

VWF & Propeptide Assay

INTENDED USE

The VWF & Propeptide Assay is a quantitative solid phase enzyme linked immunosorbent assay (ELISA) for the measurement of von Willebrand Factor (VWF) and VWF propeptide in plasma and a calculation of a propeptide: VWF ratio. An elevated Propeptide:VWF ratio in Type 1 VWD patients is indicative of a reduced VWF half-life in circulation.

For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

Von Willebrand Factor (VWF) is a multimeric, high molecular weight protein present in plasma that plays a pivotal role in successful recruitment and activation of platelets at the site of vascular injury. In addition, VWF serves as a carrier protein for the clotting factor, Factor VIII, protecting the Factor VIII from degradation.^{1,2}

Von Willebrand Disease (VWD) is caused by a deficiency in VWF. Type 1 VWD is a quantitative deficiency where reduced levels of normally functioning VWF are detected in plasma. Type 2 VWD is a qualitative deficiency where normal or reduced levels of functionally deficient VWF are detected in plasma. Type 3 VWD is a complete quantitative deficiency in VWF with plasma levels of < 1 IU/dL. Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder associated with lympho and myeloproliferative disorders, solid tumors, immunologic and cardiovascular disorders.³ AVWS is similar to VWD in regards to laboratory findings and clinical manifestations. The diagnosis and subtyping of VWD and AVWS are based on multiple test results such as Factor VIII activity, VWF antigen levels, VWF activity, multimeric analysis of VWF, as well as the patient's bleeding history.^{3,4}

The VWF propeptide is synthesized as part of a pro-VWF protein and is subsequently cleaved, stored and secreted in an equi-molar ratio with mature VWF.^{5,6} The level of VWF propeptide in the circulation can be used as a marker of VWF synthesis.⁷ In individuals with low VWF synthesis, the propeptide level is similarly decreased yielding a propeptide: VWF ratio near 1.0. In individuals with normal levels of VWF synthesis and decreased survival of VWF in circulation, an increased Propeptide:VWF ratio is observed.⁷ The quantitative deficiency observed in Type 1 VWD can be caused by ineffective synthesis and storage or by a decrease in the half-life of the VWF in the circulation. To date a number of different point mutations in VWF have been shown to cause a reduced VWF half-life.^{7,8} Clinically it is important to recognize this enhanced clearance phenotype because the increased clearance of VWF can reduce the efficacy of desmopressin treatment in these patients.^{7,9,10} The Propeptide:VWF ratio has also been used in the diagnosis of AVWS as the propeptide level is usually normal or increased and the VWF level is reduced causing an increased Propeptide:VWF ratio.^{11,12} Additionally, the propeptide levels and the Propeptide:VWF ratio in plasma can be used to assess the extent of endothelial cell activation.¹³

PRINCIPLES OF THE PROCEDURE

Diluted calibrators, controls, and sample plasma are added to microwells to which monoclonal antibodies specific for VWF or propeptide have been immobilized. Incubation of the calibrators and samples in the microwells allows for binding of the VWF or propeptide to the immobilized antibodies. A wash step removes any unbound plasma proteins from the microwells. The microwells are then incubated with a biotinylated monoclonal antibody specific for the VWF or propeptide that has been captured in the wells. A wash step then removes any unbound antibodies. Subsequently the microwells are incubated with Streptavidin-labeled Horseradish Peroxidase. After a wash step which removes any unbound Streptavidin-labeled Horseradish Peroxidase from the microwell, a fluorescent substrate is added and incubated. Following this incubation, the reaction is stopped by addition of stopping solution. The fluorescence is measured in a fluorescent plate reader with excitation wavelengths between 315 – 340 nm and emission wavelengths between 370 – 470 nm. Average values obtained from the calibrators are graphed to make a standard curve. Average values for the positive controls and the patient samples are determined from this standard curve. Reportable results are given in IU/dL for VWF and U/dL for propeptide.

This kit contains two positive control samples. The positive controls are used to confirm that the calibrator dilutions were properly made and that samples with antigen levels from different regions of the standard curve are showing proper recovery. Expected values for both of the controls are lot-specific and can be found in the included Analysis Software. Control values outside the acceptable ranges indicate an invalid assay.

REAGENTS

Maximum number of tests per kit: 41 in duplicate
All reagents should be stored as directed by the label.

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| MSVW | 1. Microwells: Black, flat-bottomed microwell strips coated with antibodies specific for VWF (color-coded yellow) or propeptide (color-coded green). Contains murine and bovine source material. Ready for use. The microwell strips are enclosed in resealable foil pouches. Store at 2-8°C. |
| MSPRP | |
| 5XCW | 2. Concentrated Wash (5x): Phosphate buffered saline solution containing Tween 20 and 0.05% ProClin 300. Dilute with deionized or distilled water before use. Store at 2-8°C. |

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| VPC | 3. Streptavidin-HRP (200x): Streptavidin labeled Horseradish Peroxidase in an HRP stabilizer. Must be diluted in Diluent before use. Store at 2-8°C. |
| VPD | 4. Diluent: Phosphate buffered saline solution containing bovine serum albumin and 0.05% ProClin 300. Ready for use. Store at 2-8°C. |
| VCAL | 5. Calibrator: Pooled Normal Human Plasma. For single use only. Assigned values can be found in the included Analysis Software. Dilute in Diluent before use. Store at ≤ -15°C. |
| VDA | 6. VWF Detection Antibody (200x): Biotinylated murine monoclonal antibody with 0.05% ProClin 300. Dilute in Diluent before use. Store at 2-8°C. |
| PDA | 7. Propeptide Detection Antibody (200x): Biotinylated murine monoclonal antibody with 0.05% ProClin 300. Dilute in Diluent before use. Store at 2-8°C. |
| QA | 8. Substrate A: Must be diluted 9 parts Substrate A to 1 part Substrate B before use. Store at 2-8°C. |
| QB | 9. Substrate B: Must be diluted 9 parts Substrate A to 1 part Substrate B before use. Store at 2-8°C. |
| QSS | 10. Stopping Solution: Ready for use. Store at 2-8°C. |
| PS | 11. Plate Sealers. |
| AS | 12. VWF & Propeptide Analysis Software. Analysis Software on CD. |

PRECAUTIONS

- Do not use reagents that are turbid or contaminated.
- Do not use reagents beyond their expiration date.
- Microwells and reagents contained in the kit are not to be used in conjunction with any other test system.
- Substitution of components other than those provided in this kit may lead to inconsistent or erroneous results.
- Discard any thawed Calibrator and used microwell strips after each run.
- Discard any unused, diluted Calibrator, diluted Detection Antibodies, diluted Strep-HRP, and diluted Substrate Solution.
- When making dilutions, follow pipette manufacturer's instructions for appropriate dispensing and rinsing techniques.
- When making dilutions, do not pipette less than 5 µL of a reagent or sample. Using volumes less than 5 µL when diluting will increase dilutional imprecision.
- Accurately calibrated pipets should be used for reagent additions.
- The enzyme substrate reaction is temperature sensitive and should be performed in a controlled area at 22 to 25°C.
- Reagents may contain more volume than stated on the container.

CAUTIONS

- All human plasma used in the Calibrator for this product have been tested and found negative for antibody to HIV, HCV and HBsAg by FDA approved methods. No test method, however, can offer complete assurance that HIV, Hepatitis C virus, Hepatitis B virus or other infectious agents are absent. Therefore, these materials should be handled as potentially infectious.
- Discard all components when completed according to local regulations.

SPECIMEN COLLECTION

Sample Collection and Preparation

NOTE: Only platelet poor plasma collected in sodium citrate may be used for this assay. See Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assay; Approved Guideline CLSI H21-A5, Volume 28, Number 5, December 2008 for details.

Platelet poor plasma collection should be performed as follows:

1. Collect blood in buffered sodium citrate (3.8% or 3.2%) tubes.

NOTE: Partial draw tubes should NOT be processed. Since the tubes are pre-calibrated to draw the specified amount of blood, the resulting sample, will not have the proper 9:1 ratio of blood to anticoagulant if a full sample is not collected.

2. After collection, store tube upright at room temperature until centrifugation.

NOTE: Blood samples should be centrifuged between fifteen minutes and two hours after blood collection for best results.

3. Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.
4. Centrifuge blood sample at room temperature in a horizontal rotor (swing-out rotor) for 15-20 minutes at 1500 to 1800 RCF (Relative Centrifugal Force).

WARNING: Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.

5. Following centrifugation, collect the top 2/3 of the plasma layer by pipetting the plasma into a clean polypropylene tube.
6. Re-centrifuge the collected plasma at 1500 to 1800 RCF for 15-20 minutes to remove any remaining red cells or platelets.
7. Transfer the top 2/3 of the plasma layer into a clean polypropylene tube, taking care not to disturb any cells at the bottom of the tube.

Sample Storage

1. Plasma should be stored at room temperature and assayed within 4 hours OR aliquoted and frozen at -15°C or colder until use.
2. Frozen plasma or Calibrator should be thawed at 37°C and then incubated at 37°C for an additional 10 minutes prior to use in order to properly solubilize the antigens. Either a 37°C waterbath or a 37°C dry incubator can be used. Thawed plasma should be stored at room temperature and assayed within 4 hours.

PROCEDURE

Materials Provided:

Vials may contain more reagent than described on the labels. Be sure to measure the reagent with an appropriate device when making dilutions.

Box A:

1. 6 x 0.5 mL vials Calibrator

Box B:

1. 12 – 1 x 8 Black Microwell Strips for VWF detection (color coded-yellow) with holder
2. 12 – 1 x 8 Black Microwell Strips for propeptide detection (color coded-green) with holder
3. 3 x 50 mL Diluent
4. 1 x 125 mL Concentrated Wash
5. 1 x 40 µL VWF Detection Antibody
6. 1 x 40 µL Propeptide Detection Antibody
7. 1 x 75 µL Streptavidin-HRP
8. 1 x 14 mL Substrate A
9. 1 x 2 mL Substrate B
10. 1 x 14 mL Stopping Solution
11. 12 Plate Sealers
12. Analysis Software on CD

Additional Materials Required:

1. Test tubes or microfuge tubes for patient sample and control dilutions and for reagent dilutions
2. Transfer pipets
3. Adjustable micropipets to deliver 1 – 10 µL, 10 – 100 µL and 100 - 1,000 µL and disposable tips
4. Timer
5. Fluorescent plate reader capable of measuring fluorescence at Excitation between 315 – 340 and Emission between 370–470 nm
6. Deionized or distilled water
7. Absorbent paper towels
8. Microplate washer or device
9. Centrifuge
10. 37°C waterbath or 37°C dry incubator
11. Computer with Microsoft®Excel® version 2003 or 2007

Test Procedure

1. Bring all assay reagents from Box B to room temperature (22 to 25°C).

NOTE: Once all reagents from Box B are at room temperature (22 to 25°C), all reagents can be diluted and stored at room temperature for the duration of the assay. No reagents are light sensitive.

- Determine the number of patient samples to be tested. Using the Recording Sheet, assign each sample to a location consisting of two (duplicate) wells. Record the identity of each sample on the Recording Sheet.
- Make Working Wash solution by diluting Concentrated Wash (5XCW). Add 1 volume of Concentrated Wash to 4 volumes of deionized or distilled water. Mix well. Working Wash solution can be stored at 2-8°C for 2 weeks.

Strips:	4 – 1x8	12 – 1 x8	24 – 1x8
Concentrated Wash	20 mL	60 mL	120 mL
DI Water	80 mL	240 mL	480 mL

- Remove the appropriate number of VWF and propeptide strips as identified on the Recording Sheet. Promptly reseal unneeded strips in the protective pouch.

NOTE: Only two frames are provided in the kit. Do not discard until all strips have been used.

- Thaw an aliquot of Calibrator stock from Box A as well as any frozen test plasma samples at 37°C.
- Once thawed the Calibrator stock and samples should be incubated at 37°C for an additional 10 minutes to allow for proper VWF solubilization prior to dilution.

NOTE: The Calibrator stock is intended to be thawed and used one time per vial. Do not re-freeze this material.

- Use the following table to make Calibrators A-E by adding the specified plasma volume to the appropriate volume of Diluent (VPD). This dilution provides enough material to run the calibrators and controls in duplicate on both VWF and Propeptide plates.

Calibrator Level	Plasma Volume	Diluent Volume
Calibrator A	20 µL calibrator stock	1,080 µL VPD
Calibrator B	200 µL Calibrator A	80 µL VPD
Calibrator C	200 µL Calibrator A	260 µL VPD
Calibrator D	200 µL Calibrator A	1,000 µL VPD
Calibrator E	----	300 µL VPD

- Use the following table to make the positive controls by adding the specified plasma volume to the appropriate volume of Diluent (VPD). This dilution provides enough material to test the sample in duplicate on both the VWF and propeptide strips.

Control Level	Plasma Volume	Diluent Volume
Positive Control High	5 µL calibrator stock	495 µL VPD
Positive Control Low	5 µL calibrator stock	995 µL VPD

- For each patient sample to be tested, make a dilution in Diluent according to the following table. A 1:200 dilution is sufficient for most samples and will allow a lower level of detection of 6 IU/dL or 6 U/dL. To confirm a low or deficient sample a dilution of 1:20 is recommended. Use of a 1:20 dilution will allow a lower level of detection of <1 IU/dL or <1 U/dL. Dilutions between 1:300 and 1:20 are appropriate for use in this assay.

Sample Dilution	Patient Plasma Volume	Diluent Volume
1:200	5 µL	995 µL VPD
1:20	13 µL	247 µL VPD

- Add 50 µL of each Calibrator (A-E) (in duplicate) to the assigned microwells of the VWF (yellow) and the propeptide (green) strips.
- Add 50 µL of the Positive Control High (in duplicate) to the assigned microwells of the VWF and the propeptide strips.
- Add 50 µL of the Positive Control Low (in duplicate) to the assigned microwells of the VWF and the propeptide strips.
- Add 50 µL of the diluted sample plasma solution prepared in step 9 (in duplicate) to the assigned microwells on the VWF and the propeptide strips.

NOTE: A set of calibrators and controls is required on each type of microwell strip used each time an assay is run.

NOTE: Label each strip to avoid errors.

14. Seal the microwells with a plate sealer and incubate for 15 minutes in a 37°C waterbath or incubator.

NOTE: Since the incubation time is only 15 minutes, it is important to add all samples to the strips as quickly as possible starting with the same well each time. All sample additions should be completed within 5 minutes.

15. Dilute the VWF detection antibody (VDA) in Diluent (VPD) as described in the following table. The VDA will be added to all of the VWF microwells.

Strips:	2 - 1x8	4 - 1x8	12 - 1x8
VDA	6 µL	10 µL	30 µL
VPD	1.2 mL	2 mL	6 mL

16. Dilute the propeptide detection antibody (PDA) in Diluent (VPD) as described in the following table. The PDA will be added to all of the propeptide microwells.

Strips:	2 - 1x8	4 - 1x8	12 - 1x8
PDA	6 µL	10 µL	30 µL
VPD	1.2 mL	2 mL	6 mL

17. WASH STEP:

- Aspirate or decant contents of each well and blot on absorbent toweling.
- Add 300 µL Working Wash solution.
- Aspirate or decant.
- Repeat steps b + c for a total of 3 washes.
- Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

NOTE: It is important to completely remove all wash solution after the final wash.

18. To each well on the VWF plate (color coded yellow) add 50 µL of diluted VWF Detection Antibody (VDA).

19. To each well on the propeptide plate (color coded green) add 50 µL diluted Propeptide Detection Antibody (PDA).

20. Seal the microwells with a plate sealer and incubate for 15 minutes in a 37°C waterbath or incubator.

21. Dilute the Streptavidin-HRP (VPC) in Diluent (VPD) as described in the following table. Use a polypropylene container. The diluted Streptavidin-HRP will be added to all the used wells on both types of microwell strips.

Strips:	4 - 1x8	8 - 1x8	24 - 1x8
VPC	10 µL	20 µL	60 µL
VPD	2 mL	4 mL	12 mL

22. WASH STEP:

- Aspirate or decant contents of each well and blot on absorbent toweling.
- Add 300 µL Working Wash solution.
- Aspirate or decant.
- Repeat steps b + c for a total of 3 washes.
- Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

23. Add 50 µL of diluted Streptavidin-HRP to all VWF and propeptide microwells.

24. Seal the microwells with a plate sealer and incubate for 15 minutes in a 37°C waterbath or incubator.

25. Prepare Substrate Solution adding 9 parts Substrate A (QA) to 1 part Substrate B (QB). Use a polypropylene container. Mix well. This material is not light sensitive. This material will be added to all used wells on both types of microwell strips.

Strips:	4 - 1x8	8 - 1x8	24 - 1x8
Substrate A Solution (QA)	1.8 mL	3.6 mL	10.8 mL
Substrate B Solution (QB)	200 µL	400 µL	1.2 mL

26. WASH STEP:

- a) Aspirate or decant contents of each well and blot on absorbent toweling.
- b) Add 300 μ L Working Wash solution.
- c) Aspirate or decant.
- d) Repeat steps b + c for a total of 3 washes.
- e) Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

NOTE: It is important to completely remove all wash solution after the final wash.

27. Add 50 μ L of the mixed Substrate Solution from step 25 to each used well on both types of microwell strips.
28. Seal the microwells with a plate sealer and incubate for 15 minutes at ROOM TEMPERATURE (22 to 25°C). It is not necessary to protect the reaction from light.
29. Stop the reaction by adding 50 μ L of Stopping Solution (QSS) to each well in the same sequence as the addition of substrate. The plate should be read in a fluorescent plate reader within 60 minutes from the addition of Stopping Solution.
30. Read the plate in a fluorescent plate reader with Excitation wavelengths between 315 – 340 nm and Emission wavelengths between 370 – 470 nm at room temperature (22 to 25°C).

DETAILS OF CALIBRATION

The Calibrator stock provided in the VWF & Propeptide Assay is frozen, pooled, normal, human plasma. The pool is made from 35 different healthy, non-medicated, non-smoking, male and female donors between the ages of 18 – 56. The Calibrator stock is assigned a VWF and propeptide value by assay relative to the SSC/ISTH Secondary Coagulation Standard Lot #3. The SSC/ISTH Secondary Coagulation Standard Lot #3 was assigned a VWF antigen level of 106 IU/dL. The SSC/ISTH Secondary Coagulation Standard Lot #3 was not assigned a propeptide level. Since there is no internationally recognized standard for propeptide measurement and since it has been shown that the average Propeptide:VWF ratio in normal donors is close to 1.0,⁷ GTI Diagnostics internally assigned the SSC/ISTH Secondary Coagulation Standard Lot #3 a propeptide level of 106 U/dL, equal to its VWF level. The VWF and propeptide values assigned to the Calibrator stock are lot-specific and are provided on the included Analysis Software.

QUALITY CONTROL

All calibrator levels, controls, and samples are tested in duplicate. The relative fluorescent units (RFU) from replicate wells should be similar. The % difference between replicate wells for Calibrators A-D, Controls, and samples should be $\leq 20\%$.

The VWF & Propeptide Assay utilizes two positive controls (Positive Control High and Positive Control Low). The Positive Control High is diluted in such a way so that the relative fluorescent values obtained are within the middle to high region of the standard curve. The Positive Control Low is diluted in such a way so that the relative fluorescent units obtained are within the middle to low region of the standard curve. The Analysis Software calculates the final reportable results for the controls by multiplying the antigen levels obtained off of the standard curve by the sample dilution used. Since both controls are dilutions of the same material, the final reportable results obtained should be similar and the values for both controls should fall within the acceptable control ranges stated in the included Analysis Software. If the control values do not fall within the acceptable ranges the assay should be repeated. If the control values do not fall within the acceptable range for one antigen (ex. VWF), but are acceptable for the second antigen (ex. Propeptide), only the antigen determination with the failed control needs to be repeated.

INTERPRETATION OF TEST RESULTS

Construct a calibration curve by plotting the average fluorescence (n=2) value for each calibration standard versus its assigned value of VWF or propeptide using the Analysis Software provided on the enclosed CD. The lot-specific assigned values for VWF and propeptide can be found in the included Analysis Software.

A calibration curve for each type of plate should be generated each time the assay is performed. Detailed instructions on the use of the Analysis Software are located on the first page of the Analysis Software. The Analysis Software calculates the average relative fluorescent values from duplicate wells for all calibrator levels, controls and samples tested. Determine the concentration of VWF and propeptide in the Controls and the test plasma samples using the Analysis Software. The Analysis Software graphs the data and solves the best-fit equation to obtain IU/dL of VWF or U/dL of propeptide for the Controls and the test plasma samples. The Analysis Software also calculates a Propeptide:VWF ratio for each sample tested.

If plasma samples give relative fluorescent values higher than those obtained for the highest level calibrator, the reportable result will be “ORR” (over reportable range) and the sample must be repeated at a higher dilution to obtain a result that is within the calibration range. If a sample is tested at a 1:200 dilution, the lower limit of the assay range is 6 IU/dL or 6 U/dL. If the result for a plasma sample is less than 6 IU/dL of VWF or 6 U/dL of propeptide, the sample should be tested at a 1:20 dilution. Using a 1:20 dilution the lower limit of the assay range is reported as <1 IU/dL or <1 U/dL. Sample dilutions between 1:300 and 1:20 are appropriate for this assay.

LIMITATIONS

Since the VWF & Propeptide Assay uses mouse monoclonal antibodies for both antigen capture and detection, the presence of human anti-mouse antibodies in a sample could result in incorrect results. Similarly, the presence of rheumatoid factor in a sample could adversely affect the results of the assay.

The results of this assay should not be used as the sole basis for a clinical decision.

EXPECTED VALUES

The normal range for VWF antigen varies according to blood type, with plasma from blood type O individuals having reduced levels when compared to the other blood types. The published normal range for VWF antigen is 50 to 150 U/dL in blood type O individuals and 90 to 200 U/dL in non-O blood types.⁴ Each laboratory should determine its own blood-type specific normal ranges for VWF antigen.

The normal range for propeptide is not affected by a donor's blood type. The published normal range for propeptide is 55 to 219 U/dL for all blood groups.⁷ Each laboratory should determine its own normal ranges for propeptide.

CLINICAL CUTOFF

Specimens from the following populations were evaluated using the VWF & Propeptide Assay in two clinical studies: One hundred-fifteen Type 1 VWD patients diagnosed on the basis of VWF antigen level, ristocetin co-factor activity, and past bleeding history were tested and 23 Type 1 VWD patients with increased clearance of VWF (Type 1C) diagnosed on the basis of VWF antigen level, ristocetin co-factor activity, past bleeding history, and the presence of a point mutation previously shown to cause increased clearance of VWF.

Receiver operator characteristics (ROC) curve analysis was used to determine the diagnostic cutoff for increased clearance of VWF based on the values for the Propeptide:VWF ratio. From the ROC analysis, a cutoff of a Propeptide:VWF of > 3.0 was selected to provide optimal clinical sensitivity and specificity when distinguishing between Type 1 VWD and Type 1 VWD with increased VWF clearance (Type 1C). Using a ratio of > 3.0 yielded 100.0% sensitivity, characterizing all known Type 1C patients correctly and yielded 95.7% specificity, where 5 Type 1 patients were characterized as Type 1C. Three of the 5 mischaracterized patients had Type O blood, one patient had Type A blood, and the blood group of the fifth sample was unknown. Since it has been demonstrated that patients with a Type O blood generally have a lower VWF level and correspondingly a higher Propeptide:VWF ratio, we suggest the use of the following grey zone. Propeptide:VWF ratios of 3.0 – 4.1 may be due to increased VWF clearance or the result of a Type 1 VWD patient with Type O blood. Ratios of > 4.1 are indicative of Type 1C VWD regardless of blood group.

SPECIFIC PERFORMANCE CHARACTERISTICS

To ensure suitable reactivity and specificity, each lot of the VWF & Propeptide Assay is tested prior to release with samples containing normal and reduced levels of both VWF and propeptide.

Precision

The within run, between run and total imprecision of the VWF & Propeptide Assay was determined. Five samples with varying levels of both VWF and propeptide were tested in the VWF & Propeptide Assay in duplicate in 20 separate assays. To obtain the imprecision of the reportable results, the data were analyzed by ANOVA according to the CLSI Document EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guidelines. The results are shown in the table below. For the measurement of VWF antigen, the total % CV for all samples was < 11.5 %. A sample that was deficient for VWF was also tested and determined to have < 1 IU/dL of VWF in all 20 assay runs. For the measurement of propeptide, the total % CV for samples 1 – 3 was < 6.0%. Sample 4 had a total % CV of 25.0 %, however the values only ranged from 1 to 6 U/dL of propeptide over the 20 assay runs. A sample that was deficient for propeptide was also tested and determined to have < 1 U/dL of propeptide in all 20 assay runs.

Sample	Average IU/dL VWF	Within Run SD	Within Run % CV	Between Run SD	Between Run % CV	Total SD	Total % CV
Sample 1 Normal	93	3.4	3.7	5.5	5.9	6.1	6.6
Sample 2 Normal	114	3.0	2.6	12.8	11.2	13.0	11.4
Sample 3 Medium	53	2.0	3.8	4.1	7.7	4.4	8.3
Sample 4 Low	18	0.9	5.0	1.4	7.8	1.6	8.9

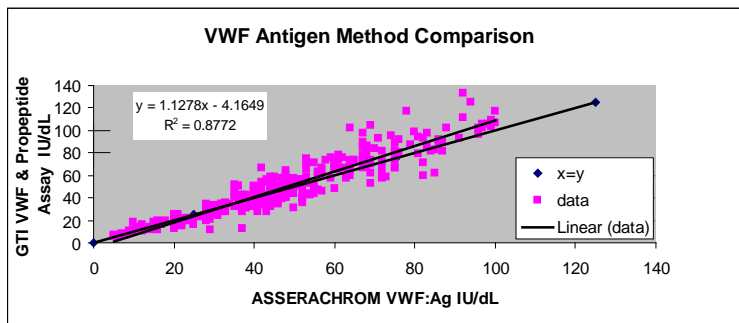
Sample	Average U/dL propeptide	Within Run SD	Within Run % CV	Between Run SD	Between Run % CV	Total SD	Total % CV
Sample 1 Normal	89	2.7	3.0	4.8	5.4	5.1	5.7
Sample 2 Normal	105	1.8	1.7	4.7	4.5	4.9	4.7
Sample 3 Medium	52	1.8	3.5	2.5	4.8	2.8	5.4
Sample 4 Low	4	0.4	10.0	1.0	25.0	1.0	25.0

Limit of Detection and Assay Range

The limit of detection is 0.021 IU/dL for VWF and 0.021 U/dL for propeptide and is confirmed on each kit lot. When a sample is tested using a 1:200 dilution the lower limit of the assay range is 4 IU/dL VWF or 4 U/dL propeptide. It is suggested that any sample with antigen values < 6 IU/dL VWF or < 6 U/dL propeptide be repeated using a sample dilution of 1:20. When a sample is tested at a 1:20 dilution, the lower limit of the assay range is 0.42 IU/dL VWF or 0.42 U/dL propeptide, however the Analysis Software will report these results as <1 IU/dL VWF or <1 U/dL propeptide. There is no known clinical significance to reporting antigen values of < 1 IU/dL VWF or <1 U/dL propeptide. The upper limit of the assay range is dependent on the specific lot of Calibrator stock used in the assay but is at least 273 IU/dL VWF or 273 U/dL propeptide.

Method Comparison: Comparison of GTI VWF & Propeptide Assay to Diagnostica Stago ASSERACHROM® VWF:Ag Assay

Three separate studies were conducted in which the VWF antigen values obtained using the GTI VWF & Propeptide Assay were compared to a previously FDA cleared device; ASSERACHROM® VWF:Ag Assay by Diagnostica Stago. Combining the results of the three studies, 298 plasma samples were tested. The following graph shows the results of the linear regression analysis for VWF where the slope of the line was 1.13 with a 95% confidence interval of 1.08 to 1.18 and the intercept was a -4.16 with a 95% confidence interval of -6.72 to -1.61.



INTERFERING SUBSTANCES

The following substances showed no interference in the VWF & Propeptide Assay at the concentrations indicated:

Hemoglobin	≤ 500 mg/dL
Bilirubin	≤ 20 mg/dL
Intralipid	≤ 500 mg/dL

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