Preface

Laboratory Diagnostics for Thrombosis and Hemostasis Testing—Part I

Kristi J. Smock, MD[1] Robert C. Gosselin, CLS[2]

[1] Department of Pathology and ARUP Laboratories, University of Utah, Salt Lake City, Utah
[2] Hemostasis and Thrombosis Center, University of California, Davis Health System, Sacramento, California

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Welcome to this themed edition of *Seminars in Thrombosis and Hemostasis* focused on laboratory diagnostics in the hemostasis/thrombosis arena. High-quality clinical laboratory testing is essential for the accurate diagnosis and care of patients with inherited or acquired disorders of the hemostatic system, or with pathologic thrombosis. Close attention must be paid to the preanalytical (specimen collection and handling),[1] analytical (the testing process),[2] and postanalytical (test interpretation contribution to diagnosis and treatment)[3] variables associated with coagulation testing to assure accurate reporting of assay result(s) and corresponding result interpretation(s). The manuscripts in this edition of the journal represent state of the art summaries of testing aspects for antiphospholipid antibodies (including lupus anticoagulant [LA][4] and solid phase testing[5]), D-dimer,[6] activated protein C (APC) resistance,[7] and von Willebrand factor (VWF),[8–13] highlighting information essential for accurate diagnosis and monitoring of common disorders that significantly impact health care systems and patient lives.

The VWF papers are a special collection of updates on VWF testing patterns and assay performance and quality contributed by authors representing five external quality assessment (EQA) programs operating in North America, Europe, and Australasia.[8–13] For those less familiar, EQA participation is an important component of quality laboratory practice and required by regulatory agencies. EQA organizations challenge laboratories to test blinded lyophilized plasma specimens representing the normal condition or a disease process, in this case primarily von Willebrand disease (VWD). Specific for VWF, once testing has been completed, the laboratories submit their results back to the EQA organization which then statistically evaluates all of the participant results for each assay with the summarized data returned to each laboratory, also reflecting how their submitted data compared with their peers. An additional benefit is the collection of information regarding use and performance of particular laboratory tests, methods, and manufacturers, both for diagnostic accuracy and between-laboratory reproducibility, essential markers of test utility. Residual EQA material also serves as an excellent source of well-characterized plasma that can be used for troubleshooting purposes as well as confirming the performance of a new VWF method prior to implementation. This collection provides a fascinating snapshot of laboratory practice in different parts of the world, presumably driven by test and method availability, regulatory approval, influence of published guidelines, and local tradition. The combined data include important information to consider when using VWF activity/antigen ratio data for VWD subtyping, recognizing that several widely used practice guidelines or diagnostic approaches recommend varying ratios (typically between 0.5 and 0.7) for discrimination between VWD subtypes.[14–18] The detailed laboratory data suggest that the diagnostic performance of static ratios is highly dependent on the specific tests being used as no single cutoff works perfectly for all VWF activity and antigen combinations. The data also show decreasing worldwide use of the traditional ristocetin cofactor activity (VWF:RCo), balanced by increasing use of the more modern glycoprotein Ib (GPIb)-based activity assays that utilize recombinant fragments or mutated gain-of-function fragments of the platelet GPIb receptor, using methodologies such as latex immunoassay or chemiluminescence.[19] A final VWF article, authored by Favaloro and Pasalic, nicely summarizes the complexity of VWD and diagnostics.[13]

The contributions to this issue begin with a thorough discussion of pertinent aspects of LA testing by Moore, with a focus on new assays that are not based on activated partial thromboplastin time or dilute Russell viper venom time, diagnostic algorithms including placement of when to perform mixing studies, approach to result interpretation, and the effects of anticoagulants on testing.[4] A highlight of this

Address for correspondence Kristi Smock, MD, Department of Pathology and ARUP Laboratories, University of Utah, Salt Lake City, UT 84112 (e-mail: kristi.smock@aruplab.com).

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manuscript is a table, which shows results patterns in 26 examples of LA testing.

Focused on antiphospholipid antibodies, the manuscript by Devreese is centered on the current state of solid phase assays including the challenges related to lack of standardization of calibrators, incompatible units between testing methods, effects of interlaboratory variability on antibody classification and titer, and use of differing cutoffs (such as > 40 GPL/MPL or > 99th percentile) with effects on sensitivity and specificity.[5] The relationship between anticardiolipin (aCL) and anti-b2-glycoprotein I (aB2GPI) antibodies is thoroughly explored, as is use of testing to detect antiphosphatidylserine/prothrombin antibodies to provide additional information on thrombotic risk, especially for patients with result combinations that do not fit the high-risk triple positive pattern (positive for LA, aCL, and aB2GPI).

The next contribution to the issue by Tachil et al explores the complexities of D-dimer testing and interpretation.[6] Especially helpful, is the discussion of how D-dimer is formed and explanation of normal D-dimer levels versus elevations seen in a range of conditions including inflammation, venous thromboembolism, disseminated intravascular coagulation, and more recently the use of this test in coronavirus disease 2019 (COVID-19) infection, where the degree of D-dimer elevation is a marker of disease severity with prognostic implications. In addition, we now know that D-dimer testing should be included in the suite of tests to screen for COVID-19 vaccine-induced thrombotic thrombocytopenia.[20]

In the fourth manuscript of the issue, Morimont et al summarize laboratory testing to evaluate phenotypic APC resistance including a review of different mechanisms resulting in this phenotype and descriptions of the functional assays that can be used for detection.[7] Particularly valuable is the description of endogenous thrombin potential (ETP) assays that provide a more global view of APC resistance in contrast to clot-based assays that are optimized for detection of APC resistance due to the factor V Leiden mutation. For instance, the paper highlights ETP-based detection of an APC-resistant phenotype due to hormonal contraceptive use.

Moving into the VWF collection, the fifth manuscript of the issue was authored by Salazar et al to summarize the College of American Pathologists (CAP) VWF Proficiency Testing Program.[8] This contribution provides a snapshot of the assays most commonly used in North American clinical laboratories, possibly driven by regulatory approval or dominant reagent-instrumentation platforms. For example, VWF collagen-binding assays, while popular in other locales, appear to be used minimally in North America. The CAP data also show how VWF assay bias may be driven by either calibrator choice or traceability of the VWF calibrator assignment. Due to the nature of the proficiency specimens, this paper was less able to comment on diagnostic performance of VWF activity/antigen ratios.

Also addressing North American laboratories performing VWF testing, Ziemba et al summarize the North American Specialized Coagulation Laboratory Association (NASCOLA) VWF EQA experience, demonstrating similar VWF assay use and test method trends to that described by the CAP group.[9] This manuscript delves into discussion of the background and rationale for laboratory guideline elements in the recently published multidisciplinary guideline for diagnosing VWD put forth by the American Society of Hematology, International Society on Thrombosis and Haemostasis (ISTH), National Hemophilia Foundation, and World Federation of Hemophilia.[14] The NASCOLA data support the guideline recommendation to use newer VWF activity assays (due to superior coefficients of variation, for instance) and these data also demonstrate misclassification potential of VWD subtypes when using suboptimal VWF activity/antigen cutoff ratios. Furthermore, important points are raised by the discussion of lack of VWF assay calibration harmonization.

In the next manuscript, Favaloro et al provide an educational summary of the 25-year history of the Royal College of Pathologists of Australasia Quality Assurance Program VWF program with a more granular update of the past 9 years.[10] The program is unique in that it includes sample types not represented in most other programs, such as acquired von Willebrand syndrome and VWF concentrates, and the program reporting is designed to capture detailed nuances for a spectrum of VWF assays. Four test panels including collagen-binding activity in addition to antigen, platelet binding activity, and factor VIII activity, are common in this locale, and the level of detail allows a close look at the diagnostic accuracy of VWF activity/antigen ratios using many different assays. Key takeaways include support for the superiority of modern activity assays to assess VWF platelet binding, in contrast to the less well-performing VWF:RCo, the added diagnostic benefit of including collagen binding assessment, and the idea that best discrimination between type 1 and type 2 VWD may come from using VWF activity/antigen ratios that are assay-specific rather than global.

An example of European VWF EQA experience comes from the contribution by Jennings et al that summarizes data from the United Kingdom National External Quality Assessment Scheme (UK NEQAS).[11] This group again emphasizes the importance of VWF assay calibration and impacts on VWF activity/antigen ratios and diagnostic cutoffs. The authors point out that international standard ISTH Lot 5 began assigning values for specific VWF activity assays, such as VWF:GPIbM and VWF:GPIbR,[21] acknowledging that different VWF assays can indeed produce different results. As shown in the data, use of assay specific calibration results in test values closer to the intended targets when international standards are tested as patients. The UK NEQAS data also include results for a VWF genetic challenge, detection of exon 28 mutations, the only example of VWF genetic testing in our collection of VWF EQA programs.

In the final VWF EQA manuscript, Hollestelle et al describe the External Quality Control for Assays and Tests Foundation VWF EQA program which includes additional European and North American laboratory participants.[12] A highlight of this paper is the analysis of a participant questionnaire regarding their local VWF testing and interpretation practices with a high number of survey respondents. That survey data showed a variety in individual laboratory approaches for VWD diagnosis which is an important consideration for the

treating physician(s) to keep in mind. Their data also demonstrated variability in diagnostic accuracy when using different VWF activity/antigen ratio cutoffs but also showing that sample misclassification comes from either individual VWF test results, combination of VWF test results reported as ratios, and the cutoffs being used for VWD classification. However, the authors note that other types of errors in interpretation can be made despite correct results and using optimal VWF activity/antigen ratio cutoffs.

To round out the VWF collection, Favaloro and Pasalic have summarized the complexity of the diagnostic landscape for VWD with a particular focus on the geographic differences due to a variety of factors.[13] Readers will be pleased to see detailed summaries of different VWF assays, making this manuscript a great resource for anyone looking to learn about the myriad different testing options for diagnosing VWD or monitoring VWD therapy. The heterogeneity of VWD, including the six currently recognized subtypes, is discussed with emphasis on the laboratory context and differences in assay performance, geographical approaches, etc. The authors correctly point out that EQA programs provide essential data that can be used to improve a more accurate diagnosis and subtyping of VWD worldwide.

In summary, we are excited to present this themed issue of *Seminars in Thrombosis and Hemostasis* focused on laboratory diagnostics and VWF quality performance. We hope the readership learns as much from reading this excellent issue as we did in the editing process and would like to warmly thank the contributing authors for their efforts in compiling important state of the art summaries and VWF EQA data.

Conflict of Interest
None declared.

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