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ZYMUTEST™ vWF

REF RK030A

96 tests

ELISA assay of von Willebrand Factor

English, last revision: 07-2020

INTENDED USE:

The ZYMUTEST™ vWF kit is a sandwich ELISA method for the *in vitro* quantitative determination of von Willebrand Factor (vWF) on human plasma.

SUMMARY AND EXPLANATION:

Technical:

vWF is a multimeric protein produced in endothelial cells and megakaryocytes. It circulates in blood as multimers ranging from 500 to more than 20,000 kDa.
vWF mediates platelet adhesion to subendothelium of the damaged blood vessel and, by complexing to Factor VIII, extends its half-life into the bloodstream.
Ultra-large multimers are proteolytically cleaved by ADAMTS13 into less active vWF forms. The biological function of vWF depends largely on the size of its multimers. Larger multimers are more likely to bind to platelets and collagen, and to promote platelet adhesion in circulating blood[1,2].

Clinical:

vWF functional or quantitative deficiency leads to von Willebrand disease (vWD), which can be divided into 3 groups:

- Type 1: vWD is characterized by a partial quantitative deficit of vWF (most frequently).
- Type 2: vWD is characterized by an abnormal vWF adhesion activity. It is divided into 4 subtypes: 2A, 2B, 2M and 2N, depending on the multimers functional abnormality.
- Type 3: vWD is characterized by a severe quantitative deficit of vWF.

vWF deficiencies can be associated to different other pathologies, thus constituting an acquired von Willebrand disease.
When vascular endothelium is affected, the vWF concentration can be increased in relation to inflammatory processes[3,4].

PRINCIPLE:

ZYMUTEST™ vWF is and ELISA method, based on antigen-antibody reaction: vWF antigen of the sample reacts with anti-vWF polyclonal antibodies, stabilized. The tested plasma is introduced into a microwell coated. The vWF is captured onto the solid phase. Following a washing step, the vWF fixed on the plate is revealed by immunoconjugate, polyclonal antibody coupled to horse radish peroxidase (HRP), witch binds to free epitopes of immobilized vWF. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H_2O_2), is introduced in microwell plate and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human vWF in the tested sample.

REAGENTS:

1. **COAT ELISA microplate :** 12x8 contaning 12 strips of 8 wells, coated with a rabbit polyclonal antibody, specific for human vWF, stabilized and packed in an aluminium pouch in presence of a desiccant. Contains small amounts of sodium azide (0.9 g/L).
2. **SD ELISA Sample Diluent :** 2 vials of 50 mL, ready to use. Contains Proclin and BSA.
3. **CAL vWF vWF calibrator :** 3 vials of 2 mL, lyophilized. Each vial should be reconstituted by 2 mL of sample diluent to obtain a standard containing a concentration **"C%"** of human vWF (**already diluted at 1:50**), precisely determine for each lot. This concentration "C" Is between 120 and 160% according to the lot. The standard is referenced to the NIBSC international standard. Contains BSA.
4. **CI vWF vWF Control high :** 1 vial of 0.5 mL, lyophilized.
5. **CII vWF vWF Control low :** 1 vial of 0.5 mL, lyophilized.
6. **IC ANTI-(h)-vWF HRP Anti-(h)-vWF-HRP immunoconjugate :** 3 vials of 7.5 mL, a polyclonal rabbit antibody, specific for vWF and coupled to Horse-Radish-Peroxidase (HRP), lyophilized. Contains BSA.
7. **CD ELISA Conjugate diluent :** 1 vial of 25 mL, ready to use. Contains Proclin and BSA.
8. **WS ELISA Wash solution :** 1 vial of 50 mL, 20x 20 fold concentrated. Contains Proclin.
9. **TMB 3,3',5,5'-Tetramethylbenzidine:** 1 vial of 25 mL of substrate, ready to use. Contains hydrogen peroxide.
10. **Stop 0.45M Sulfuric acid:** 1 vial of 6 mL, ready to use.

The calibrator and controls concentrations may vary slightly from one batch to the next. For the assay, see the exact values provided on the flyer provided with the kit used.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Allow the strips and reagents to stabilize for at least 30 min at room temperature before use. Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

COAT Open the aluminum pouch and take off the required amounts of strips for the test series. The strips must be used within 30 minutes.

Reconstitute the contents of each vial with exactly:
CI vWF ➔ **0.5 mL of distilled water** at least 30 min before use. Shake vigorously until complete dissolution.
CII vWF ➔ **0.5 mL of distilled water** at least 30 min before use. Shake vigorously until complete dissolution.
CAL vWF ➔ **2 mL of SD ELISA** at least 30 min before use **in order to obtain a solution containing** "C" % **of vWF (already diluted at 1:50)**. Shake vigorously until complete dissolution.
IC ANTI-(h)-vWF HRP ➔ **7,5 mL of CD ELISA** at least 15 minutes before use. Shake gently until complete dissolution.

SD ELISA **TMB** **Stop** **CD ELISA**
Reagent ready to use.

WS ELISA Shake the vial and dilute the wash solution **1:20 in distilled water** (the 50 mL of concentrated solution allow to prepare 1 litter of wash solution after dilution).
Incubate, if necessary, the vial in a water bath at **37°C**, until complete dissolution of solids.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

COAT Unused strips can be stored at 2-8°C **for 4 weeks** in their original aluminum pouch (hermetically closed, in presence of the desiccant), stored in the provided plastic microplate storage bag (minigrip), protected from any moisture.

Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

CAL vWF
➔ **8 hours** at room temperature (18-25°C).

CI vWF **CII vWF**
➔ **24 hours at** 2-8°C.
8 hours at room temperature (18-25°C).
2 months frozen at -20°C or less*

IC ANTI-(h)-vWF HRP
➔ **4 weeks** at 2-8°C.
24 hours at room temperature (18-25°C).

*Thaw only once, as rapidly as possible at 37°C and use immediately.

Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

SD ELISA **CD ELISA** **TMB**
➔ **4 weeks** at 2-8°C.

WS ELISA ➔ **4 weeks** at 2-8°C.
7 days at 2-8°C for the diluted solution.

Stop ➔ **8 weeks** at 2-8°C.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.

Materials:

- 8-channel or repeating pipette allowing dispensing volumes of 50-300 µL.
- Pipettes at variable volumes from 0 to 20 µL, 20 to 200 µL and 200 to 1000 µL.
- Micro ELISA plate washing equipment and shaker.
- Micro ELISA plate reader with a wavelength set up at 450 nm.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI GP44-A4[5] (and CLSI H21-A5[6]) guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references.

PROCEDURE:

Assay method:

1. Specimens and controls should be diluted using **SD ELISA** as described in the table below:

Specimens	Dilution
CI vWF and **CII vWF**	**1:50**
Specimens	**1:50**

For expected vWF concentrations above "C"%, dilute specimens at **1:100** (D=100), or more.

2. Using the Calibrator **CAL vWF** with a concentration "C" in %, prepare the calibration range as described in the table below:

Concentration of vWF (%)	C	C:2	C:4	C:10	C:20	0
Vol. of **CAL vWF**	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Vol. of **SD ELISA**	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Mix for homogenization.
The dilutions are stable for **6 hours** at room temperature (18-25°C).

3. Put strips in the frame provided. Introduce the reagents in the micro ELISA plate wells and perform the assay as indicated on the following table:

Reagent	Volume	Procedure
CAL vWF or **CI vWF** or **CII vWF** or **Specimens to test diluted** or **SD ELISA** (blank)	**200µL**	Introduce the standard solutions or the tested specimen in the corresponding micro ELISA plate well
Incubate for 2 hours at room temperature (18-25°C) (a)		
WS ELISA	**300µL**	Proceed to 5 successive washings **(b)**
IC ANTI-(h)-vWF HRP	**200µL**	Introduce the **IC ANTI-(h)-vWF HRP** in the micro ELISA plate wells
Incubate for 1 hour at room temperature (18-25°C) (a)		
WS ELISA	**300µL**	Proceed to 5 successive washings **(b)**
TMB	**200µL**	Immediately after the washing, introduce the substrate into the wells **(b,c)**. **Nota:** The substrate distribution, raw by raw, must be accurate and at exact time intervals.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (a)		
Stop	**50µL**	Following exactly the same time intervals, raw to raw, than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid **(c)**.
Wait for 10 minutes in order to allow the colour to stabilize then measure absorbance at 450 nm. Subtract the blank values (d).		

Distribute calibrator dilutions, controls and specimens as rapidly as possible, in order to obtain homogeneous kinetics of the dosage. A too long delay (>10 min) between the first and the last distribution wells may have incidence on immunological kinetics and produce inaccurate results (underestimated value for the last wells).

(a) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.
(b) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components and reduce the reactivity plate. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could damage coating and lower plate reactivity.
(c) For addition of the substrate, the time interval between each row must be accurate and exactly determined.
(d) For bichromatic readings, a reference wavelength at 620 nm or at 690 nm can be used.

QUALITY CONTROL:

Using quality controls allows validating the method compliance, as well as the homogeneous of assays for a same lot of reagents.
Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out for each test series.
Each laboratory can establish acceptance ranges and verify expected performances in its analytical system.

RESULTS:

- Obtained OD450 can vary according to the effective temperature during the assay run.
- Plot the calibration curve with the OD 450 nm along the Y-axis and the vWF concentration in %, along the X-axis by choosing the "best fit" interpolation mode (refer to the flyer in the kit).
- Results are expressed with the obtained OD450 for specimens and controls using the calibration curve.
- The concentration of vWF (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution 1:50 is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- Alternatively, a specific software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.
- For **CI vWF** and **CII vWF**, the concentrations are directly deduced from the calibration curve.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific colour development, check that the washing step is performed efficiently.

EXPECTED VALUES:

The vWF concentration in normal human plasma is of about 10 µg/mL. vWF presents a large distribution (from 50 to 160%) in the general population.
This level is highly influenced by the ABO histo group (decreased of about 25% in type O subjects), the gender (level higher in women), and the ethnic origin (level lower in Caucasians). It is positively associated with diabetes, and it increases with age.

PERFORMANCES:

- Dynamic range: 0 to 150%.
- Detection threshold ≤ 5%.
- Intra-assay variability: 3-8 %.
- Inter-assay variability: 5-10 %.
- Interferences: No interference was observed with the molecules and up to following concentrations:

Heparin	Bilirubin	Hemoglobin
2 IU/mL	0.05 mg/mL	10 mg/mL

REFERENCES:

1. Luo GP. et al. von Willebrand Factor: more than a regulator of hemostasis and thrombosis. Acta Haematol, 2012.
2. Peyvandi F. et al. Role of von Willebrand Factor in the haemostasis. Blood Transfus. 2011.
3. Schwameis M. et al, vWF excess and ADAMTS13 deficiency: a unifying pathomechanism linking inflammation to thrombosis in DIC, malaria, and TTP. Thrombosis and Haemostasis. 2015.
4. Farkas P. et al, Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies. Immunobiology. 2017.
5. CLSI Document GP44-A4: "Procedures for the handling and processing of blood specimens for common laboratory tests".
6. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Stop H290 : May be corrosive to metals.
SD ELISA **CD ELISA** **WS ELISA** H317 : May cause an allergic skin reaction.