Laboratory testing for von Willebrand disease (VWD) - Assessment of von Willebrand factor (VWF) level and activity

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The Basics - von Willebrand factor (VWF)

- Large and complex molecule
- Plasma VWF – multimers of core protein (monomer of 2050 AA) ranging in size from 250kDa to >20000kDa
- Has binding sites for many ligands and these describe various functions of VWF
- The larger the size, the more ‘overall functionality’ or adhesiveness.
  - ‘High molecular weight’ (HMW) multimers more adhesive than intermediate or low molecular weight multimers
- Absent, deficient, or defective VWF = von Willebrand ‘disease’ (or ‘disorder’ [VWD]; congenital) or acquired von Willebrand ‘syndrome’ (aVWS)
The Basics - von Willebrand factor (VWF)
The Basics - von Willebrand factor (VWF)
The Basics - von Willebrand disorders

- VWD separated into six types: 1, 2A, 2B, 2N, 2M, 3
- Quantitative deficiencies (type 1 = partial; type 3 = total)
- Qualitative defects (+/- quantitative deficiency)
  - type 2A = loss of HMW VWF
  - type 2B = hyper-adhesive VWF (loss of HMW VWF & mild thrombocytopenia)
  - type 2N = loss of FVIII binding (loss of FVIII stability and loss of FVIII activity from plasma)
  - type 2M = ‘the rest’ (dysfunctional VWF not associated with loss of HMW VWF)
The Basics – Clinical diagnosis of VWD

- **Patient**
  - **Physical Examination**
    - **History**
      - **Personal**
      - **Family**
        - **Blood tests**
          - DDAVP trial
            - FBC/CBC ([plt], Hct, Hb)
            - Routine coags (PT, APTT, Fib)
            - FVIII:C
            - VWF:Ag
            - VWF:RCO
            - VWF:CB
              - (VWF multimers)
              - (RIPA)
              - (VWF:FVIIIIB)
              - (VWF:pp)
              - (PFA-100, PFS)
            - Repeat for confirmation
  - **Bruising - extent, range, size**
  - **Muscle hematomas, joints involvement?**
    - VWD - 2N or 3? Hemophilia?
  - **Bleeding history (epistaxis, menorrhagia, surgery, gums, etc)**

- Diagnosis and management plan
PFA-100/200

• Flow based assay & very sensitive to presence or absence of functional plasma VWF
• Therefore, sensitive to VWD
• 100% sensitive to Types 2A, 2M, 2B, and 3 VWD.
• ~80% sensitive to Type 1 VWD, with increasing sensitivity according to reduction in plasma VWF
PFA-100/200

• Reasonable negative predictor
  – Normal PFA = VWD very unlikely
  – Normal PFA = VWD types 3, 2A, 2M, 2B can probably be excluded (>99% ‘certain’)

• Prolonged PFA-100 CT ‘less’ informative
  – May be VWD
  – May be platelet dysfunction,
  – May be low platelet count, medication (eg aspirin, NSAIDs) or low haematocrit

• Either case, further testing required, because
  – PFA is not a diagnostic test
  – Normal PFA can still occur with clinical bleeding, so need specific testing
  – Abnormal PFA does not define any specific disorder, so need specific testing
PFA-200*

* Twice as good as the PFA-100?
Ligands and assay principles used for functional assays of VWF

- **VWF**
  - D
  - D3
  - A1 (GPIb\(\alpha\) (Mab))
  - A2
  - A3 (Collagen)
  - D4
  - B1-3
  - C1
  - C2

- **Ligand**
  - FVIII
  - GPIb\(\alpha\)
  - Collagen
  - GPIIbIIIa

- **Laboratory Assay**
  - VWF:FVIIIIB
  - VWF:RCo
  - GPIb binding assays (VWF ‘Activity’ or VWF:Act)
  - VWF:CB
  - (Flow or platelet function assays)

Measurement of FVIII level

- Can measure level of factor VIII (FVIII) protein by immune-related assay (= FVIII ‘antigen’ = FVIII:Ag), but now rarely performed
- More typical to measure activity by:
  - One stage assay of ‘coagulant’ activity (FVIII:C) – simple and most labs assess FVIII with this method using an automated analyser
  - Two stage assay of ‘coagulant’ activity (FVIII:C) – more complex and rarely performed
  - Chromogenic assay – less commonly performed, and generally only available in haemophilia centers.
**Measurement of FVIII level**

- **Advantages/disadvantages of different procedures:**
  - **One stage** –
    - **Advantages:** common; reagents widely available; easy to automate; well suited to STAT testing.
    - **Disadvantages:** may underestimate severity of haemophilia in some patients (genetic mutation related); may underestimate level of FVIII activity in some FVIII concentrates.
  - **Two stage** –
    - **Advantage:** Better marker of severity of haemophilia in some patients (genetic mutation related).
    - **Disadvantage:** more complex; reagents not widely available; less easy to automate; less well suited to STAT testing.
  - **Chromogenic** –
    - **Advantage:** Better marker of severity of haemophilia in some patients (genetic mutation related) (similar to two stage assay).
    - **Disadvantages:** more complex; reagents less widely available; less easy to automate; not commonly performed; less suited to STAT testing; may over-estimate level of FVIII activity in some concentrates.
Measurement of VWF level

- Measure level of VWF protein by immune-related assay (= VWF ‘antigen’ = VWF:Ag):
  - typically measured by ELISA (typically polyclonal antibodies used) – either automated or manual, or
  - by LIA (turbimetric; typically [multiple] monoclonal antibodies used) – automated (most modern haemostasis analysers), or
  - by other techniques
    - gel based systems in the past
    - fluorescence based procedures (VIDAS; Acustar)
    - flow cytometry described
Measurement of VWF level (VWF:Ag) - ELISA

- Extent of color reaction proportional to level of VWF

Color reaction

Substrate

HRP

Labeled (Rabbit) α-VWF

VWF

(Rabbit) α-VWF
Measurement of VWF level (VWF:Ag) - LIA

Latex aggregation proportional to level of VWF

Latex particle $\alpha$-VWF VWF

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Measurement of VWF ‘activity’ – VWF:RCo (1)

- Measure level of VWF ‘activity’ by ristocetin induced platelet agglutination:
  - ristocetin binds to VWF and causes conformational change (‘unfolding’) which leads to exposure of VWF epitopes that then subsequent bind to platelet via platelet GPIb
  - in vivo – unfolding of VWF occurs by blood flow shear stress
  - VWF:RCo assay designed to be sensitive to VWF defects (VWF conformational changes; e.g. 2A, 2B, 2M) and HMW VWF
  - agglutination measured ‘visually’ (semi-quantitative) or with an aggregometer (semi-automated) or on an automated analyser (Stago, Siemens, IL-Werfen)
Measurement of VWF activity (VWF:RCo) (2)

Platelet

GpIb

ristocetin

‘unfolded’ VWF

Latex aggregation proportional to level of ‘active’ and HMW VWF
Measurement of VWF activity (VWF:RCo) (3)

- Still employs ristocetin
- Latex particles replace platelets (theoretically more stable & reproducible assay)
- Recombinant GPIb bound to latex replaces native GPIb bound to platelet.

Latex aggregation proportional to level of ‘active’ and HMW VWF

Latex

ristocetin

rGPIb

‘unfolded’ VWF
Measurement of VWF ‘activity’ – VWF:CB

- Measure level of VWF ‘activity’ by collagen binding:
  - Collagen binds to VWF (natural adhesive activity in vivo)
  - ELISA assay can be designed to be sensitive to specific VWF defects (VWF conformational changes; e.g., 2M) and HMW VWF
  - Requires assay optimisation
    - Type III collagen binds VWF too avidly; poor discrimination of HMW VWF if used at [high] to coat wells
    - Type I collagen binds VWF too poorly; poor anchoring of VWF
    - Type III collagen used at [lower] or mixture of Type I/III collagen (~95%/5%) seems to provide optimal discrimination of HMW VWF.

Measurement of VWF activity (VWF:CB) - ELISA

Extent of color reaction proportional to level of adhesive (HMW) VWF

Substrate

Color reaction

Labeled (Rabbit) α-VWF

VWF

Collagen
Measurement of VWF ‘activity’ (VWF:Act) – LIA (IL-Werfen)

- Measure level of VWF ‘activity’ using monoclonal antibody directed against functional site on VWF that otherwise binds to platelet GPIb:
  - Originally developed (non-Werfen) as an ELISA assay
  - Werfen developed into a LIA assay
  - Assay can be designed to be sensitive to specific VWF defects (VWF conformational changes that affect GPIb binding site on VWF; e.g., 2M) and HMW VWF.
Measurement of VWF ‘activity’ (VWF:Act) - ELISA

Extent of color reaction proportional to level of HMW VWF and also sensitive to VWF structural changes (e.g., 2A, 2B, 2M)

Color reaction

Substrate

Labeled (Rabbit) \(\alpha\)-VWF

VWF

Monoclonal \(\alpha\)-VWF

Favaloro EJ, et al. Thrombosis & Haemostasis, 2000; 84: 541-7
Measurement of VWF ‘activity’ (VWF:Act) – LIA (IL-Werfen)

Latex aggregation proportional to level of HMW VWF and also sensitive to VWF structural changes (e.g., 2A, 2B, 2M)
Assay inter-relationships:

- High Molecular Weight VWF
- Intermediate Molecular Weight VWF
- Low Molecular Weight VWF

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<td>Type 2A</td>
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Favaloro EJ, Sem Thromb Hemost, 2007; 33: 727-44
Measurement of VWF ‘activity’ – VWF:FVIIIb

- Measure level of VWF – FVIII binding ‘activity’:
  - Typically ELISA assay – used to discriminate 2N VWD and haemophilia A
  - VWF anchored to ELISA wells by (polyclonal) antibody
  - any bound FVIII removed by high [CaCl]
  - addition of standard amount of recombinant or purified FVIII, which then binds VWF if functional
  - FVIII bound then detected by either labeled antibody vs FVIII or by FVIII chromogenic assay.
Measurement of VWF FVIII binding activity (VWF:FVIIIIB) - ELISA

Extent of color reaction proportional to level of FVIII bound to VWF

Color reaction

Substrate

Labeled α-FVIII

FVIII

VWF

(Rabbit) α-VWF

HRP
Measurement of VWF – assay limitations

- VWF:Ag protein level – no measure of functionality
- VWF:RCo – just one measure of activity; poor precision (high variability); poor sensitivity to low levels of VWF.
- VWF:CB - just one measure of activity; better precision (lower variability) & better sensitivity to low levels of VWF
- VWF:Ag & both activity assays (VWF:RCo + VWF:CB) needed to identify all known forms of VWD.
- In general, VWF:Ag + VWF:CB yields better utility than VWF:Ag + VWF:RCo, but will miss some forms of VWD with either panel
Assessment of VWD – assay limitations

Laboratory errors

2B VWD misidentified as normal or type 1 VWD


RCPA QAP data 2006

VWF functional assays compared:
Type 2B VWD (FVIII:C plus…)

Error rate (%)

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Assessment of VWD – assay limitations

Laboratory errors vs. RCPA QAP data 1998-2005
VWF functional assays compared:
(VWF:Ag plus FVIII:C plus…)

Error rates & cause (RCPAQAP)

RCPA QAP (2007 - 2013 inclusive)

1 mild/mod = Mild/moderate quantitative deficiencies (‘type 1 VWD’)

1 severe = Severe quantitative deficiencies (‘type 1 VWD’)

2 = Qualitative defect (loss of HMW VWF; ‘type 2 VWD’)

Error rates & cause (RCPAQAP)

RCPAQAP (2007 - 2013 inclusive)

- False activity/Ag concordance
- False activity/Ag discordance
- Insufficient test panel
- Lab misinterpretation

Types of defect/deficiency:
- 1 mild/mod = Mild/moderate quantitative deficiencies (‘type 1 VWD’)
- 1 severe = Severe quantitative deficiencies (‘type 1 VWD’)
- 2 = Qualitative defect (loss of HMW VWF; ‘type 2 VWD’)

Error rates & cause (RCPAQAP)

RCPA QAP (2007 - 2013 inclusive)

Adjusted error rate (%)

- VWF activity/Ag false concordance/discordance error rates

1 mild/mod = Mild/moderate quantitative deficiencies ('type 1 VWD')
1 severe = Severe quantitative deficiencies ('type 1 VWD')
2 = Qualitative defect (loss of HMW VWF; 'type 2 VWD')

Type of defect/deficiency

- VWF:RCo
- VWF:CB
- Other VWF:Act

Measurement of VWF – assay limitations

Assay variability

Measurement of VWF – assay limitations

Assay variability

VW08-02a & VW10-03a = normal plasma

Measurement of VWF – assay limitations

Lower limit assay sensitivity

VW9-03b = VWF def plasma

VW10-08b = VWF def plasma

Discrimination of type 1 vs 2 VWD (RCPAQAP)

Normal samples

VWF deficient samples (‘type 1 VWD’)

HMW VWF deficient samples (‘type 2A VWD’)  

Measurement of VWF – assay limitations

Assay sensitivity to HMW VWF

Assay sensitivity to HMW VWF

Assay sensitivity to HMW VWF

The Basics – Laboratory ‘diagnosis’ of VWD

- Basic VWD assay panel
  - VWF:Ag
  - VWF:CB
  - VWF:RCo
  - FVIII:C

- Additional screening assays:
  - PT
  - APTT
  - FBC/CBC
  - PFA-100

- Assess abnormal results / consider alternate diagnoses:
  - Factor deficiencies
  - Thrombocytopenia
  - Platelet function defect

1. VWF:Ag low, but RCo/Ag & CB/Ag normal:
   - Quantitative deficiency or Type 1 VWD
   - Repeat for confirmation / assess severity:
     - Ag < 15U/dL = severe type 1 VWD
     - Ag 16-35U/dL = moderate/mild type 1 VWD
     - Ag >35U/dL = ‘low VWF’

2. RCo/Ag & CB/Ag both low:
   - Type 2A or 2B or PT VWD
   - Repeat for confirmation / perform RIPA:
     - Response to low dose RIPA = 2B or PT VWD
     - Response only to ‘high’ dose RIPA = 2A VWD

3. RCo/Ag low but CB/Ag normal:
   - Type 2M VWD
   - Repeat for confirmation / perform RIPA:
     - Response only to high dose RIPA

4. VWF:Ag <2 U/dL:
   - Type 3 VWD
   - Repeat for confirmation
   - VWF:CB & VWF:RCo should also be <2 U/dl

5. FVIII:C/Ag low:
   - Type 2N VWD or haemophilia A/carryer
   - Repeat for confirmation / perform VWF:FVIIIIB:
     - Low FVIIIIB/Ag = 2N VWD
     - Otherwise haemophilia A /carrier (assess FVIII:C level)

6. None of the above / all normal:
   - Repeat for confirmation
   - Not VWD?
   - Perform platelet function studies
Assay changes over the years

- **VWF:Ag**
  - Laurell gel then EIA then ELISA then LIA
  - Flow cytometry described – uptake in the future?
  - Fluorescence procedures available

- **VWF:RCo**
  - Slide to aggregometer/chart to aggregometer/computer to automated
  - Flow cytometry described – uptake in the future?
  - Platelets can be replaced by latex (theoretically better more standardized and reproducible assay)
  - Fluorescence procedures available
Assay changes over the years

- VWF:CB
- ELISA
- Flow cytometry described – uptake in the future?
- Fluorescence procedure ‘in development’
- LIA procedure?
- VWF – GPIb binding assays
  - New Siemens Innovance VWF activity assay
## Automation

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Techniques</th>
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<tbody>
<tr>
<td>Mid-late 1970s</td>
<td>Laurell gels (VWF:Ag); Aggregometry (VWF:RCo)</td>
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<tr>
<td>Late 1980s</td>
<td>ELISA (VWF:Ag)</td>
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<td>1990s</td>
<td>ELISA (VWF:Ag &amp; VWF:CB); Aggregometry/computers (VWF:RCo)</td>
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<td>2000s</td>
<td>LIA (VWF:Ag); automated agglutination (VWF:RCo); PFA-100 (flow system)</td>
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<tr>
<td>2010s</td>
<td>automated ELISA workstations (VWF:Ag &amp; VWF:CB); Acustar; Siemens GPIb VWF binding; PFA-200; Flow cytometry (VWF:Ag, VWF:CB &amp; VWF:RCo).</td>
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</table>
New Siemens VWF ‘activity’ assay

- No ristocetin
- Not a VWF:RCo assay
New Siemens VWF ‘activity’ assay

Excellent correlation to VWF RCo activity

High agreement for VWF ratio activity/antigen
New Siemens VWF ‘activity’ assay


50 healthy normal subjects, 80 patients with VWD (12 VWD type 1, 60 VWD type 2, 6 VWD type 3, 1 unclassified and 1 acquired von Willebrand Syndrome)
New Siemens VWF ‘activity’ assay

600 samples, comprising nearly 100 VWD, nearly 60 individual normal samples, post DDAVP & Biostate, with the rest mostly non-VWD but for VWF/VWD workups.

Favaloro EJ, Mohammed S. Thromb Res 2014 in press
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DDAVP studies

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DDAVP studies

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DDAVP studies

Favaloro EJ, Mohammed S. Thromb Res 2014 in press
VWF:Ac – Summary 1

- Very similar test patterns to VWF:RCo
- Like VWF:RCo = GPIb binding assay
- Unlike VWF:RCo does NOT use ristocetin, so NOT a VWF:RCo assay
- Nevertheless, seems ‘interchangable’ with VWF:RCo with respect to VWD diagnostics, at least from our experience & rest of current literature.
- Not a replacement for VWF:CB, which is not a GPIb binding assay
VWF:Ac – Summary 2

- VWF:Ac & VWF:CB, or VWF:RCo and VWF:CB, with VWF:Ag plus FVIII:C, would permit detection of 1, 2A, 2M, 2B, 3 VWD.
- Add VWF:FVIIIB for 2N VWD
- Add repeat testing for additional confirmation/diagnostic clarity
- Add DDAVP trial data for additional confirmation/diagnostic clarity
- Add genetic analysis sparingly for less clear cases, selective type 2 VWD & type 3 VWD.
Werfen IL - ACL AcuStar

- Chemiluminescent technology
- “Superior range and sensitivity compared to ELISA or immunoturbidimetric assays”
- Fully automated
- Self-contained, ready-to-use reagent cartridges, stable up to twelve weeks onboard
- Assays onboard and available 24 hours/day, 7 days/week
- Assay calibration on lot change only
- Random access: no batching required

* Not currently 510(k) cleared.
** In development. Not currently saleable.
New AcuSTAR VWF assays

Figure 1
AcuStar VWF:Ag and VWF:RCo Assay Description

RCPA QAP data – trends in VWF testing
Pre-analytical issues – plasma vs filtered plasma

Pre-analytical issues – plasma vs serum

Analytical issues – method issues

Favaloro EJ, Mohammed S. Thromb Res 2014 in press
The Basics – Laboratory ‘Diagnosis’ of VWD

- Multiple assays required to properly diagnose VWD and assign VWD type (implications for therapy)
- Must perform FVIII:C plus VWF:Ag plus more than one functional VWF assay, otherwise VWD (or type) will be misdiagnosed
- New assays promise easier test performance, reduced variability, better low VWF level sensitivity and similar HMW VWF sensitivity to VWF:RCo, so potential future replacements to reduce diagnostic errors?
Case study 1

46 year-old female; ? VWD results are as follows:

- VWF:Ag = 38 U/dL (NRR = 45-200)
- VWF:RCo = 4 U/dL (NRR = 35-350)
- VWF:CB = 31 U/dL (NRR = 50-250)
- FVIII:C = 35 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.82 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.11 (NRR = 0.7 – 2.0)

* NRR= normal reference range

Question: What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 1

46 year-old female; repeat and extended results:

- VWF:Ag = 22 U/dL (NRR* = 45-200)
- VWF:RCo = 7 U/dL (NRR = 35-350)
- VWF:CB = 20 U/dL (NRR = 50-250)
- FVIII:C = 76 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.91 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.32 (NRR = 0.7 – 2.0)

*NRR= normal reference range

Full blood count normal, including platelet count (191; reference interval 150 – 400 x 10⁹).

Platelet aggregation results were normal except for an abnormal response to ristocetin (threshold response was 2.0 mg/mL; normal threshold response is between 1.0 – 1.5 mg/mL).

**Question:** Conclusion/ further testing?
Case study 2

Sample for confirmatory VWF testing for ?VWD on 4 year-old female who requires a tonsillectomy; results from another pathology provider have suggested possible type 2A VWD. No personal history of bleeding, but patient’s maternal grandmother has previously been diagnosed with VWD (subtype not clear). Your lab’s results:

- VWF:Ag = 86 U/dL (NRR = 45-200)
- VWF:RCo = 23 U/dL (NRR = 35-350)
- VWF:CB = 37 U/dL (NRR = 50-250)
- FVIII:C = 48 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.43 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.27 (NRR = 0.7 – 2.0)

* NRR = normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 2

4 year-old female; repeat and extended results:

- VWF:Ag = 81 U/dL (NRR* = 45-200)
- VWF:RCo = 32 U/dL (NRR = 35-350)
- VWF:CB = 27 U/dL (NRR = 50-250)
- FVIII:C = 52 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.91 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.32 (NRR = 0.7 – 2.0)

Full blood count normal, including platelet count (184; reference interval 150 – 400 x 10⁹).

Platelet aggregation results were normal except for an abnormal response to ristocetin (threshold response was 0.3 mg/mL; normal threshold response is between 1.0 – 1.5 mg/mL).

**Question:** Conclusion/ further testing?

*NRR= normal reference range*
Case study 2

A. Control PRP RIPA

B. Patient PRP RIPA

C. Patient platelets/control plasma

D. Control platelets/patient plasma
Case study 3

Pregnant female with personal & family history of bleeding investigated for von Willebrand disease gives the following test results:

- VWF:Ag = 81 U/dL (NRR* = 45-200)
- VWF:RCo = 10 U/dL (NRR = 40-200)
- VWF:CB = 10 U/dL (NRR = 50-250)
- FVIII:C = 60 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.12 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.12 (NRR = 0.7 – 2.0)

*NRR= normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 4

Pregnant female with personal & family history of bleeding investigated for von Willebrand disease gives the following test results:

- **VWF:Ag = 71 U/dL (NRR = 45-200)**
- **VWF:RCo = 60 U/dL (NRR = 35-350)**
- **VWF:CB = 65 U/dL (NRR = 50-250)**
- **FVIII:C = 85 U/dL (NRR = 45-180)**
- **CB/Ag ratio = 0.92 (NRR = 0.7 – 2.0)**
- **RCo/Ag ratio = 0.85 (NRR = 0.7 – 2.0)**

*NRR= normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 5

Female with a personal and family history of bleeding investigated for von Willebrand disease gives the following test results:

- VWF:Ag = 42 U/dL (NRR = 45-200)
- VWF:RCo = 42 U/dL (NRR = 35-350)
- VWF:CB = 41 U/dL (NRR = 50-250)
- FVIII:C = 7 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.98 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 1.00 (NRR = 0.7 – 2.0)

*NRR= normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 6

Male with CLL on dialysis without any personal or family history of bleeding develops mucosal bleeding. Previous APTT was normal, but is now prolonged. Mixing studies show correction. Additional testing yields the following results:

FVIII:C = 25 U/dL (NRR = 45-180)
FIX = 95 U/dL (NRR = 60-150)
FXI = 110 U/dL (NRR = 50-140)
FXII = 122 U/dL (NRR = 40-190)

*NRR= normal reference range

Question: What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 6

Additional testing:

VWF:Ag = 23 U/dL (NRR* = 45-200)
VWF:RCo = 11 U/dL (NRR = 35-350)
VWF:CB = 3 U/dL (NRR = 50-250)
CB/Ag ratio = 0.13 (NRR = 0.7 – 2.0)
RCo/Ag ratio = 0.48 (NRR = 0.7 – 2.0)

* NRR: normal reference range

Question: What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 7

Young male patient with a vague family history of bleeding – father and paternal grandfather deceased. Previously investigated externally, identified to have a factor VIII level of 4 U/dL (NRR = 45-180) and diagnosed with moderate haemophilia A. Patient requires surgery.

**Question:** What therapeutic support might be considered appropriate for this patient, how would you monitor this therapy and would you recommend any additional testing prior to surgery.
Case study 7

Additional testing:

- VWF:Ag = 1 U/dL (NRR* = 45-200)
- VWF:RCo = 2 U/dL (NRR = 35-350)
- VWF:CB = 1 U/dL (NRR = 50-250)
- FVIII:C = 5 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.5 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 2.5 (NRR = 0.7 – 2.0)

*NRR= normal reference range

Question: What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 8

Male with personal history of bleeding, but no family, investigated for von Willebrand disease gives the following test results:

- VWF:Ag = 4 U/dL (NRR* = 45-200)
- VWF:RCo = 10 U/dL (NRR = 35-350)
- VWF:CB = 2 U/dL (NRR = 50-250)
- FVIII:C = 5 U/dL (NRR = 45-180)
- CB/Ag ratio = 0.5 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 2.5 (NRR = 0.7 – 2.0)

*NRR= normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 9

Male patient with personal & family history of bleeding, investigated for von Willebrand disease gives the following test results:

- VWF:Ag = 69 U/dL (NRR = 45-200)
- VWF:RCo = 20 U/dL (NRR = 35-350)
- VWF:CB = 70 U/dL (NRR = 50-250)
- FVIII:C = 44 U/dL (NRR = 45-180)
- CB/Ag ratio = 1.01 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = 0.29 (NRR = 0.7 – 2.0)

Question: What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 10

55y male patient with personal & family history of bleeding, investigated for von Willebrand disease gives the following test results:

- VWF:Ag = 191 U/dL (NRR = 45-200)
- VWF:CB = 201 U/dL (NRR = 50-250)
- FVIII:C = 180 U/dL (NRR = 45-180)
- CB/Ag ratio = 1.05 (NRR = 0.7 – 2.0)

*NRR= normal reference range

**Question:** What could you conclude from these results, and what further testing/investigation would you recommend?
Case study 10

55 year-old male; repeat and extended results:

- VWF:Ag = >200 U/dL (NRR* = 45-200)
- VWF:RCo = 1 U/dL (NRR = 35-350)
- VWF:CB = >200 U/dL (NRR = 50-250)
- FVIII:C = >200 U/dL (NRR = 45-180)
- CB/Ag ratio = ~1.0 (NRR = 0.7 – 2.0)
- RCo/Ag ratio = <0.1 (NRR = 0.7 – 2.0)

*NRR= normal reference range

Full blood count normal, including platelet count.

PFA CT >300sec both C/ADP & C/Epi.

Platelet aggregation results were normal except for an abnormal response to ristocetin (no response to 1.5 mg/mL).

**Question:** Conclusion/ further testing?
Haemostasis = Love

Everybody talks about it, nobody understands it.
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