

FEATURED REAADS PRODUCTS

REAADS von Willebrand Factor Antigen Test Kit

For *In Vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Sample matrix -	Citrated human plasma
Sample dilution -	1:26
Capture antigen-	rabbit anti-human von Willebrand Factor (VWF)
Detection antibody-	Horseradish peroxidase conjugated rabbit anti-human VWF
Chromogenic substrate -	TMB (single component)
Stopping solution -	0.36N sulfuric acid
Assay incubations	
Sample -	15 min @ room temperature
Conjugate -	15 min @ room temperature
Substrate -	10 min @ room temperature
Wavelength -	450 nm
Assay calibration -	six point reference curve Prepared from Reference Plasma provided in kit.
Assay sensitivity -	≤ 5% of normal
Product number -	034-001

REAADS Collagen Binding Assay*

For Investigational Use Only

Assay format -	96-well microtiter plate (6 x 16 strips) with breakaway wells
Sample matrix -	Citrated human plasma (3.2% sodium citrate)
Sample dilution -	1:40
Capture antigen-	Collagen (equine)
Detection antibody-	Horseradish peroxidase (HRP) conjugated rabbit anti-human VWF
Chromogenic substrate -	TMB (single component)
Stopping solution -	2M sulfuric acid
Assay incubations	
Sample -	60 min @ room temperature
Conjugate -	60 min @ room temperature
Substrate -	5 min @ room temperature
Wavelength -	450 nm
Assay calibration -	Reference curve prepared with Normal Standard provided in kit.
Normal range -	50–400%
Product number -	11160

*CE marked for distribution in the European Community

UPCOMING CONFERENCES

September 18-20, 2008: The 2008 Scientific Assembly, an annual CME event sponsored by the American Academy of Family Physicians (AAFP), will convene at the Convention Center in San Diego. Corgenix will feature the AspirinWorks® Test at booth #4109 during the Exposition, where company representatives will be available to discuss the latest research on aspirin resistance.

September 24-25, 2008: A Case-Oriented Symposium on Bleeding and Thrombosis, sponsored by Nichols Institute for Coagulation, will be held at the Hyatt Regency in Reston, VA. We invite you to stop by the **Corgenix** display at the Exhibitor Showcase to learn about our newest products, the AspirinWorks® and the IgG Anti-AtherOx® Tests.

October 30–November 4, 2008: The Liver Meeting 2008, the 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD) will be held at the Moscone West Convention Center in San Francisco. Corgenix representatives look forward to meeting you at booth #610 during the exhibition where we are featuring the Hyaluronic Acid (HA) Assay. The test

(which is available for research use only in the US) has been proposed as a non-invasive fibrosis marker in chronic liver disease.

November 8-12, 2008: Scientific Sessions 2008, sponsored by the American Heart Association, will be held at the Ernest M. Morial Convention Center in New Orleans, LA. The meeting is billed as the premier cardiovascular meeting in the world. Visit the Corgenix booth (#2536) in the exhibit hall for details about the AspirinWorks® Test for assessing aspirin effect in healthy individuals on low dose aspirin therapy, and the IgG Anti-AtherOx® Test, recently cleared by the FDA for the detection of IgG antibodies to complexes formed by oxidized low-density lipoprotein (oxLDL) with B2 glycoprotein I (B2GPI) in individuals with SLE and lupus-like disorders such as the antiphospholipid syndrome (APS).

Corgenix, Inc.
11575 Main Street, Suite 400
Broomfield, CO 80020, USA

Phone: 303-457-4345
Toll Free: 800-729-5661
Fax 303-457-4519
Website: www.corgenixonline.com
Email: techsupport@corgenix.com

**Specialists in ELISA
diagnostic technology**

corgenix



THE READER

September 2008
Volume 18, Number 3

Guidelines for the Diagnosis, Evaluation and Management of von Willebrand Disease

The National Heart, Lung, and Blood Institute (NHLBI), a division of the National Institutes of Health (NIH), recently published a comprehensive set of clinical practice guidelines for von Willebrand disease (VWD). These guidelines for diagnosis and management of VWD represent four years of effort by a panel of experts, chaired by Dr. William Nichols of the Mayo Clinic. The Expert Panel reviewed the literature for clinical trials, multicenter studies, meta-analyses, prospective studies, academic reviews, validation studies, technical reports, case reports, etc., that were published between 1990 and 2006, as well as earlier landmark references. These references, in combination with the Panel's expertise and consensus, form the basis for the guidelines. This paper will review the guidelines for the initial evaluation and laboratory diagnosis of VWD.

VWD is an inherited bleeding disorder characterized by either deficiency or dysfunction of von Willebrand Factor (VWF). VWF is a plasma protein that mediates platelet adhesion to sites of vascular injury, and binds to activated factor VIII (FVIII), protecting it from proteolytic cleavage in circulation. VWD is a heterogeneous disease. It is classified based on distinct pathophysiologic mechanisms with unique clinical features and therapeutic strategies (outlined in Table 1). The classification criteria include the changes recommended in 2006 by the ISTH Subcommittee on VWF, and are based on the VWF protein phenotype of the patient rather than genotypic data, as protein characteristics are accessible through commonly available laboratory tests, while the availability of genotypic data is limited.

Type 1 VWD is a partial quantitative deficiency of VWF due to decreased synthesis. Approximately 75% of symptomatic patients have Type 1 VWD. These patients have concordant decreases in VWF protein concentration (VWF:Ag) and function. In addition, Factor VIII levels may be reduced secondary to reduced VWF.

Type 2 VWD subjects have a qualitative (functional) VWF defect. Type 2 VWD is further classified into four variants (2A, 2B, 2M, 2N) with distinct, specific therapeutic needs, based on details of the phenotype:

Type 2A variants have decreased VWF-dependent platelet adhesion related to a deficiency of high molecular weight multimers. Levels of VWF:Ag and FVIII may be normal or modestly decreased (typically <50 IU/dL), but VWF function is disproportionately lower. Type 2A deficiency may result from defective assembly or secretion of large multimers, or increased susceptibility of VWF multimers to proteolysis in circulation.

In **Type 2B** variants, their VWF has an increased affinity for platelet Glycoprotein Ib associated with the proteolytic degradation and depletion of large MW multimers in plasma. The mutant VWF also coats circulating platelets, interfering with their ability to adhere at sites of injury. Laboratory findings are similar in Type 2A and 2B patients (low to normal antigen levels with reduced function), however Type 2B patients may have mild thrombocytopenia.

Type 2M VWD variants have a mutation that causes decreased VWF-dependent platelet adhesion, not associated with an absence of high MW multimers. The interaction of VWF with platelet GPIb or with connective tissue is reduced, while multimer assembly is not substantially impaired. These patients can be distinguished from Type 2A variants in the laboratory using multimer gel electrophoresis.

Type 2N VWD variants have VWF mutations that impair binding to FVIII, typically lowering FVIII levels to less than 10 percent, mimicking an autosomal recessive form of hemophilia A.

(continued on page 2)

Table 1: Classification of VWD

Type	Description
1	Partial quantitative deficiency of VWF
2	Qualitative VWF defect
2A	Decreased VWF-dependent platelet adhesion with selective deficiency of high-molecular weight multimers
2B	Increased affinity for platelet GPIb
2M	Decreased VWF-dependent platelet adhesion without selective deficiency of high-molecular-weight multimers
2N	Markedly decreased binding affinity for FVIII
3	Virtually complete deficiency of VWF

Table adapted from *The Diagnosis, Evaluation and Management of von Willebrand Disease*

VWF:Antigen and VWF:Activity levels are typically normal. FVIII-VWF binding assays can be used to discriminate Type 2N from hemophilia A.

Type 3 VWD is a virtual absence of VWF, and is characterized by undetectable VWF protein and activity. FVIII levels are typically very low in these patients. A variety of mutations throughout the VWF gene can cause type 3 VWD; most are unique to the family in which they were first identified.

In reality it is not always easy to distinguish between the various VWD subtypes through laboratory testing. Some patients have compound mutations that cause VWD by different mechanisms, while some mutations have multiple phenotypic expressions. Vicenza VWD is a good example. Vicenza variants have VWF levels usually <15 IU/dL, with VWF multimers larger than normal. These individuals have a specific mutation that promotes rapid clearance of VWF from circulation. Due to rapid clearance, their multimers have less of an opportunity for cleavage by ADAMTS13, which may account for the increased multimer size. Depending on the interpretation of lab results, Vicenza VWD can be classified as either Type 1 or Type 2M VWD.

Patient Evaluation: The new NHLBI guidelines emphasize the importance of a detailed assessment of a patient’s clinical history, including a personal history of excessive bleeding or a positive family history of a bleeding disorder, prior to initiating a laboratory evaluation for VWD.

Suggested questions for screening asymptomatic patients prior to surgical or interventional procedures are included in the guidelines, along with a decision tree for identifying patients needing further evaluation. These patients are subjected to a set of more definitive questions to screen for bleeding disorders. The initial patient screening also includes a physical examination to confirm evidence of a bleeding disorder (such as ecchymoses, hematomas, and petechiae), and to look for other potential causes of increased bleeding. If the history and physical examination is suggestive of VWD, a laboratory evaluation should be performed.

Laboratory Evaluation: Unless the patient has a strong bleeding history, the initial hemostasis screening tests recommended in the guidelines include a CBC and platelet count, PTT, PT, and either a fibrinogen or Thrombin Time (TT). A bleeding time is no longer recommended. The initial screening tests may potentially identify other causes for bleeding including thrombocytopenia, an isolated abnormal PT, low fibrinogen, or abnormal TT. These patients should be referred for other appropriate evaluation.

Patients with an isolated prolonged PTT that corrects on 1:1 mixing study or with a strong bleeding history should be evaluated for VWD by testing 1) the amount of VWF antigen present in plasma (VWF:Ag), 2) the function of VWF protein using ristocetin cofactor (VWF:RCo) or other assays, and 3) FVIII levels.

VWF:Ag assays measure the concentration of VWF protein in plasma by immunoassay, such as enzyme-linked immuno-sorbent assay (ELISA) or automated latex immunoassay (LIA). The standard reference plasma used in the assay is critical. It should be calibrated against the World Health Organization (WHO) Standard. The guidelines recommend reporting results in international units (IU/dL or IU/mL). IU/dL is most commonly used due to the similarity to the conventional manner of reporting factor assays as a percentage of normal.

VWF:RCo and other functional assays:

VWF:RCo is the most widely used functional test for VWF. It measures the ability of VWF to agglutinate and aggregate normal platelets in the presence of ristocetin. Several different methods are used to measure ristocetin-induced platelet aggregation including 1) time to visible platelet clumping, 2) slope of aggregation, 3) automated turbidometric tests, and 4) ELISA assays that assess direct binding of patient VWF to platelet GP1b in response to ristocetin. Some drawbacks to the ristocetin assays are the high intra- and interlaboratory variation, the inability of some methods to quantitate extremely low levels, and the fact that the test does not measure physiologic function. 5) vWF:Activity ELISA or LIA assay (not involving ristocetin) to assess the binding of a monoclonal antibody to a conformational epitope of the VWF A1 loop, which contains the GP1b binding site.

FVIII coagulant assay measures the ability of VWF to bind and maintain the level of FVIII in circulation. It is typically run as a one-stage clotting assay which measures the ability of plasma FVIII to shorten the clotting time of FVIII-deficient plasma. This test is important for discriminating between VWD and hemophilia A.

If all of the initial VWD test results are normal, the patient should be referred for other appropriate evaluation. In a patient with a strong bleeding history, it may be necessary to retest the patient to identify low levels of VWF. VWF is an acute phase reactant - VWF levels can be elevated during conditions of stress, including surgery, exercise, anxiety, systematic inflammation, pregnancy or the administration of estrogen/oral contraceptives, which could mask lower baseline levels. In addition, pre-analytic variables associated with traumatic venipuncture or improper sample processing or storage can influence test results.

See **Table 2** on the following page for cutoff values suggested by the Expert Panel for a definitive VWD diagnosis.

If any of the initial VWD tests are abnormal, the patient is generally referred to a hemostasis specialist for more specialized VWD studies to diagnose and classify VWD. The initial VWD tests may be repeated for confirmation.

(continued on page 3)

September 1, 2008: A moderated poster, “Incomplete inhibition of thromboxane biosynthesis by ASA: determinants and effect on cardiovascular risk” presented by John Eikelboom at the 2008 Congress of the European Society of Cardiology confirmed that elevated urinary 11-dehydro thromboxane B2 (11dhTxB2) concentrations in ASA-treated patients were associated with an increased risk of stroke, myocardial infarction (MI) or cardiovascular (CV) death.

In a prospective multi-center study, baseline urinary 11dhTxB2 levels were measured in a population of 3261 high vascular risk patients from the CHARISMA trial who were on ASA therapy. The patients were followed for the occurrence of stroke, myocardial infarction or cardiovascular death. The results showed that urinary 11dhTxB2 concentrations in the highest quartile were associated with an increased risk of stroke, MI or CV death compared with the lowest quartile (Adjusted Hazard Ratio [HR] 1.66 to 2.61, p=0.03). Factors independently associated with higher urinary 11dhTxB2 levels included increasing age, female gender, history of peripheral artery disease, current smoking, and oral hypoglycemic or angiotensin converting enzyme (ACE) inhibitor therapy. ASA dose ≥ 150 mg.d, history of treatment with NSAIDS, history of hypercholesterolemia, and statin treatment were associated with lower 11dhTxB2 levels.

Based on the study results, the authors concluded that, “urinary concentrations of 11dhTxB2 are an externally valid and potentially modifiable determinant of stroke, MI or CV death in patients at risk of atherothrombotic events. The potential for higher doses of ASA and statins to reduce urinary 11dhTxB2 concentrations and CV risk should prompt randomized evaluation of the clinical efficacy of titrating doses according to 11dhTxB2 concentrations, and the clinical efficacy of other treatments that reduce thromboxane production.”

Reference: Incomplete inhibition of thromboxane biosynthesis by ASA: determinants and effect on cardiovascular risk. J. Eikelboom, GJ Hankey, DL Bhatt, et al. European Heart Journal (2008) 29(Abstract Supplement), 404.

VWF:Ag is calculated to see if the decrease in activity and antigen levels is proportional. A ratio of <0.6 or <0.7 indicates dysfunctional VWF (i.e. possible type 2 VWD).

A VWF multimer test may be performed to determine the variable concentrations of the different sized VWF multimers, preferably using a frozen aliquot of the same plasma sample used for the initial round of VWD tests. The VWF Collagen Binding assay (CBA) may also be used to differentiate type 1 VWD from types 2A, 2B, or 2M. This test measure the binding of VWF to collagen, which is dependent on multimeric size. The largest multimers bind more avidly than smaller forms. Other specialized VWD tests used to classify VWD patients include low dose ristocetin platelet aggregation (RIPA), and the VWF-platelet binding assay (VWF:PB assay), used to diagnose type 2B VWD. The VWF:FVIII binding assay is useful for diagnosing type 2N VWD. It measures the ability of VWF to bind added exogenous FVIII. Additional studies for selected patients may include Gene sequencing, assays for antibodies to VWF, and platelet-binding studies.

In summary, the laboratory diagnosis of VWD is challenging, and in some cases, the testing may need to be tailored to individual patients. An accurate diagnosis and classification is critical as treatment depends on the specific subtype of the disease. For more information and VWD Management recommendations, see the full report, “The Diagnosis, Evaluation, and Management of von Willebrand Disease” which is available on the NHLBI Website (www.nhlbi.nih.gov) and from the NHLBI Health Information Center.

Condition	VWF:Ag (IU/dL)	VWF:RCo (IU/dL)	FVIII	Ratio of VWF:RCo/VWF:Ag
Type 1	30*	<30*	↓ or Normal	>0.5 - 0.7
Type 2A	<30 - 200*†	<30*	↓ or Normal	<0.5 - 0.7
Type 2B	<30 - 200*†	<30*	↓ or Normal	Usually <0.5 - 0.7
Type 2M	<30 - 200*†	<30*	↓ or Normal	<0.5 - 0.7
Type 2N	30 - 200	30 - 200	↓↓	>0.5 - 0.7
Type 3	<3	<3	↓↓↓ (< 10 IU/dL)	Not applicable
Normal	50 - 200	50 - 200	Normal	>0.5 - 0.7

*<30 IU/dL is designated as the level for a definitive diagnosis of VWD; there are some patients with type 1 or type 2 VWD who have levels of VWF:Ag and/or VWF:Rco of 30-50 IU/dL

†The VWF:Ag level in the majority of individuals with type 2A, 2B, or 2M VWD is <50 IU/dL