Von Willebrand Factor (VWF) Testing

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Topics for Discussion

- Introduction to VWF
- Screening Tests for VWD
  (FVIII Activity, VWF Antigen, VWF Function Using Ristocetin, Other VWF Functions, and VWF Structure)
- Confirmatory Tests for VWD
- Clinical Examples
Introduction to VWF
Von Willebrand Factor History

- **1926**: Described by Eric von Willebrand
- **1928-49**: Vessel wall & platelet defect
- **1952-53**: Plasma FVIII deficiency
- **1953-70**: Search for VWF
- **1970**: FVIII/VWF protein (Zimmerman)
- **1980**: Platelets in VWD
- **1985**: VWF gene cloned (Sadler)
- **1987**: First mutation described (Mannucci & Sadler)

Happy 80th Birthday!
VWF Gene / Protein Structure

Functional domains

- FC VIII: Heparin
- GPIb: Collagen VI Heparin
- Collagen Types I & III
- α_{IIb}β_3 (GPIIb/IIIa)

Multimerization

- Propolypepetide (741 aa)
- Mature VWF Monomer (2050 aa)

Dimerization

Signal (22 aa)

Signal

Localization of VWD

Type 2 mutations

- Type 2A (IIC)
- Type 2A (IIE)
- Types 2A, 2B
- Type 2N
- Type 2A (IIA)
- Type 2M
- Type 2A (IID)

Type 2 mutations

- VWF gene, located on chromosome 12, is 178 kB in length.
VWF Roles in Hemostasis

Primary Hemostasis

Secondary Hemostasis

Monroe DM, et al. ATVB 2006;26:41-8
Von Willebrand Disease (VWD)

- **Most common bleeding disorder in humans**
- **Autosomal inheritance**
- **~ 0.8 - 1.3% of population has a detectable, inherited defect in Von Willebrand Factor (VWF)**
  - Low VWF levels, bleeding, and family history (the “holy” three)
- **Types of bleeding**
  - **Mucocutaneous bleeding**
    - Epistaxis, menorrhagia, ecchymoses & hematomas, gingival and gastrointestinal bleeding
    - Results from defect in primary hemostasis
  - **Soft tissue bleeding (after trauma/injury)**
    - Dental extraction, wounds, post-operatively, post-partum
    - Results from defect in secondary hemostasis
      - VWF is carrier (protector) protein for FVIII
**VWD Categories**

- **Type 1: partial quantitative deficiency of VWF**
  - Of patients with VWD, 73% have Type 1

- **Type 2: qualitative deficiency of VWF (21% of VWD patients)**
  - Type 2 variants
    - VWD Type 2A (synthesis or stability defect)
      - Decreased platelet dependent function due to loss of large, functional polymeric forms (high molecular weight multimers [HMWM])
    - VWD Type 2B (gain of function defect)
      - Increased affinity for platelet GPIb
    - VWD Type 2M (“multimer”)
      - Qualitative defects with decreased platelet dependent function not caused by loss of HMWM
    - VWD Type 2N (FVIII binding defect)
      - Decreased affinity for FVIII

- **Type 3: total deficiency of VWF (6% of VWD patients)**
Screening Tests for VWD
**Initial Tests**

- **Personal and family history is vital!**

- Activated partial thromboplastin time (APTT)
  - Degree of prolongation depends on FVIII levels (~ <40%)

- **Bleeding Time**
  - *in vivo* test – performed directly on patient
  - Fallen into disrepute and replaced by instruments that perform “in vitro” bleeding times

- **PFA closure time**

- **Platelet count**
  - Excludes disorders of primary hemostasis due to thrombocytopenia that is unrelated to VWD
VWF is an adhesive molecule that exerts its function in areas of the vasculature with high shear forces (stress & rate). Test systems (perfusion chambers) that subject denuded endothelium or collagen to high shear forces are only available in research laboratories. Two devices, available to clinical laboratories, are presented in the following slides.
"In vitro" Bleeding Time Device

- Dade Behring PFA-100® (Platelet Function Analyzer)
- High shear (rates = 5,000 - 6,000 s\(^{-1}\)) flow system
- Assess platelet-collagen and platelet-platelet interactions
- Platelets occlude an aperture within membranes coated with:
  - Collagen/epinephrine (used for primary screening)
  - Collagen/ADP (differentiates dysfunction due to aspirin)
Cone and Plate Device

- Citrated whole blood applied to polystyrene plates is exposed to arterial shear rates (1800 seconds^{-1}) for 2 minutes using a cone & plate device
  - Induces a laminar flow with uniform shear stress over the entire plate surface that is covered by the rotating cone
  - System is dependent on plasma VWF with its receptor (GPIb) and fibrinogen with its receptor (GPIIb/IIIa)
    - Assess platelet-platelet interactions
  - Adherent platelets are stained and percentage of surface covered is noted

Matis Medical Cone and Plate(let) Analyzer (DiaMed Impact)
Confirmatory Tests for VWD
Confirmatory Armamentarium

- Factor VIII procoagulant activity (FVIII:C)
  - APTT clot-based or chromogenic methods

- Von Willebrand Factor antigen (VWF:Ag)
  - ELISA or lateximmunoassay (LIA) methods

- Von Willebrand Factor (VWF) function
  - Ristocetin-dependent
    - VWF Ristocetin Cofactor activity (VWF:RCo)
      - Aggregometric, turbidimetric, and ELISA methods
    - Ristocetin induced platelet aggregation (RIPA)
      - Titratable aggregometric method (low shear environment)
  - Ristocetin-independent
    - Collagen binding (VWF:CB)
    - FVIII binding (VWF:FVIIIb)

- VWF structure (VWF multimer analysis)
Pre-Analytical Concerns

- Refrigerated (2–4°C) transport of citrate-anticoagulated whole blood or storage of specimens at this temperature prior to centrifugation & subsequent testing and/or freezing can adversely affect samples to be tested for VWF function (VWF ristocetin cofactor activity and collagen binding) and VWF structure (VWF multimer analysis)
  - Refrigerated temperatures cause *in vitro* progressive loss of high molecular weight multimers (most functional forms) within 3-6 hours
    - VWF test results mimic VWD Type 2
  - Phenomenon may be caused by cold-induced binding of VWF to platelets or susceptibility of VWF to certain proteases such as leukocyte elastases, plasmin, or calpain
  - Reversed by rewarming or retarded if collected in EDTA
Choice of reference plasma used in preparation of reference curves for FVIII, VWF:Ag, and VWF:RCo affects accuracy

- Improper assignment of values for reference plasma can result in gross over or under estimation of patient FVIII and VWF
- Reference material must be traceable to WHO (World Health Organization) international calibrator plasma

<table>
<thead>
<tr>
<th>WHO International Biological Reference Preparations</th>
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<tbody>
<tr>
<td><strong>PREPARATION</strong></td>
</tr>
<tr>
<td>Blood Coagulation factor VIII and von</td>
</tr>
<tr>
<td>Willebrand factor, plasma, human. Lyophilized.</td>
</tr>
<tr>
<td>0.68 IU/ampoule Factor VIII:C; 0.94 IU/ampoule</td>
</tr>
<tr>
<td>factor VIII. Antigen; 0.91 IU/ampoule VWF:antigen;</td>
</tr>
<tr>
<td>0.78 IU/ampoule VWF:ristocetin cofactor; 0.94</td>
</tr>
<tr>
<td>IU/ampoule VWF:collagen binding</td>
</tr>
<tr>
<td>Blood Coagulation factor VIII concentrate, human.</td>
</tr>
<tr>
<td>Lyophilized. 11.0 IU/ampoule</td>
</tr>
</tbody>
</table>

- Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis secondary FVIII plasma standard
  - Calibrated by multiple laboratories against WHO standard
Assessing FVIII Activity

Example

Patient value is determined by interpolating time, 50.1 seconds using a 1:10 dilution, from the reference curve to yield a FVIII activity of 38%. This indicates that the patient’s FVIII activity level is only 38% that of normal activity.

<table>
<thead>
<tr>
<th>Reference Dilution</th>
<th>% FVIII</th>
<th>Seconds</th>
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<tbody>
<tr>
<td>1:10</td>
<td>92</td>
<td>41.4</td>
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<tr>
<td>1:20</td>
<td>46</td>
<td>48.1</td>
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<tr>
<td>1:40</td>
<td>23</td>
<td>53.8</td>
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<tr>
<td>1:80</td>
<td>11.5</td>
<td>60.5</td>
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<tr>
<td>1:160</td>
<td>5.7</td>
<td>65.7</td>
</tr>
<tr>
<td>1:320</td>
<td>2.9</td>
<td>71.0</td>
</tr>
<tr>
<td>1:640</td>
<td>1.4</td>
<td>76.2</td>
</tr>
</tbody>
</table>
Factor VIII Procoagulant Activity

- Measured by clot-based or chromogenic methods
- Low plasma levels seen in VWD types 1, 2, and 3
  - Levels generally mirror those of VWF:Ag except in VWD Type 2N where FVIII levels are disproportionately lower
- Generally FVIII values are slightly higher than VWF:Ag values (saturation of VWF sites by normal amount of FVIII)
  - Patients with VWD Type 3 may show 3-5% FVIII activity though no VWF:Ag is present (represents free, unbound FVIII)
One-Stage Assay for FVIII

- Most widely used FVIII assay
  - First described by Langdell, Wagner, & Brinkhous in 1953

- Based on the APTT
  - Requires both contact activation (APTT reagent activator) and recalcification (Ca$^{+2}$ ions)
  - Design assumes that FVIII supplied by patient plasma is rate-limiting and that all other components (other clotting factors, phospholipid, and other cofactors) are present at saturating levels

- Assay is a “Mixing Study” that uses diluted patient plasma to “correct” the deficiency of FVIII (complete absence) in a FVIII deficient substrate plasma
One-Stage FVIII Assay

Prepare 1:10 dilution of reference plasma or patient plasma in buffer.

Prepare serial dilutions of plasma from 1:10 starting dilution.

Mix each serial dilution with factor deficient substrate plasma.

Perform APTT at 37° C.
Analytical Variables

- Diluent used for making dilutions
- Composition of FVIII deficient plasma
- Instrument clot detection method
- Reagent sensitivity to individual factors
- Nature of reference material used for preparing the reference (calibration) curve (see slide #17)
  - Impacts FVIII assays irrespective of method (clot-based or chromogenic)
- Criteria for acceptability of reference curve
Laurell electroimmunoassay was originally used to quantify VWF antigen. An agarose gel containing anti-VWF antibody is subjected to an electrical current and VWF antigen is precipitated (“rocket”). Since VWF is polymeric, this technique over-estimates protein levels in patients with higher concentrations of smaller multimers (VWD variants). Currently, VWF is quantified by ELISA (enzyme-linked immunosorbent assay) or by LIA (lateximmunoassay).
**VWF:Ag – ELISA Method**

- Microtiter well coated with rabbit anti-human VWF
- Patient VWF antigen attaches to capture antibody
- Immunoconjugated tagged antibody added to form a “sandwich”
- Bound enzyme is revealed by acting upon a substrate
  - Color which is released is directly proportional to antigen concentration
- Laboratory sensitivity is to less than 1% VWF:Ag
VWF:Ag – LI A Method

- Particles coated with polyclonal rabbit anti-human VWF antibodies
- VWF antigen is measured by increase in turbidity (increase in absorption or decrease in transmitted light) produced by agglutination of the latex particles
- Degree of agglutination is directly proportional to patient VWF antigen (the higher the OD, the greater the amount of antigen)
- Method limitations
  - Cloudiness of a plasma sample leads to under-estimation of VWF level
  - Presence of rheumatoid factor leads to over-estimation of VWF level
- Linearity
  - Generally, levels below 10% should be verified by ELISA
Regulation of VWF Blood Levels

- Levels influenced by
  - ABO type (affects clearance)
  - Age
  - Race

- Transient increases noted with beta-adrenergic stimuli
  - Exercise
  - Trauma or surgery

- Sustained elevation in chronic conditions
  - Inflammatory states, malignancy, renal failure, liver disease

<table>
<thead>
<tr>
<th>ABO Type</th>
<th>Mean VWF Antigen</th>
<th>-2SD</th>
</tr>
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<tbody>
<tr>
<td>O</td>
<td>74.8</td>
<td>35.6</td>
</tr>
<tr>
<td>A</td>
<td>105.9</td>
<td>48.0</td>
</tr>
<tr>
<td>B</td>
<td>116.9</td>
<td>56.8</td>
</tr>
<tr>
<td>AB</td>
<td>123.3</td>
<td>63.8</td>
</tr>
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</table>
Ristocetin (ristomycin) was used as an antibiotic in the late 1950s.

Introduced in 1971 as a diagnostic tool for VWD by Firkin & Howard.

In 1972 Weiss and colleagues outlined assay for measuring the deficiency of a plasma factor necessary for normal platelet function.

Procedure enhanced by Macfarlane in 1975 by stabilizing/fixing the washed platelets with formalin.

Ristocetin as a Testing Agent

Ristocetin Induced Platelet Aggregation (RIPA)

Measures ability of patient VWF and patient platelet GpIb to aggregate in the presence of high (1.5 mg/ml) and low concentrations (0.5 mg/ml) of ristocetin.

Ristocetin Cofactor (VWF:RCo)

Quantitative assay that determines VWF function by measuring, in patient platelet poor plasma, ability of patient VWF to agglutinate formalin-fixed platelets in the presence of ristocetin.

Assay methods
- Macroscopic slide technique
- Aggregometry
- Turbidity
- ELISA

Tracing at right is normal. In VWD Type 2B aggregation would be observed for low dose ristocetin.

Please see lecture entitled Von Willebrand Factor (VWF) Functional Assays for further information.
The VWF / GPIb interaction can also be assessed in the absence of ristocetin by either using monoclonal antibodies directed to the glycoprotein Ib (GPIb) binding site on VWF or using other modulators such as botrocetin, a snake venom protein that alters VWF conformation and increases its binding affinity for GPIb.
Monoclonal-Based ELISA

Assay utilizes monoclonal antibody (MAb RFF-VIII:R/1)
- MAb inhibits ristocetin-induced platelet aggregation and reportedly recognizes a conformationally specific epitope on VWF (molecule is in “active” state) where VWF binds to GPIb
- Subsequent studies have shown that the MAb does not, per se, assess a functional property of VWF (binding to collagen, FVIII, etc) but rather that it is an inhibitor of VWF function
- Appears that MAb, which serves to capture plasma VWF onto microtiter plate, preferentially captures high molecular weight multimers because these forms provide a greater number of antibody binding sites
  • Results more closely parallel those of VWF:Ag ELISA than those obtained from aggregometric (functional) method

When initially released in early 1990s, it was thought that the assay could potentially replace aggregometric method
- New version of assay was released in 2000 in which the detection system was modified (HRP-conjugated to monoclonal anti-VWF versus conjugated to polyclonal anti-VWF) to improve sensitivity
Monoclonal-Based LIA

- Functional immunoassays can be adapted to analyzers by using latex particles in place of microtiter plate wells.
- Particles are coated with purified anti-VWF mouse monoclonal antibody directed against the GPIb binding site on VWF.
- VWF activity is measured by increase in turbidity (decrease of transmitted light) produced by agglutination of the latex particles.
  - Degree of agglutination is directly proportional to patient VWF activity (the higher the OD, the greater the activity).
- No ristocetin is used in test system.
VWF interacts with numerous other proteins. An important localized function, *in vivo*, is the interaction between VWF released from platelets & endothelial cells and the platelet GPIIb/IIIa receptor, a critical event related to platelet aggregation. Additionally, VWF interacts with collagen, FVIII, and heparin.
VWF binds via two domains to collagen
- Binding by the VWF A1 domain to collagen type VI exposed by perturbed subendothelium leads to a conformational change in VWF that permits it to bind to GPIb
- Subendothelial collagens type I & III bind to VWF A3 domain

ELISA assays for collagen binding had initially been considered as replacements for the ristocetin cofactor assay (both tests are sensitive to a reduction in high molecular weight multimers)
- Can not serve as substitutes since collagen binding assays do not reflect interaction between VWF and GPIb
- Performing both collagen binding and ristocetin cofactor assays increases ability to identify VWD Type 2 variants
  - Aids in the discrimination of VWD types 2A or 2B from Type 2M
Collagen Binding Assays-2

- Assays have reproducibility issues
  - Collagens are prone to forming fibrils or aggregates at neutral pH
  - Variability in types of collagen and concentrations used for coating microtiter plates
    - Equine tendon [type I, type III, or type I/III mixture], bovine tendon or skin, human placenta [type III, type VI]

- Most recent information obtained from the VWF Scientific and Standardization Committee of the ISTH indicates that a collagen mixture of types I/III from equine tendon is the most sensitive to loss of the highest molecular weight multimers
**FVIII Binding Assay**

- Used for identifying patients with VWD Type 2N
- Assay design
  - Plasma at various dilutions is added to microtiter wells coated with polyclonal anti-VWF antibodies
  - Endogenous FVIII from captured plasma VWF is removed by incubating with CaCl₂
  - Recombinant FVIII added and amount of bound FVIII quantified by a chromogenic assay for FVIII
  - Amounts of immobilized VWF in each well are measured by ELISA
  - FVIII binding to VWF is plotted against concentration of VWF bound to microtiter plate well

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time (minutes)</td>
<td>9</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>FVIII (U/ml)</td>
<td>0.12</td>
<td>0.50-1.50</td>
</tr>
<tr>
<td>VWF:Ag (U/ml)</td>
<td>0.49</td>
<td>0.50-1.50</td>
</tr>
<tr>
<td>RIPA (1.2 mg/ml)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>


Normal binding by VWF for FVIII

Patient
Qualitative abnormalities of the VWF molecule were initially determined by crossed-immunoelectrophoresis (protein is first separated by molecular charge and then identified immunologically).

In 1980 Ruggeri & Zimmerman and Hoyer & Shainoff published papers describing the use of agarose gels and an ionic detergent to separate the protein according to size and to do so without any purification steps.
Study VWF internal structure and distribution of polymeric forms using SDS agarose gel electrophoresis and visualized by autoradiography.

Separate protein into high, intermediate, and small molecular weight multimers (MWM).

- Loss of high and/or intermediate molecular weight forms leads to a loss in function (for example, decrease in VWF ristocetin cofactor activity).

Gel System: 1.2% Low Gelling Temperature (LGT) Agarose
NPP = Normal Pooled Plasma; IIC = IIC Miami variant
Autoradiogram – 1.0% Gel

- Low resolution gel (1.0% LGT agarose)
- Gel of high porosity which allows all multimers to enter running gel
  - Internal triplet structure not discernable
- Used to study platelet VWF, post-DDAVP plasma samples or VWF in TTP/HUS
- Autoradiogram shows a full complement of VWF multimers from normal pooled plasma (NPP) and presence of unusually large molecular weight VWF multimers (UL MWM) in plasma from a patient with TTP

NPP = Normal Pooled Plasma; TTP = Thrombotic Thrombocytopenic Purpura
Intermediate resolution gel (1.2% LGT agarose)

- Gel of intermediate porosity allowing for visualization of internal triplet structure for each multimer
- Used for screening since all multimers, except UL MWM, are seen

Discerns normal from pathologic patterns

- Normal patterns seen in normalcy and VWD types 1, 2M, 2N
- Abnormal patterns seen in VWD types 2A & 2B, acquired VWD, Pseudo VWD, and improper sample handling

PLT  NPP  1  2A  IIC  2B  3

NPP = Normal Pooled Plasma; PLT = Platelet (VWF)
Autoradiogram – 2.0% Gel

- High resolution gel (2.0% LGT agarose)
- Gel of low porosity
  - Six to eight of fastest moving, lowest molecular weight multimers enter running gel
- Used to study internal multimer structure
  - Triplet pattern now seen as a quintuplet (one central with 2 satellite bands on either side)
- Internal multimer patterns differ in various subtypes of VWD such as in Type 2A and Type 2B

NPP = Normal Pooled Plasma
## Physiologic Variables Affecting VWF Multimer Analysis

**Pathogenetic Mechanism**

<table>
<thead>
<tr>
<th>Disorder</th>
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<tbody>
<tr>
<td>Specific or non-specific autoantibodies forming immune complexes that enhance VWF clearance</td>
</tr>
<tr>
<td>Adsorption of VWF onto malignant cell clones or other cellular surfaces</td>
</tr>
<tr>
<td>Increased proteolytic degradation of VWF</td>
</tr>
<tr>
<td>Enhanced shear stress</td>
</tr>
<tr>
<td>Decreased synthesis</td>
</tr>
</tbody>
</table>

**Disorder**

- Lymphoproliferative Disorders, Neoplastic Diseases, Immunologic Disorders
- Lymphoproliferative Disorders, Neoplastic Diseases, Myeloproliferative Disorders
- Myeloproliferative Disorders, Enhanced Shear Stress, Uremia, Ciprofloxacin, Primary & Secondary Hyperfibrinolysis, Fibrinolytic Therapy
- Congenital Cardiac Defects, Aortic Stenosis, Endocarditis, Malformation of Vessels, Severe Atherosclerosis, Sickle Cell Disease
- Hypothyroidism

Clinical Examples
Diagnostic Application

- Ratios of VWF function to antigen
  - VWF:RCo to VWF:Ag
    • Ratio ~1.0 indicates normalcy or VWD Type 1
    • Ratio < 0.7 indicative of VWD types 2A, 2B, or 2M
      - Disparity exists between amount of protein and its functionality
      - Overestimation of VWF activity will falsely increase ratio
  - VWF:CB to VWF:Ag
    • Ratio < 0.7 in VWD types 2A & 2B
    • Ratio > 0.7 in VWD Type 2M

- Ratios of FVIII activity to VWF antigen
  - Very low ratios are seen in VWD Type 2N
  - Ratio ~1.0
    • Normalcy
    • VWD Type 1 due to increased clearance of FVIII/VWF complex
  - Ratio >1.0
    • Decreased synthesis of VWF (VWD Type 1 due to a VWF null allele)

### VWD Type 2A

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Interval</th>
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<tbody>
<tr>
<td>APTT (sec)</td>
<td>35.2</td>
<td>27.7-37.2</td>
</tr>
<tr>
<td>Bleeding Time (min)</td>
<td>&gt; 30</td>
<td>3-8</td>
</tr>
<tr>
<td>FVIII:C (U/ml)</td>
<td>0.34</td>
<td>0.70-1.70</td>
</tr>
<tr>
<td>VWF:Ag (U/ml)</td>
<td>0.46</td>
<td>0.49-1.69</td>
</tr>
<tr>
<td>VWF:RCo (U/ml)</td>
<td>&lt;0.20</td>
<td>0.45-1.49</td>
</tr>
<tr>
<td>VWF:RCo / VWF:Ag</td>
<td>0.43</td>
<td>&gt; 0.70</td>
</tr>
<tr>
<td>FVIII:C / VWF:Ag</td>
<td>0.74</td>
<td>1.0</td>
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# VWD 2A (IIC Miami)

<table>
<thead>
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<tbody>
<tr>
<td>APTT</td>
<td>32.5</td>
<td>27.7 - 37.2 seconds</td>
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<tr>
<td>Bleeding Time</td>
<td>14.5</td>
<td>3 – 8 minutes</td>
</tr>
<tr>
<td>FVIII</td>
<td>1.29</td>
<td>0.70 - 1.70 U/ml</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>2.97</td>
<td>0.49 - 1.69 U/ml</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>0.59</td>
<td>0.45 - 1.49 U/ml</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>276,000</td>
<td>150,000 – 400,000</td>
</tr>
<tr>
<td>Blood Group</td>
<td>O</td>
<td>------</td>
</tr>
</tbody>
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**Luminography – 1.2% LGT Gel**

Red = Normal Plasma
Black = IIC Miami Plasma
Diagnostic testing for VWD should answer the following questions:

- How much protein is present?
  - Quantify VWF antigen

- How well does the protein function?
  - Quantify VWF function in order to assess the physiologic interaction between VWF & GPIb or VWF & collagen

- What is the structural appearance of the protein?
  - Qualitative (can be semi-quantitative) assessment of the protein’s structure and distribution of its polymeric forms

A composite “picture” that includes answers to these questions is required to make a diagnosis of VWD

- Consideration of multiple assays and their relationships to each other
  - Ratios comparing VWF antigen to a particular function or various functions to each other
VWF test results and diagnosis of VWD are significantly impacted by:

- Patient biological variables
- Pre-analytical testing issues
- Analytical variables
  - Calibrators used for establishing reference curves
  - Laboratory sensitivities of assays (lower limits of detection)
- Post-analytical concerns
  - Lower limits of reference intervals used to discriminate normal from abnormal values
  - Cut-off limits for various ratios

<table>
<thead>
<tr>
<th>Clinical Sensitivity of VWD Assays</th>
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<tbody>
<tr>
<td>High</td>
</tr>
<tr>
<td>PFA CT</td>
</tr>
<tr>
<td>VWF:RCo</td>
</tr>
<tr>
<td>Multimer Analysis</td>
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</tbody>
</table>


ISTH SSC Database for VWF at http://www.sheffield.ac.uk/vwf/index.html


