possibilities for analysis (ie, 5% for the preceding example). To undertake fairer method-based evaluations, individual extreme outliers (>3 SD from the group mean) were deleted from group values. Analysis of group data (eg, mean, SD, coefficient of variation [CV]) was performed independently using Microsoft Excel (Microsoft, Redmond, WA) and GraphPad Prism (version 2.0c, GraphPad Software, San Diego, CA; PowerMac, Apple Computer, Cupertino, CA) for validation of results.

Evaluation of Interpretive Data

To help assess diagnostic efficacy of test panels, laboratories were asked to comment on the possibility of vWD, as well as the particular subtype suggested. The interpretive choices comprised 1 of 6 choices: (1) normal/not vWD, (2) equivocal (neither clearly normal nor clearly vWD), (3) mild type 1 vWD, (4) severe type 1 vWD, (5) type 2 vWD, or (6) type 3 vWD. However, participants could specify an alternative conclusion or choose not to complete this section of the survey.

Given recognized survey limitations,11,12 a fairly liberal approach to the evaluation of interpretation data was used. For example, given the nature of the samples and the aforementioned approximate test values, it would not be unreasonable for samples QAP-2a and QAP-2b to result in an interpretation of normal/not vWD (especially if normal values, eg, >50%, were obtained on testing) or mild type 1 vWD (especially if slightly abnormal values, eg <50%, were obtained on testing and if vWF:Ag vs vWF functional concordance was evident). Sample QAP-6 also might result in such alternative interpretive predictions. Similarly, sample QAP-3 might result in several interpretations of severe type 1 vWD. Nevertheless, we generally anticipated consensus interpretations of the samples as follows: QAP-1a and QAP-1b, normal/not vWD; samples QAP-2a and QAP-2b, equivocal; QAP-3, type 3 vWD; QAP-4, type 2 vWD; QAP-5, normal/not vWD; and QAP-6, equivocal.

Results

Numeric Data Analysis

Of 47 laboratories sent samples for testing, 44 (94%) returned test results.

Methods in Use

Tests performed were as follows: vWF:Ag, 43 (98%); vWF:RCo, 32 (73%); vWF:CB, 21 (48%); FVIII:C, 44 (100%); and vWF:multimers, 3 (7%) (Figure 1). vWF:Ag usually was measured using an ELISA procedure (21/43 laboratories [49%]) or LIA (20/43 laboratories [47%]); only 1 laboratory (2%) performed EID. vWF:RCo typically was performed by platelet aggregometry (29/32 laboratories [91%]), with only 3 laboratories (9%) performing an alternative ELISA procedure. vWF:CB invariably was performed by ELISA (in-house method, 9/21 laboratories [43%]; commercial kit, 11/21 laboratories [52%]). In regard to a diagnostic screen test panel, 42 (95%) of 44 laboratories performed FVIII:C and vWF:Ag, and most of these also performed vWF:RCo, vWF:CB, or both. Two laboratories performed FVIII:C and vWF:RCo without vWF:Ag and vWF:CB.

Assay Variation

Interassay variation was similar for all assays, with CVs around 15% to 25% (Table II), although those for vWF:RCo generally were a little higher than those for vWF:Ag, FVIII:C, and vWF:CB. Within-method data analysis suggested similar intra-assay variation for most methods.
Summary data are given in Table 1. Comparative results for FVIII:C are also given in Figure 2. vWF:RCo results were less than 50% on all test occasions, for all vWF assays. For sample QAP-6, vWF:RCo and QAP-5 resulted in a range of results above and below 50% for sample QAP-4. Samples QAP-2a, QAP-2b, and QAP-4, 2 laboratories reported outlier results of more than 50% for samples QAP-3 and QAP-4, 2 laboratories reported outlier results of more than 50% for sample QAP-1. Results from all laboratories testing vWF:CB for sample QAP-1a also were consistently reported as more than 50%. Similarly, for samples QAP-3 and QAP-4, all laboratories reported results for vWF:Ag and vWF:CB as less than 50%. Although most laboratories also reported vWF:RCo results of less than 50% for samples QAP-3 and QAP-4, 2 laboratories reported outlier results of more than 50% for sample QAP-4. Samples QAP-2a, QAP-2b, and QAP-5 resulted in a range of results above and below 50% for all vWF assays. For sample QAP-6, vWF:RCo and vWF:CB results were less than 50% on all test occasions, and vWF:Ag results were less than 50% on most occasions. Comparative results for FVIII:C are also given in Figure 2. Summary data are given in Table 1.

Calculated assay ratios (vWF:Ag vs functional assay results) are shown in Figure 3. Functional vs antigen concordance generally was obtained for testing of samples QAP-1 and QAP-2, consistent with the nature of the tested samples. In contrast, functional discordance was generally observed in testing of QAP-4, again consistent with the nature of the test sample (mimicking a qualitative type 2 vWD defect). Functional discordance for QAP-4 was observed more consistently for comparisons of vWF:Ag with vWF:CB (ie, ratios most generally >2.0) than for comparisons of vWF:Ag with vWF:RCo (about one third of laboratories reported ratios of <2.0 and would not have identified the functional discordance for this sample). vWF assay concordance generally was obtained for QAP-5, again consistent with the nature of the tested sample. However, functional discordance was observed for QAP-6 by a large number of laboratories reporting vWF:RCo results.

Range of Results for Normal Samples vs Samples Mimicking vWD

Despite the similar assay variation noted, slight differences could be noted in the range of assay results obtained for plasma sets using different assays. For example, vWF:Ag results for samples QAP-1a and QAP-1b always were reported as more than 50% in Figure 2. However, although all laboratories testing vWF:RCo reported sample QAP-1a as more than 50%, values occasionally fell below 50% for sample QAP-1b. Results from all laboratories testing vWF:CB for sample QAP-1a also were consistently reported as more than 50%. Similarly, for samples QAP-3 and QAP-4, all laboratories reported results for vWF:Ag and vWF:CB as less than 50%. Although most laboratories also reported vWF:RCo results of less than 50% for samples QAP-3 and QAP-4, 2 laboratories reported outlier results of more than 50% for sample QAP-4. Samples QAP-2a, QAP-2b, and QAP-5 resulted in a range of results above and below 50% for all vWF assays. For sample QAP-6, vWF:RCo and vWF:CB results were less than 50% on all test occasions, and vWF:Ag results were less than 50% on most occasions. Comparative results for FVIII:C are also given in Figure 2. Summary data are given in Table 1.

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Multimer Analysis

Results of multimer analysis from all 3 laboratories were consistent with quantitative vWF assay results. Thus, a normal level and a normal multimer pattern were reported for QAP-1, and a slightly reduced level with a normal multimer pattern was reported for QAP-2. vWF was reported as absent for QAP-3, and loss of high-molecular-weight vWF was reported for QAP-4. Results for QAP-5 and QAP-6 were essentially normal, although a slight reduction in vWF intensity could be identified in QAP-6.

Interpretation of Laboratory Results and Prediction of vWD

All 20 laboratories providing an interpretation correctly identified sample QAP-1a as a normal sample in Figure 4. Of 19 laboratories giving an interpretation, 15 (79%) also correctly identified sample QAP-1b as a normal sample (Figure 4). Two laboratories (11%) identified this sample as equivocal and 2 (11%) as indicating type 2 vWD (Figure 4). The unexpected interpretations and low assay results were not always connected (Table 2).

Most laboratories providing an interpretation identified samples QAP-2a (16/19 [84%]) and QAP-2b (13/19 [68%]) as equivocal or indicating mild type 1 vWD (Figure 4). Two laboratories identified sample QAP-2a as indicating type 2 vWD, and 4 laboratories identified sample QAP-2b as indicating type 2 vWD (Figure 4; Table 2).

Most laboratories providing an interpretation identified sample QAP-3 as indicating type 3 vWD or severe type 1 vWD (Figure 4). One laboratory identified this sample as type 2 vWD (Figure 4; Table 2).

Most laboratories providing an interpretation identified sample QAP-4 as indicating type 2 vWD or severe type 1 vWD (Figure 4). Two laboratories identified this sample as indicating type 3 vWD and 3 laboratories as mild type 1 vWD (Figure 4; Table 2). There were 2 outlier results with a vWF:RCo value of more than 50%, but these were not from the 2 laboratories that provided the unexpected interpretations.

Most laboratories providing an interpretation correctly identified sample QAP-5 as normal/not vWD or equivocal (Figure 4). Three laboratories identified this sample as
Summary of all von Willebrand factor (vWF) and factor VIII coagulant (FVIII:C) assay results. Assay values are shown as percentages of normal for each sample tested. **A** and **B**, Quality assurance program (QAP) samples 1a and 1b (normal level control plasma samples; SAC-1, Helena Laboratories, Melbourne, Australia). **C** and **D**, QAP samples 2a and 2b (level 2 control plasma samples; SAC-2, Helena Laboratories). **E** and **F**, QAP samples 3 (vWF-deficient control plasma samples; type 3 von Willebrand disease [vWD]) and 4 (4× cryosupernatant; type 2 vWD). **G** and **H**, QAP samples 5 and 6 (normal individual plasma samples derived from citrate-anticoagulated whole blood, stored at 22°C and 4°C, respectively, for 3.5 hours before centrifugation and processing). Arrows indicate outlier data points (>3 SD). Data for the collagen binding activity assay (CB) for vWF (vWF:CB) in **B** and **D** (QAP samples 1b and 2b) are not shown because only 2 laboratories performed the test. Ag, antigen; RCo, ristocetin cofactor.
indicating mild type 1 vWD, and 3 laboratories identified this sample as indicating type 2 vWD (Figure 4; Table 2).

Sample QAP-6 provoked a range of interpretive comments, but most identified it as indicating mild type 1 vWD or type 2 vWD (Figure 4). No laboratory identified this normal sample as being from a healthy person.

**Discussion**

Compared with the results of previous surveys, some substantial changes to test methods used by laboratories in this geographic location are evident. Otherwise, the findings of the present study relating to methods and assay variability largely confirm those of previous surveys. In brief, interassay and within-method

**Figure 3** Summary of ratio results. The ratio of vWF:Ag/vWF:RCo (Ag/RCo, squares) or vWF:Ag/vWF:CB (Ag/CB, circles) for each sample tested is shown (see the legend for Figure 2 and the text for descriptions of the samples). A, Quality assurance program (QAP) samples 1a and 2a. B, QAP sample 4. Some ratios have been normalized to a maximum of 15. Ag/RCo ratios using the vWF:Act (alternative) (Shield Diagnostics, Dundee, England) enzyme-linked immunosorbent assay are circled. C, QAP samples 5 and 6. Ag, antigen; CB, collagen binding activity assay; RCo, ristocetin cofactor; vWF, von Willebrand factor.

**Figure 4** Summary of interpretations provided by participants. For descriptions of the samples, see the legend for Figure 2 and the text. *Expected consensus data, based on sample information and pretesting in the host laboratory and as further explained in text. QAP, quality assurance program. Types are types of von Willebrand disease.
Table 2
Summary of Unexpected Laboratory Interpretations*

<table>
<thead>
<tr>
<th>QAP Sample/Plasma Type and Expected Consensus</th>
<th>Participant Interpretation</th>
<th>Assay</th>
<th>Possible Explanation</th>
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<tbody>
<tr>
<td>Interpretation/Laboratory No. †</td>
<td>Factor VIII</td>
<td>vWF:Ag</td>
<td>vWF:RCo</td>
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<tr>
<td>1b/Normal</td>
<td>Equivocal</td>
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<tr>
<td>8</td>
<td>Type 2 vWD</td>
<td>46.0</td>
<td>52.0</td>
</tr>
<tr>
<td>9</td>
<td>Type 2 vWD</td>
<td>34.0</td>
<td>51.0</td>
</tr>
<tr>
<td>3/WF deficient/type 3 vWD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Type 2 vWD</td>
<td>6.0</td>
<td>33.0</td>
</tr>
<tr>
<td>4/Cryosupernatant (4x) /type 2 vWD</td>
<td>Mild type 1 vWD</td>
<td>26.0</td>
<td>20.0</td>
</tr>
<tr>
<td>11</td>
<td>Mild type 1 vWD</td>
<td>27.0</td>
<td>45.0</td>
</tr>
<tr>
<td>12</td>
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<td>Type 3 vWD</td>
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<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>Type 3 vWD</td>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5/Normal (22°C)</td>
<td>Mild type 1 vWD</td>
<td>57.0</td>
<td>38.0</td>
</tr>
<tr>
<td>14</td>
<td>Mild type 1 vWD</td>
<td>76.0</td>
<td>31.0</td>
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<tr>
<td>7</td>
<td>Mild type 1 vWD</td>
<td>51.0</td>
<td>65.0</td>
</tr>
<tr>
<td>2</td>
<td>Type 2 vWD</td>
<td>74.0</td>
<td>77.0</td>
</tr>
<tr>
<td>8</td>
<td>Type 2 vWD</td>
<td>86.0</td>
<td>82.0</td>
</tr>
<tr>
<td>9</td>
<td>Type 2 vWD</td>
<td>66.0</td>
<td>65.0</td>
</tr>
<tr>
<td>Ag, antigen; CB, collagen binding activity assay; EID, electroimmunodiffusion; LIA, latex immunoassay; NA, not applicable; ND, not done; QAP, quality assurance program; RCo, ristocetin cofactor; vWD, von Willebrand disease; vWF, von Willebrand factor.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>* Values are given as percentages or ratios. For further details about the samples, test methods, and procedures, refer to the text and Table 1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>† Expected consensus interpretations are based on knowledge of the plasma type and presurvey testing in the host laboratory. The laboratory number (which is randomized) indicates participants providing unexpected results.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‡ The interpretation given by the participant with data returned. It is possible that some laboratories indicating type 2 vWD might have been suggesting type 2N for low relative factor VIII levels.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Possible explanations suggest potential reasons for participant interpretations. Reasons were not always clear.</td>
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variation was similar for all assays, with CVs around 15% to 25% (Table 1), although those for vWF:Ag, FVIII:C, and vWF:CB generally were a little lower than those for vWF:RCo.

Despite similar assay variation, slight differences were noted in the range of assay results obtained for plasma sets using different assays. For example, for the normal plasma sample QAP-1a, all laboratories reported FVIII:C, vWF:Ag, vWF:RCo, and vWF:CB values as being more than 50% (Figure 2) and correctly identified the sample as normal (Figure 4). However, for normal plasma sample QAP-1b, a few laboratories recorded vWF:RCo and FVIII:C values of less than 50% (Figure 4), and 2 laboratories identified sample QAP-1b as equivocal and 2 as indicating type 2 vWD. Low assay results were not necessarily linked to these unexpected interpretations (Table 2), and, therefore, the reasoning behind some responses was unclear. Thus, although one of the laboratories providing an equivocal response also reported some equivocal results (notably a vWF:RCo value of 55% and an Ag/RCo ratio of 1.9), the other laboratory reported normal assay values. Similarly, although one of the laboratories providing the type 2 vWD response reported a high Ag/RCo ratio of 2.8 (and despite a normal vWF:Ag value of 171% by EID and a normal vWF:RCo value of 62%), the other laboratory reported normal vWF values.

Similarly, while samples QAP-2a and QAP-2b were identified by most laboratories as equivocal or indicating mild type 1 vWD (Table 2), the reasoning for 2 type 2 vWD (QAP-2a) and 4 type 2 vWD (QAP-2b) responses was unclear, as all results reported by these 6 laboratories were quite consistent with mild type 1 vWD or equivocal. Only 2 of these 6 laboratories performed vWF:CB.

Most laboratories identified sample QAP-3 as indicating type 3 vWD or severe type 1 vWD and sample QAP-4 as indicating type 2 vWD or severe type 1 vWD (Figure 4). All laboratories reported results for vWF:Ag and vWF:CB for samples QAP-3 and QAP-4 as less than 50% (Figure 2). Although most laboratories also reported vWF:RCo results of less than 50% for samples QAP-3 and QAP-4, 2 laboratories reported outlier results of more than 50% for sample QAP-4 (Figure 2). Interestingly, these 2 laboratories were not the laboratories that reported the unexpected interpretations for this plasma sample as shown in Table 2. It is not clear why QAP-4 yielded 3 mild type 1 vWD responses and 2 type 3 vWD responses or why 1 laboratory interpreted QAP-3 as indicating type 2 vWD. Low assay results were not necessarily linked to these unexpected interpretations (Table 2), and, therefore, the reasoning behind some responses was unclear. Thus, although one of the laboratories providing an equivocal response also reported some equivocal results (notably a vWF:RCo value of 55% and an Ag/RCo ratio of 1.9), the other laboratory reported normal assay values. Similarly, although one of the laboratories providing the type 2 vWD response reported a high Ag/RCo ratio of 2.8 (and despite a normal vWF:Ag value of 171% by EID and a normal vWF:RCo value of 62%), the other laboratory reported normal vWF values.

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Transcription error, use of a limited test panel, lack of use of vWF:CB, and potential inexperience in assay interpretations may explain many of these unexpected findings. Indeed, we believe each of the 4 gross outliers noted in Figure 2 (samples QAP-3 and QAP-4) to be the result of non–test-specific random events such as transcription error. For sample QAP-3, most laboratories reported the vWF:RCo value as less than 5%.

The laboratory reporting a value for vWF:RCo of 36% used an agglutination procedure and also reported 36% for sample QAP-6; thus, a transcription error owing to double reporting of the result for sample QAP-6 or, perhaps, an
intended value of 3.6% is a distinct possibility. For sample QAP-3, most laboratories reported a value of less than 5% for vWF:Ag. The laboratory reporting a value of 33% for vWF:Ag used an LIA and reported 33% for sample QAP-4; thus, a transcription error owing to double reporting of the result for sample QAP-4 or, perhaps, an intended value of 3.3% again is feasible. For sample QAP-4, most laboratories reported a value of less than 10% for vWF:RCo. The 2 laboratories reporting 51% and 55% values for vWF:RCo also used an agglutination procedure; transcription error also is possible.

Most laboratories correctly identified sample QAP-5 as normal/not vWD (Figure 4). Given the range of assay results reported, it is not disturbing to have some laboratories identify this sample as equivocal. However, 3 laboratories identified this sample as indicating mild type 1 vWD, and 3 laboratories identified this sample as indicating type 2 vWD. None of these 6 laboratories performed vWF:CB. In most cases, the unexpected interpretation probably resulted from a limited test panel and reliance on procedures with higher variability such as EID or vWF:RCo. Sample QAP-5 was derived from the same person tested in a previous survey,\textsuperscript{11} in which it was identified as sample 7. In that survey, most laboratories also correctly identified this sample as being normal, although a few unexpected interpretations similarly were identified on that occasion.

Sample QAP-6 provoked a range of interpretive comments, but most identified it as indicating mild type 1 vWD or type 2 vWD (Figure 4). Notably, no laboratory identified sample QAP-6 as a normal sample, despite it having derived from a healthy person (and, in fact, from the same collection as sample QAP-5) and despite both samples having been processed in a manner consistent with current NCCLS guidelines.\textsuperscript{13} These guidelines recommend that specimens for hemostasis assays in addition to prothrombin time and aPTT be kept at 2°C to 4°C or 18°C to 24°C until they are centrifuged and that they be tested within 4 hours from the time of collection. Sample QAP-6 was kept at approximately 4°C for 3.5 hours before centrifugation and processing, whereas sample QAP-5 was kept at approximately 22°C for 3.5 hours before centrifugation and processing. It has been noted recently that storage of whole blood at 4°C can lead to artifactual vWD results in a substantial proportion of samples.\textsuperscript{14} The person providing the present sample also was tested in this previous study, and 4°C treatment was noted to provide such a finding for the sample.\textsuperscript{14} In the present survey, presurvey lyophilization testing by the host laboratory for sample QAP-6 provided test results similar to the mean survey results. Overall results thus confirm that transport of samples at a low temperature (eg, 4°C) may not be appropriate for samples destined for vWF testing, despite NCCLS guidelines, and further support the view that a review of these guidelines be undertaken.

Loss of vWF function after low-temperature storage also has been noted in other studies.\textsuperscript{15,16}

The present survey comprised 44 participant laboratories, twice the number of previous surveys.\textsuperscript{11,12} Thus, compared with previous surveys, general increases in the number of tests performed were evident (Figure 5A). Of particular interest, however, is the changing test repertoire in some laboratories. Notably, performance of EID has almost ceased in this geographic region (Figure 5B; only 1 user remaining). Given the previously reported wide and variable test results using this method,\textsuperscript{11,12} this is not necessarily a bad trend. In the present study, the laboratory using this procedure provided occasional outcomes that suggested it was time to consider alternatives (Table 2). Contrasting the decline in the use of this method is the apparent increase in the use of the LIA procedure, with at least a doubling of the user base during the 6-year period (Figure 5). This method will continue to gain favor owing to automation. However, an evident limitation of the procedure seems to be its sensitivity to the presence of rheumatoid factor.\textsuperscript{17,18} Another assay apparently in declining use is the vWF:multimer assay, a time-consuming and technically complex procedure. The number of users for the vWF:RCo ELISA “alternative” (marketed as a “vWF:Act” assay [Shield Diagnostics, Dundee, Scotland]) remains reasonably small and steady. In previous studies, including the previous surveys, the vWF:Act ELISA did not generally provide as much evidence of functional discordance for type 2 vWD compared with vWF:RCo by agglutination and vWF:CB.\textsuperscript{5,6,11,12} Thus, in type 2 vWD, assay results tended to be higher for vWF:Act than for vWF:RCo by agglutination or vWF:CB, and Ag/RCo ratios tended to be lower and closer to one. In the present survey, only 1 type 2 vWD–like plasma sample was tested (sample QAP-4).

Of additional interest is the apparent decline in overall use of the vWF:RCo assay, from more than 90% in previous surveys to just less than 75% in the present survey. It is unclear whether this is because laboratories are replacing this assay with the vWF:CB; although absolute user numbers for the vWF:CB assay have increased compared with those in past surveys,\textsuperscript{11,12} the vWF:RCo assay apparently in declining use is the vWF:multimer assay, a time-consuming and technically complex procedure. The number of users for the vWF:RCo ELISA “alternative” (marketed as a “vWF:Act” assay [Shield Diagnostics, Dundee, Scotland]) remains reasonably small and steady. In previous studies, including the previous surveys, the vWF:Act ELISA did not generally provide as much evidence of functional discordance for type 2 vWD compared with vWF:RCo by agglutination and vWF:CB.\textsuperscript{5,6,11,12} Thus, in type 2 vWD, assay results tended to be higher for vWF:Act than for vWF:RCo by agglutination or vWF:CB, and Ag/RCo ratios tended to be lower and closer to one. In the present survey, only 1 type 2 vWD–like plasma sample was tested (sample QAP-4).

A consistent finding between the present study and previous surveys is that, on occasion, the reported interpretation did not match the reported assay results. It seems that some inexperience may continue to explain many of the unexpected interpretations. However, in contrast with previous surveys,\textsuperscript{11,12} in which most laboratories misinterpreted on at least 1 occasion, in the present survey, most
laboratories provided expected interpretations for all test cases, and it was only a small number of laboratories that provided most of the unexpected interpretations. Given the overall limitations of the testing analysis of our study (eg, inability to perform RIPA, inability to repeat some tests, lack of clinical information), generating occasional misinterpretations would not be surprising. Such findings serve to emphasize that a final diagnosis of vWD and subtype analysis require much more than 1-time plasma testing. Accordingly; consideration always should be given to clinical bleeding and family history, test results always should be repeated for confirmation, and the test panel must be complete and comprehensive. We hope that one reason that many laboratories participating in the present survey did not misinterpret is that their personnel have learned from their experience of previous surveys. However, it also is clear that personnel in some laboratories require continued education. We look forward with interest to the planned international vWF/vWD test survey to be conducted by the Working Party on the standardization of vWD diagnosis on behalf of the International Society on Thrombosis and Haemostasis.21

Conclusions

It is clear from this and previous surveys that in most test cases, a laboratory usually will correctly identify a normal plasma sample as having derived from a healthy person and a vWD plasma sample as having derived from a person with vWD. Furthermore, when vWD can be identified, the laboratory usually will identify the correct vWD subtype. However, it also is clear that in around 5% to 10% of test cases, an incorrect diagnosis or incorrect subtype will be defined during initial laboratory testing. A number of reasons can be identified for this, including transcription errors, sample identification errors, laboratory test interpretation errors, inexperience, and assay test limitations (individual assay limitations, test panel limitations, or method-based limitations). Although it may be impossible to completely eliminate such errors, it usually is possible to reduce error rates and improve interpretation outcomes. At a basic level, laboratories should put measures into place to reduce transcription and sample identification errors. In addition, it is important to understand the limitations of the laboratory process as undertaken in each laboratory and to put in place additional measures to avoid potential errors due to limitations in test methods and test panels. For example, it is clear from these studies that certain methods such as EID are problematic. In addition, functional vWF assays have their limitations, in particular the vWF:RCo.7,19 Most problems can be overcome by simply requesting repeated samples for confirmation of initial test results, using further more extensive testing panels as required, and reviewing test results in line with clinical and family histories.5,7

Thus, given preliminary test findings and a measure of clinical suspicion, it may be necessary to extend the subsequent test panel used according to case-by-case need. For specific vWD investigations, a strong recommendation can be made for assessing platelet count, FVIII:C, vWF:Ag, and vWF:CB and/or vWF:RCo in the preliminary screen. If a bleeding disorder other than vWD is feasible, the screen could include routine coagulation assays and a platelet-function screening process (eg, PFA-100). Based on initial findings and clinical suspicion, a repeated test for confirmation often is required. This repeated testing can be followed by performance of additional tests on a case-by-case basis. Such additional tests might include RIPA, vWF:factor VIII binding assay, and vWF:multimers. It also is important not to place excessive confidence in certain test procedures, since ultimately, no test procedure is infallible.5-7 Finally, it is important to recognize the many pretest variables (eg, sample collection, processing, transport, and storage) that might influence assay results for vWF and factor VIII.5,7 Consequently, the resultant test findings generally will relate to the measure of vWF in the sample, but this may be affected by the quality of the sample received and tested and might not always reflect the actual condition.

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References

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