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IMUBIND®
Von Willebrand Factor (vWF) Activity ELISA

REF 885

IVD

8°C
2°C

Σ 96

EC REP Obelis s.a
Bd. Général Wahis 53, 1030 Brussels, BELGIUM

INTENDED USE

The IMUBIND® vWF Activity ELISA is a quantitative direct enzyme-linked immunosorbent assay (ELISA) for the detection of von Willebrand Factor (vWF) activity in citrated human plasma. It is intended for the assessment of vWF activity in patients where this is deemed useful in the diagnostic process, particularly as an aid in the differential classification of von Willebrand's disease. vWF activity represents one parameter in a multicriterion diagnostic process.

INTRODUCTION

Von Willebrand Factor (vWF) is a complex multimeric adhesive glycoprotein synthesized by endothelial cells and megakaryocytes. Its principle, apparently unrelated, functions are as a carrier/stabilizer for the pro-coagulant protein FVIII:C, which circulates in serum as a non-covalently linked complex, and it appears to be an adhesive protein in haemostasis. vWF binds collagen and possibly other endothelial structures; it mediates the adhesion of platelets to the sub-endothelium (binding surface receptor glycoprotein Ib), it may also play a role in platelet-platelet interaction through binding glycoprotein IIb/IIIa.[1,2]

Von Willebrand first described this congenital bleeding disorder in 1926. In its mildest form, von Willebrand's disease is the most common bleeding disorder; the reported frequency is 1 in 100. It is a heterogeneous disorder, caused by vWF defects or reduced levels of vWF. Sub-classifications of the disease are based upon clinical observation, patient history and laboratory analysis such as bleeding times, vWF antigen and vWF activity. Measurement of both antigen and activity aids in the differential diagnosis of the two predominant classes of the disease, types 1 and 2. Classification of the disease is important as clinical management may vary with type[3].

Increased levels of vWF antigen/activity indicate endothelial damage in vascular disease[4]; preliminary studies suggest endothelial damage may be important in the vascular complications of hypertension[5]. The short-term vWF increases observed after exercise, adrenaline, DDAVP infusion (1-deamino (8-d-arginine)-vasopressin) and during pregnancy[6], however, may indicate activation/stimulation of endothelial cells[7] rather than epithelial damage. Decreased vWF levels are found in hypothyroidism and systemic lupus erythematosus. Acquired forms of von Willebrand's disease have been described in autoimmune disease, Waldenström's disease, benign monoclonal gammopathies, myeloproliferative adrenal carcinoma, rheumatic vasculitis and diabetes[8]. Due to the diversity of clinical conditions associated with vWF abnormalities, a more appropriate descriptor might be von Willebrand's Syndrome.

PRINCIPLE OF THE ASSAY

The wells of the microtiter strips are coated with a preparation of purified murine anti-vWF monoclonal IgG antibody, the characteristics of which have been documented[9,10], which recognizes a functional epitope of vWF. During the first incubation, specific antigen in diluted plasma binds to the antibody-coated surface. The wells are then washed to remove unbound components. In the second incubation, the Conjugate, a horseradish peroxidase-labelled murine monoclonal antibody to vWF, binds any surface-bound antigen. After further washing, specifically bound antibody is traced by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a colored end-product. The amount of Conjugate bound is measured in absorbance units. The von Willebrand Factor activity can be estimated by interpolation from a dose-response curve based on dilutions of the Calibrator. The Calibrator is referenced against the 5th International Standard for Factor VIII and von Willebrand Factor in Plasma, code 02/150, supplied by NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK.

KIT COMPONENTS

R1	Anti-Human vWF IgG Coated Microwells and Strip Holder	96 microwells (12 × 8 well strips)	Coated with murine anti-vWF monoclonal antibody, in a resealable foil pack with desiccant.
R2	vWF Calibrator*	2 × 0.5 mL	vWF antigen in human plasma, freeze-dried. Reconstitute and dilute before use*. An assigned activity in IU/mL and % is given on the label.
R3	vWF Control 1*	2 × 0.5 mL	vWF antigen in human plasma, freeze-dried. Reconstitute and dilute before use*. The acceptable mean and range in IU/mL and % is given on the label and represents a 'normal' activity level.
R4	vWF Control 2*	2 × 0.5 mL	vWF antigen in human plasma, freeze-dried. Reconstitute and dilute before use*. The acceptable mean and range in IU/mL and % is given on the label and represents an 'abnormal' activity level.
R5	vWF Diluent Concentrate (5X)	1 × 25 mL	Phosphate buffer, bovine albumin, 0.1% (w/v) Bronopol. **Dilute before use.**
R6	vWF Wash Buffer Concentrate (16X)	1 × 25 mL	Borate buffer, 0.32% (w/v) Bronopol. **Dilute before use.**
R7	vWF Conjugate	1 × 15 mL	Horseradish peroxidase-labelled murine monoclonal antibody to human vWF, stabilisation buffer. Ready-to-use.
R8	TMB Substrate	1 × 11 mL	3,3',5,5'-tetramethyl benzidine (TMB) Ready-to-use.
R9	Stop Solution	1 × 15 mL	2N H_2SO_4 Ready-to-use.
	Instruction booklet		

* The values printed on the labels of the Calibrator and Controls represent the activity after reconstitution in 0.5 mL distilled/deionized water and further dilution of 1 in 20 as described in Preparation for the Assay. Also note that 1 IU/mL = 100% activity.

STORAGE OF REAGENTS

Handling and Procedural Notes

1. Store kit components at 2°-8°C and use until the expiry date on the labels. Do not use expired reagents.
2. Do not mix different lot numbers.
3. Wash Buffer Concentrate, Diluent Concentrate, Calibrator and Controls 1 and 2 must be diluted before use. All other reagents are ready-to-use.
4. Diluted Wash Buffer and diluted Diluent are stable at 2°-8°C for up to 6 months if microbial contamination is avoided.
5. Reconstituted and diluted Calibrator and Controls are stable at 2°-8°C for up to 24 hours.
6. Replace surplus microtiter strips in the foil pack and store within the resealable polythene bag with the desiccant at 2°-8°C, until required.
7. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.
8. Do not modify the handling or storage conditions for kit reagents or plasma samples.

Indications of Deterioration

Blue coloring or precipitation indicates the TMB Substrate is contaminated and should be discarded.

Sample Collection and Storage

The assay is for citrated plasma samples. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition", CLSI Document H21-A5, Vol. 28, No.5, 2008. Collect nine volumes of blood in 0.109M (3.2%) trisodium citrate anticoagulant (one volume). Centrifuge the capped specimen tube at a speed and duration required to consistently produce platelet-poor plasma (platelet count <10 x 10^9/L).

Plasma may be stored at 2°-8°C and assayed within 4 hours or -20°C or lower for four weeks. Frozen plasmas must be thawed rapidly at 37°C before testing. Thoroughly mix thawed samples before assay and avoid repeated freeze/thawing. Samples at 1:20 dilution in diluted Diluent should be assayed within 24 hours of dilution.

WARNINGS and SAFETY PRECAUTIONS

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2. Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.
3. Do not pipette by mouth.

4. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
5. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
6. Safety data sheets for all components contained in this kit are available on request from BioMedica Diagnostics.

Wash Buffer 16X	Warning		H319, P264, P280, P305+P351+P338, P337+P313
vWF Conjugate	Warning		CONT | 2-methyl-4-isothiazol-3 one H317, P261, P272, P280, P302 + P352, P333 + P313
TMB	-	-	Observe good laboratory practices.
Stop Solution	Warning		CONT | Sulfuric acid H315, H319, P264, P280, P302 + P352, P305 + P351 + P338, P333 + P313, P337 + P313

Other kit components do not possess CLP-GHS-qualified hazards.

Hazard Statements:
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.

Precautionary Statements:
P261 Avoid breathing mist or vapor.
P264 Wash thoroughly after handling.
P272 Contaminated work clothing must not be allowed out of the workplace.
P280 Wear eye protection/face protection. Wear protective gloves.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313 If eye irritation persists: Get medical advice/attention.

PREPARATION

Materials/Equipment Required but not Provided

1. 96 well plate/strip reader with 450 nm filter.
2. Precision pipettes to dispense 50 µL, 100 µL, 500 µL, 1 mL. Automatic pipette to dispense 100 µL. Automatic pipette to dispense 200 µL for manual washing, automatic plate washer optional.
3. Glass/plastic measuring cylinders: 1×100 mL, 1×500 mL.
4. 1 mL containers for Calibrator/Control/sample dilutions.
5. Distilled/deionised water.
6. Paper towels.
7. Timer for 15 and 60 minute intervals.

Preparation for the Assay

Allow all kit components, including the microtiter strips, to warm up to 18°-25°C for a minimum of 30 minutes before use. Mix liquid reagents by gentle inversion.

Dilute vWF Wash Buffer Concentrate by adding the contents of one vial to 375 mL distilled/deionised water in a clean vessel and mixing thoroughly.

Dilute vWF Diluent Concentrate by adding the contents of one vial to 100 mL distilled/deionised water in a clean vessel and mixing thoroughly.

Reconstitute one vial of Calibrator and each Control by adding 0.5 mL distilled/deionised water and swirling gently until all particulate matter is in solution. DO NOT VORTEX. Allow to stand for 30 minutes at 18°-25°C with intermittent gentle swirling before dilution.

Dilute the reconstituted Calibrator 1 in 10 (one part Calibrator to nine parts diluted vWF Diluent). The 1 in 10 dilution activity is equivalent to twice the value given on the label; i.e. if the value given on the Calibrator label is 0.85 IU/mL (85%), then the value for the Calibrator at a 1 in 10 dilution is 1.7 IU/mL (170%). From the 1 in 10 stock, prepare a series of doubling dilutions using diluted vWF Diluent to give a decreasing range of either five or eight Calibrator values. Use diluted vWF Diluent as a Zero Calibrator.

Dilute the reconstituted Controls 1 in 20 by adding 50 µL of Control to 950 µL diluted vWF Diluent and mixing gently.

Dilute patient samples 1 in 20, and 1 in 100 if further dilutions are required. Add 50 µL sample to 950 µL diluted vWF Diluent (1:20) and mix gently; if required, add 200 µL of the 1 in 20 dilution to 800 µL diluted vWF Diluent (1:100) and mix gently.

Calculate the number of microtiter strips required for the current assay, and retain these in the microtiter strip holder. Return surplus strips to the foil pack with the desiccant, and store in the resealable plastic bag at 2°-8°C until required. Ensure that all strips are securely held within the microtiter strip holder, with the assay identification tab lying along the bottom edge below row H.

ASSAY PROTOCOL

1. Reference wells for identification.
2. Pipette 100 µL of each reconstituted Calibrator dilution in duplicate, pre-diluted reconstituted Controls 1 and 2, and pre-diluted patient samples (at 1 in 20) into appropriate wells. Addition should be in a continuous operation and should not exceed ten minutes for any one set of Calibrators. Should further dilutions of patient samples be required, a 1 in 100 dilution is recommended.
3. Incubate 60 ± 10 minutes at 18°-25°C.

4. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials, bearing in mind the potential infective hazard of the samples. Blot inverted strips well with paper towels.
5. Wash wells five times with 200 µL diluted vWF Wash Buffer. Decant and blot after each wash step.
6. Add 100 µL vWF Conjugate to each well.
7. Incubate 15 ± 2 minutes at 18°-25°C.
8. Repeat steps 4 and 5.
9. Add 100 µL of TMB Substrate to each well.
10. Incubate 15 ± 2 minutes at 18°-25°C. **Do not decant.**
11. Add 100 µL Stop Solution to each well, in the same order and rate as the Substrate. Tap wells gently to mix.
12. Read strips within 30 minutes at 450nm.

CALCULATION AND INTERPRETATION OF RESULTS

Consider each assay separately when calculating and interpreting results.

Calculate the mean absorbance value of each Calibrator dilution and linear plot against Calibrator activity or concentration on suitable graph paper. Alternatively, a suitable computer and curve-fitting program may be used. The following standard curves have been evaluated.

8-point curve: Seven doubling dilutions of the Calibrator from 1 in 10 to 1 in 640, plus a zero standard.

5-point curve: Four dilutions of the Calibrator of 1 in 10, 1 in 20, 1 in 40 and 1 in 160, plus a zero standard.

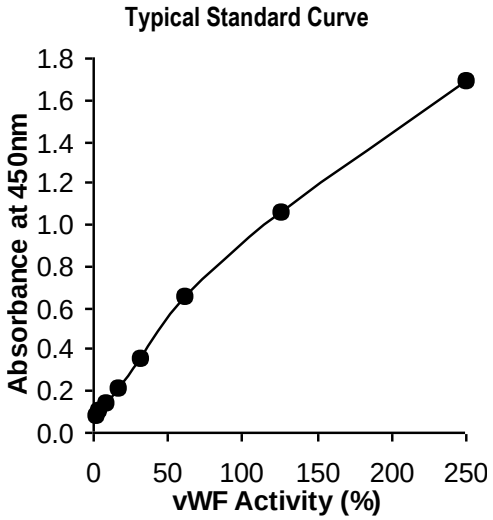
The vWF activity for each of the dilutions can be calculated from the vWF activity given on the Calibrator label using the following formula.

$$\text{Activity of diluted Calibrator} = \text{vWF activity given on label} \times \frac{20}{\text{Dilution Factor}}$$

The following curve-fitting programs have been found to be suitable:
Smoothed Spline; 4-parameter logistic; 5-parameter logistic; log/logit; lin/linit.

Control/sample activities can be read by interpolation from the Calibrator curve; a sample plot is shown below for reference purposes, it must not be used for interpreting results.

The activity for Controls/samples should be calculated from the 1 in 20 Control/sample dilution result. DO NOT CORRECT FOR THIS DILUTION FACTOR. If the activity for the samples at a 1 in 20 dilution is greater than the top Standard, results can be calculated using the 1 in 100 dilution if this has been included in the assay run. Results calculated using the 1 in 100 dilution must be multiplied by 5 to correct for this further sample dilution. N.B. 1 IU/mL = 100% activity.



QUALITY CONTROL

Ensure that adequate maintenance and calibration of the plate-reader is performed according to the manufacturer's instructions, and that the correct wavelength is employed.

Users should ensure they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section, and the Handling and Procedural Notes. Users should demonstrate that they can obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results. Two Controls representing normal (Control 1) and abnormal (Control 2) vWF activity are supplied for monitoring the quality of the test procedure. It is recommended by the manufacturer that the pre-diluted reconstituted Controls are run in duplicate in each sample test run. The acceptable mean and range in IU/mL and % of each control is given on the label (at 1 in 20 dilution when read from the calibrator curve).

Assuming the precision specifications described by the manufacturer are met, failure of either Control to meet the Control specifications given on the label renders the assay invalid and patient results should not be reported. The operator may repeat the assay, having reviewed their procedure, or contact the distributor/manufacturer. If repeating the assay, prepare a fresh dilution of each Control and sample. Reconstituted Calibrator and Controls are stable at 2°-8°C for up to 24 hours. Laboratories may wish to include in-house controls in each assay run. Store such control material at or below -20°C. Directly thaw at 37°C and avoid repeat freeze/thaw cycles. Do not freeze/thaw samples more than once.

Levels of analytes identified in particular diseases are those established by the manufacturer for specific populations, and may not necessarily mirror the literature. Incidence levels, their relationship to specific diseases, reference ranges, and appropriate cut-off points should all be calculated for the specific populations serviced by users.

EXPECTED VALUES

Asymptomatic Reference Range: 180 citrated plasma samples from asymptomatic apparently healthy Caucasian donors with an age range of 18-71 years were assayed with the IMUBIND vWF Activity ELISA. The distribution of results is given in the following table. 162/180 (90%) samples gave values greater than 50%, with no statistically significant differences observed with age or gender. See Limitations of Use number 2 regarding vWF level fluctuations in the normal population. It is recommended that users establish reference ranges for the populations served by their laboratories.

Control Group	n	% Activity Range									
		≤30		>30-50		>50-60		>60-180		>180	
Asymptomatic Healthy	180	2	1.1%	16	8.9%	17	9.4%	133	74.0%	12	6.6%

Clinical Samples: 72 samples from clinically typed 1, 2 and 3 vWF patients were assayed with the IMUBIND vWF Activity ELISA. Patients known to be on treatment were excluded from analysis. The distribution of results is given in the following table.

Clinical Samples	n	% Activity Range									
		≤10		>10-20		>20-30		>30-50		>50	
Type 1	20	7	35%	5	25%	3	15%	2	10%	3	15%
Type 2	49	26	53%	14	29%	4	8%	4	8%	1	2%
Type 3	3	2	67%	1*	33%	-	-	-	-	-	-

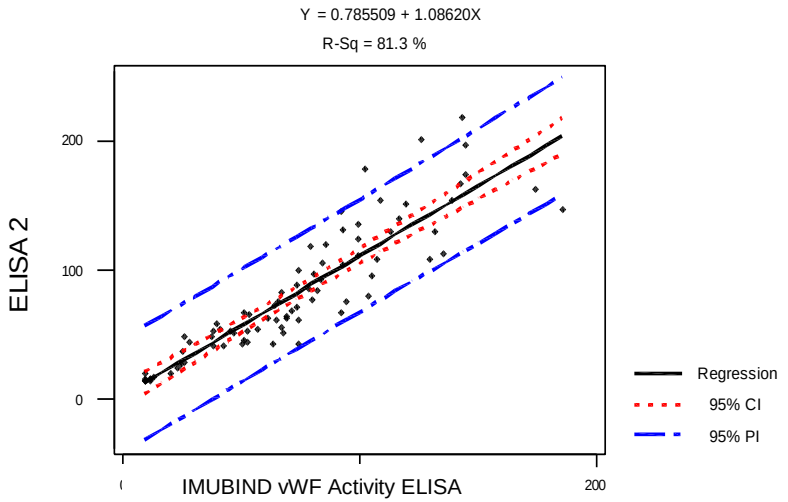
* Activity = 11%

PERFORMANCE DATA

External Study

An evaluation of the IMUBIND vWF Activity ELISA with another commercially available activity ELISA was carried out in the hemostasis department of a large medical center. Eighty samples with a putative vWF diagnosis were analyzed. Samples included those from patients on treatment.

Correlation between IMUBIND vWF Activity ELISA and ELISA 2



Y = 0.78551 + 1.08620X
R - Sq = 81.3%
R = 0.902 where X = IMUBIND ELISA
and Y = ELISA 2

Dilution Characteristics

Five dilutions of three patient samples were assayed using two kit batches. The following table shows the mean values obtained and the dilution-corrected recoveries of the initial dilution.

Sample	Dilution	Mean Value %	Dilution Corrected % Recovery (1/20)
1	1/20	83.3	100
	1/40	39.7	95
	1/80	20.9	100
	1/160	10.8	104
	1/320	6.1	117
2	1/20	40.8	100
	1/40	19.8	97
	1/80	10.3	101
	1/160	6.0	118
	1/320	2.8	110
3	1/20	24.4	100
	1/40	12.1	99
	1/80	7.0	115
	1/160	3.7	121

Imprecision

1. **Intra-assay imprecision** determined by testing two Controls with replication of four in 26 assays, using three operators and three kit batches, over a period of three days.

Control	Mean Value %	Root Mean Square %CV
1	80	8.0
2	16	11.1

2. **Inter-assay imprecision** determined by testing two Controls with replication of four in 26 assays, using three operators and three kit batches, over a period of three days.

Controls	Mean Value %	SD	%CV
1	80	13.4	17.0
2	16	3.45	21.5

Lower Limit of Detection

The lower limit of detection, calculated from the zero standard plus 2 standard deviations run in duplicate in 9 separate assays using 3 kit batches, was 1.6%.

Interferences

Hemoglobin up to 400 mg/dL, bilirubin up to 0.2 mg/mL and intralipid up to 15 mg/dL do not interfere with vWF activity results. Rheumatoid factor at a 1/640 titer showed no interference.

LIMITATIONS OF USE

1. Although the presence of low vWF activity levels is indicative of von Willebrand's Disease, the information must be considered in light of other clinical and laboratory findings, particularly bleeding times. Decreased levels of vWF have been reported in hypothyroidism and systemic lupus erythematosus.
2. vWF levels fluctuate widely in the normal population and in those affected by von Willebrand's disease. The cause of variation is not always apparent but physical exercise, stress, pregnancy, use of the contraceptive pill and age may contribute to these fluctuations. These factors should be taken into consideration when evaluating the significance of von Willebrand activity results.

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DEFINITION OF SYMBOLS

IVD	For In vitro diagnostic use
REF	Catalog number
LOT	Batch code / Lot number
Σ_n	Contains sufficient for <n> tests
⚠	Caution, refer to SDS
	See instructions for use
	Use By / Expiration Date
2°C 8°C	Temperature limitation: *Store at 2°C to 8°C*
CE	CE Mark
	Manufactured by
EC REP	European Authorised Representative

SUMMARY OF PROTOCOL

1. Prepare dilutions of reconstituted Calibrator to provide a Calibrator curve range.
2. Reconstitute and dilute Controls 1 and 2, 1 in 20 (1+19).
3. Dilute patient samples 1 in 20 (1+19) and 1 in 100 (1+99) if required.
4. Add 100 µL of prepared Calibrators in duplicate, prepared Controls and pre-diluted patient samples into referenced wells of the microtiter strip.
5. Incubate 60 ± 10 minutes at 18°-25°C.
6. Wash strips 5 times.
7. Add 100 µL of vWF Conjugate to each well.
8. Incubate 15 ± 2 minutes at 18°-25°C.
9. Wash strips 5 times.
10. Add 100 µL of vWF TMB Substrate to each well.
11. Incubate 15 ± 2 minutes at 18°-25°C.
12. Add 100 µL of vWF Stop Solution to each well.
13. Read absorbance at 450nm.