

## Collagen Binding Activity Assay for von Willebrand Disease

### Background Information

Von Willebrand disease (VWD) is the most common inherited bleeding disorder with a prevalence of approximately 1% in the general population. It also can occur as an acquired bleeding disorder. VWD is a clinically heterogeneous disorder with several subtypes due to deficiency and/or dysfunction of von Willebrand factor (VWF). VWF is a multimeric adhesive glycoprotein that plays a major role in primary hemostasis and coagulation. VWF mediates adhesion of platelets to injured sub-endothelium and to the platelet surface receptor GPIb and serves as the specific carrier protein for coagulation factor VIII (fVIII) in plasma preventing proteolytic degradation. The revised classification of VWD identified two major categories, quantitative and qualitative defects.

The quantitative VWF defects include type 1 (partial deficiency of VWF) and type 3 (complete absence of VWF) in plasma and/or platelets. Type 2 is a qualitative VWF defect that is further classified as four subtypes by different pathophysiologic mechanisms.

Accurate laboratory diagnosis and classification of VWD using both quantitative (antigenic) and qualitative (functional) assays based on the VWD diagnostic algorithm (see page 3) are crucial because the presenting biological activity of VWF determines both the hemorrhagic risk and subsequent clinical management.

### Clinical Indications

The functional activity of VWF traditionally has been assessed using the ristocetin cofactor activity (RiCof) assay, which measures the VWF-mediated agglutination of platelets

in the presence of ristocetin. However, the usefulness of this assay has limitations due to poor reproducibility and lack of calibration. The collagen binding activity (CBA) assay has been proposed as a supplemental test for VWF activity.

The CBA assay is based on the ability of multimeric forms of VWF to bind collagen, and its greatest strength lies in the ability to selectively detect primarily high molecular weight (HMW) forms of VWF, which are known to be most functional and adhesive.

The CBA assay is a useful adjunctive to the RiCof assay for the diagnosis of VWD and to differentiate VWD with deficiency of HMW multimer forms in type 2A and type 2B from type 1. It also can differentiate very low levels of VWF in severe type 1 from complete absence of VWF in type 3 and has been reported as a better marker for therapeutic efficacy of treatment with DDAVP<sup>®</sup> (desmopressin) and fVIII concentrate.

### Interpretation

CBA results are reported as % of the reference value for CBA. CBA to VWF:Ag ratio is calculated to provide a ratio of VWF activity to protein amount.

1. Type 1 VWD patients have concordantly decreased CBA and VWF:Ag levels.
2. Type 3 VWD patients have markedly decreased or nearly absent CBA and VWF:Ag levels.
3. Type 2A VWD and type 2B VWD patients have discordantly decreased CBA and VWF:Ag levels with markedly decreased CBA level, normal or decreased VWF:Ag level and loss of HMW multimers.

4. Type 2M VWD patients have a discordantly decreased CBA level with a normal or decreased VWF:Ag level but without the loss of HMW multimers.
5. Type 2N VWD patients have normal CBA and VWF:Ag with discordantly decreased FVIII coagulant activity.
6. CBA values are known to be lower in O blood groups compared with non-O blood groups. However, as VWF:Ag levels show similar blood group dependence, the ratio of CBA/VWF:Ag is not affected.

#### Methodology

The CBA assay is an enzyme immunoassay (REAADS® Collagen Binding Assay ELISA kit, Corgenix, Inc., Broomfield, Colo.) that quantitates the binding of VWF to a collagen-coated microwell plate. After binding peroxidase-conjugated anti-VWF antibodies to VWF multimers, the resulting color intensity is determined photometrically, which is proportional to HMW forms of VWF present in the plasma. *In situ* evaluation for precision and accuracy of the CBA assay shows low coefficient of variation (6.3-11.1%) with lower limit of detection 0.2% (linearity 1-530%).

#### Specimen Collection and Handling

Collection of blood by routine venipuncture in a 3.5ml light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant.

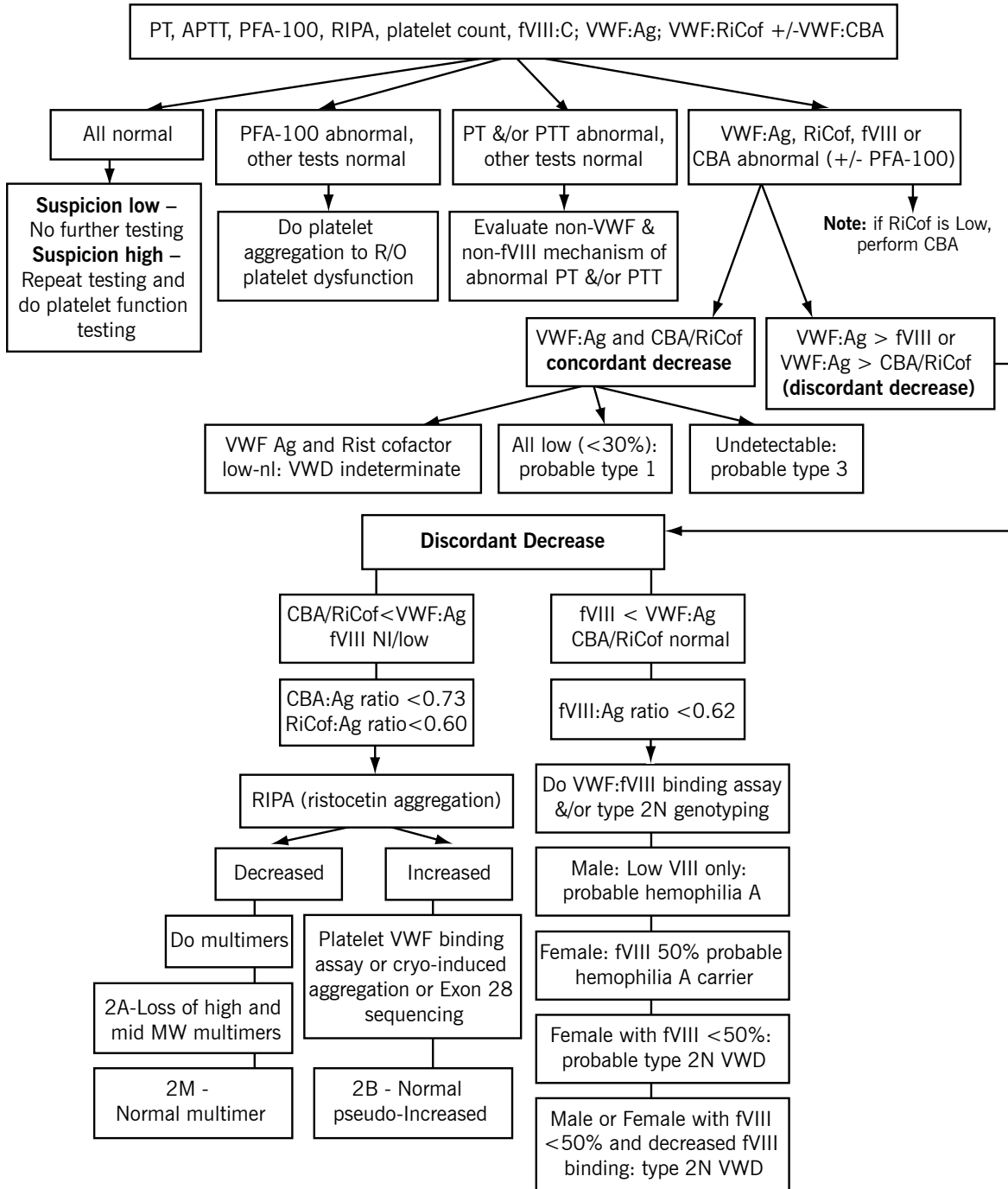
Pediatric volume of 2.5ml with an appropriate ratio of anticoagulant is acceptable.

Specimens other than 3.2% trisodium citrate plasma and those that are improperly collected, stored, misidentified or of insufficient volume are unacceptable. Also refer to "Criteria for rejection and special handling of coagulation specimens."

#### Suggested Reading

1. Favaloro EJ. Von Willebrand factor collagen-binding (activity) assay in the diagnosis of von Willebrand disease: a 15-year journey. *Semin Thromb Hemost.* 2002;28:191-202.
2. Nichols WL *et al.* The diagnosis, evaluation, and management of von Willebrand disease. U.S. Department of Health and Human Services. NIH Publication No. 08-5832, December 2007.
3. Castaman G, Federici AB, Rodeghiero F, Mannucci PM. Von Willebrand disease in the year 2003: toward the complete identification of gene defects for correct diagnosis and treatment. *Haematologica.* 2003;Jan;88(1):94-108.
4. Kalla A, Talpsep T. The von Willebrand factor collagen-binding activity assay: clinical application. *An Hematol.* 2001;80:466-471.
5. Favaloro EJ. Toward a new paradigm for the identification and functional characterization of Von Willebrand disease. *Semin Thromb Hemost.* 2009;35:60-75.

## DIAGNOSTIC ALGORITHM FOR VON WILLEBRAND PANEL



**Abbreviations:** Ag - antigen; CBA - collagen binding activity; fVIII:C - factor VIII: coagulant; MW - molecular weight; NL - normal; PFA-100 - platelet function screen; PT - prothrombin time; PTT - partial thromboplastin time; RiCof - ristocetin cofactor; RIPA - ristocetin-induced platelet aggregation; VWF - von Willebrand factor

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**Test Overview**

<b>Test Name</b>	Collagen Binding Activity Assay
<b>Reference Range</b>	CBA: 41-161%; Ratio of CBA/VWF:Ag $\geq$ 0.73
<b>Specimen Requirements</b>	Testing Volume/Size: 2 mL; Type: Plasma; Tube/Container: Sodium citrate (lt. blue); Transport Temperature: Centrifuge, aliquot and freeze.
<b>Specimen Collection and Handling</b>	Collection of blood by routine venipuncture in a 3.5ml light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5ml with an appropriate ratio of anticoagulant is acceptable. Specimens other than 3.2% trisodium citrate plasma and those that are improperly collected, stored, misidentified or of insufficient volume are unacceptable. Also refer to "Criteria for rejection and special handling of coagulation specimens."
<b>Test Ordering Information</b>	3.2% sodium citrate is the preferred anticoagulant recommended by CLIS. Order VWF panel for further evaluation and classification.
<b>Billing Code</b>	87899
<b>CPT Code</b>	83520

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