



COLLAGEN BINDING ASSAY

PACKAGE INSERT
INSTRUCTIONS AND INFORMATION

Store at 2-8°C

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1. INTENDED USE

This kit is an *in-vitro* laboratory assay for the determination of von Willebrand Factor function in human plasma.

2. INTRODUCTION

Von Willebrand syndrome is probably the most common condition in the community responsible for excessive bleeding or bruising. It is caused by deficiency of or defects in von Willebrand factor (VWF), an adhesive protein in normal blood plasma. VWF is necessary for the correct functioning of platelets, especially for their binding to collagen exposed at wound sites. There are several subtypes of VWD (1). In the type II disease VWF is present and detectable by regular ELISA but it lacks high molecular weight multimers which are the forms which bind most strongly to collagen.

Brown and Bosak (2) originally developed a simple ELISA-like method to assess the binding of VWF to collagen. Instead of using an antibody against VWF coated on microwells, they used collagen. An improved version of this method has been used extensively by Favalaro et al (3, 4) It is increasingly being shown in publications that VWF detectable by the CBA method is more closely related to the main biological activity of VWF than other methods (4, 5).

3. PRINCIPLE OF THE TEST

Plasma samples are diluted 1/50 and applied to the collagen-coated microwells for 1 hour. The most adhesive forms of VWF bind to the collagen while other forms are washed away. Bound VWF is then tagged by applying diluted HRP-conjugated anti VWF antibody for an hour. Unbound HRP antibody is washed away. TMB substrate solution is then applied to quantitate the bound HRP antibody-VWF complex. The reaction is stopped after a set time (usually 15 minutes) by adding dilute sulphuric acid which changes the colour of the product from blue to yellow and increases its intensity.

Plasma samples should be tested in parallel with a regular ELISA or other method for VWF. In most cases VWF quantitated by CBA and by ELISA are similar. If CBA is significantly less than VWF antigen a qualitative defect in the multimers may be expected.

4. KIT COMPONENTS

1. Microplate coated with collagen (equine type 1)* :12 breakapart 8-well snap-off strips coated with collagen (equine type 1) ; vacuum sealed, in resealable aluminium foil.
2. Reference plasma *: 1 bottle containing lyophilised plasma. Reconstitute with 1ml distilled water.
3. Abnormal control plasma * : 1 bottle containing lyophilised plasma. Reconstitute with 0.5ml distilled water.
4. Sample Diluent * : 1 bottle containing 60 ml of buffer for sample dilution; pH 7.0 ± 0.2; ready to use; amber cap.
5. Conjugate : 1 vial containing HRP conjugated anti VWF antibody; red cap. To be diluted 1/2500 with sample diluent.
6. Washing Solution (10x conc.)* : 2 bottles containing 52ml of a 10-fold concentrated buffer (pH 7.0 ± 0.2) for washing the wells; clear cap.
7. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; amber cap. Colourless solution.
8. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.5 mol/l; ready to use; clear cap
9. 1 Strip holder
10. 1 Package Insert

* These products contain 0.05% Sodium Azide

5. OTHER EQUIPMENT REQUIRED BUT NOT SUPPLIED

1. MICROWELL PLATE READER (capable of reading at 450 / 620 nm).
2. WASH BOTTLE - 500mL or suitable MICROWELL PLATE WASHER.
3. MEASURING CYLINDERS
4. MULTICHANNEL PIPETTE
5. REAGENT TROUGHS
6. VARIABLE VOLUME PIPETTORS (10µL – 2mL) AND DISPOSABLE TIPS
7. INCUBATOR OR WATER BATH 37°C
8. DISTILLED WATER
9. VORTEX TUBE MIXER
10. TIMER
11. DISPOSABLE TUBES

6. ASSAY PROCEDURE

- Review all instructions thoroughly before testing.
- Room temperature incubations should be performed at 20 – 24°C.
- All reagents, samples and controls should be brought to room temperature before use.
- Unused strips are to be returned to the foil pouch along with the desiccant and resealed.
- The kit components should not be left at room temperature for longer than the procedure requires. Store kits and kit components at 2 – 8°C when not in use.
- Kit components from different lot numbers should not be interchanged.
- The WASH BUFFER CONCENTRATE (10x) may crystallise at 4°C. Incubate in a 37°C water bath until crystals have dissolved.
- To ensure accurate quantification, it is essential that all pipettes used in the assay are calibrated and a fresh tip is used for controls and samples.
- Human citrated plasma is to be used in this assay. If the assay is completed within 48 hours of the specimen being collected then store the specimen at 2-8°C. Otherwise aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.
- The recommended format for this assay

1 well for blank
16 wells for reference plasma
2 wells for abnormal control
2 wells per test plasma

- Once started, finish the assay without interruption.

1. Perform a 1/50 dilution on the test plasma and abnormal control
e.g. 10ul of plasma plus 490uL of diluent

For the calibration curve, mix 40ul of reference plasma with 460ul of diluent to give 500ul of a 1/12.5 starting concentration and then serially dilute 250ul volumes through 8 wells in a separate plate.

2. Remove the required number of microwells from the sealed bag and place them in the holder. Ensure unused wells are resealed and stored at 4°C.
3. Pipette 100µl of controls (or calibration curve) and diluted samples to the appropriate wells.
4. Incubate for 60 MINUTES ± 5 MINUTES AT ROOM TEMPERATURE
5. Dilute the WASH BUFFER CONCENTRATE (10x) 1/10 with distilled water. Place in either a wash bottle or plate washer reservoir.

6. Wash Procedure: Aspirate the samples from the wells and then fill all wells with diluted WASH BUFFER (350µL/well) soak for 10 seconds on the first wash. Repeat the above for 2 more cycles but with no soak time.
7. Add 100µL of diluted CONJUGATE to all microwells.
8. Incubate the plate for 60 MINUTES ± 5 MINUTES at ROOM TEMPERATURE.
9. Wash the plate as in step 6. NB no soak time is required in this wash step.
10. Add 100µL/well of TMB SUBSTRATE to all microwells. Incubate the plate at ROOM TEMPERATURE in the dark for 15 MINUTES.
11. Stop the reaction by adding 100µL/well of STOP SOLUTION to the microwells.
12. Read the plate at 450/620 nm within 15mins of stopping the reaction
13. Subtract the blank from all wells and then calculate the mean O.D. of the controls and samples.

7. CALCULATION AND INTERPRETATION OF RESULTS

Plot final absorbance or optical density at 450nm against concentration of CBA (assuming 1/12.5 as 400%, 1/25 as 200%, 1/50 as 100%, etc) on a log-log plot.

Interpolate mean absorbance results from samples onto the curve of best fit and read off CBA values.

Normal range (Mean +/- 2SD) should be approximately 50-200%. Note that blood group "O" individuals tend to have lower value.

A CBA value below the normal range suggests von Willebrands disease increasing in significance with the degree below the lower limit of normal.

If the CBA is significantly lower than VWF assessed by regular ELISA then a deficiency of high molecular weight forms may be assumed. This suggests VWD type 2.

8. PRECAUTIONS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC, the use of this in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
2. All components containing material of human origin have been tested and found negative for HBsAg, anti-HCV and antibodies to HIV. They should still, however, be treated as though they were potentially infectious.

3. Do not substitute any component for the ones supplied with the kit.
4. All components that contain preservatives must have care exercised in handling/disposing of these products.
5. Heat inactivation or repeated freezing and thawing of serum samples may cause erroneous results.
6. Performing the assay at temperatures other than those stated may cause erroneous results.
7. Do not use components after the expiry date stated on the label.
8. Care should be taken to reduce microbial contamination of reusable kit components.
9. To avoid cross-contamination do not interchange screw caps of components.
10. It is recommended that external or "in-house" controls be included with each assay.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

9. REAGENT STORAGE AND SHELF LIFE

- All kit components must be stored at 2 – 8°C. All reagents are stable until labelled expiration date when stored at 2 – 8°C.

10. SPECIMEN COLLECTION

- Blood should be collected by venepuncture.
- If the assay is completed within 48 hours of the specimen being collected then store the specimen at 2-8°C. Other wise aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.

11. LIMITATIONS

- As with other diagnostic test procedures, the results obtained serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
- CBA may increase with age and vary according to patient condition, ie. Trauma, DDAVP treatment, pregnancy and hormonal status.
- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values

12. REFERENCES

1. Sadler JE. A revised classification of von Willebrands disease. *Thromb Haemost.* 1994; 71; 520-5.
2. Brown JE, Bosak JO. An ELISA test for the binding of von Willebrand antigen to collagen. *Thromb Res.* 1986; 43; 303-11.
3. Favalaro EJ, Grispo L, Exner T, Koutts J. Development of a simple collagen-based ELISA assay aids in the diagnosis of and permits sensitive discrimination between types I and II von Willebrands disease. *Blood Coagul Fibrinolysis.* 1991; 2; 285-91.
4. Favalaro EJ. Von Willebrand factor collagen-binding (activity) assay in the diagnosis of von Willebrand disease: A 15 year journey. *Semin Thromb Hemost.* 2002; 28; 191-202.
5. Popov J, Zhukov O, Ruden S, Zeschmann T, Sferruzza A, Sahud M. Performance and clinical utility of a commercial collagen binding assay for laboratory diagnosis of von Willebrands disease. *Clin Chem.* 2006; 52; 1965-7.

13. LIMITED EXPRESS LIABILITY

The manufacturer makes no express warranty other than the diagnostic kit will measure von Willebrand Factor function when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the intended use of this product.

The manufacturer disclaims any and all implied merchantability, fitness for use or implied utility for any other purpose. Any or all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

In some jurisdictions the law makes these disclaimers unenforceable and, accordingly all or part of the disclaimer may not apply to all users.

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