corgenix

Collagen Binding Assay

For Research Use Only – Not for use in Diagnostic Procedures

INTENDED USE

Enzyme immunoassay for the measurement of von Willebrand Factor (VWF) to collagen, which is associated with high molecular weight VWF multimers.

SUMMARY AND EXPLANATION

VWF is an important blood clotting protein, involved in both assisting platelet adhesion and stabilization of clotting factor VIII.

In von Willebrand Disease (VWD) there is typically a partial quantitative deficiency (classified as VWD Type 1) or a qualitative deficiency (classified as VWD type 2). VWD Type 3 is rare and characterised by virtually complete deficiency of VWF. Higher molecular weight multimers of VWF serve to bind activated platelets through specific membrane glycoproteins to connective tissue fibers exposed at wound sites and thus promote blood clotting and wound sealing[2].

The incidence of VWD worldwide is estimated at 1% to 3% but may be more common as mild cases may remain undetected. The CBA is an ELISA procedure that quantitates the collagen binding capacity (VWF:CB) of VWF to collagen coated microtitre wells[3]. Collagen binding of VWF is associated with the higher molecular weight (HMW) forms of VWF.

TEST PRINCIPLE

During the first incubation step the VWF multimers present in the sample bind to the collagen which is attached to the surface of the microwell plate. Unbound plasma proteins are then removed by washing and in a second reaction, peroxidase conjugated anti-VWF antibodies bind to the captured VWF multimers. Excess antibody is washed off and the bound activity is determined by the addition of substrate. The color development of the substrate is stopped by the addition of acid. The resulting color intensity, which is proportional to high molecular weight VWF multimers present in the sample, is determined photometrically. The supplied calibrated standards can be used to quantify the high molecular weight VWF multimers.

REAGENTS

Composition

Microwell plate (1 x 96 well. 6 x 1 6 well strips); coated with collagen.
40x Wash buffer concentrate: Tris buffer solution, preservatives and dye.
Normal standard / Cryoprecipitate / Cryosupernatant controls:
Lyophilized human plasma, sodium azide and dye.
Conjugate: Horseradish peroxidase (HRP) conjugated anti-human VWF and dye.
Substrate:TMB and solvent
Stopping Solution: 2M H_2S0_4

Warnings and precautions

For Research Use Only. Not for use in Diagnostics Procedures.
Treat as potentially infectious. All human plasmas prepared for lyophilization have been tested and confirmed as negative for HBsAg and antibodies to HIV-1, HIV-2 and HCV using FDA approved assays.
When disposing of azide, always flush with large volumes of water to avoid the possibility of an explosive residue forming in metal plumbing.
Avoid skin and eye contact with stopping solution and concentrated wash buffer.

Preparation for use

Bring all kit components to room temperature prior to use.

40x Wash Buffer concentrate: Prepare a wash buffer solution by diluting the concentrate 1 in 40 with purified water. Ensure sufficient wash buffer solution is prepared for dilution of samples, controls and standard in addition to washing the plate, (ie. For a full plate assay, dilute 22 ml of concentrate to 880 ml with distilled water). Wash buffer should be brought to room temperature and mixed well before use.

NOTE: If particles are present in 40X Wash Buffer Concentrate, mix solution well before dilution.

Normal Standard: Reconstitute the normal standard with 2ml of wash buffer solution. (This is now equivalent to a 200% standard). Allow to stand at room temperature for 10-15 minutes before use. Using the wash buffer solution, prepare 100%, 50%, 25% and 12.5% standards, (ie. Make 1/2, 1/4, 1/8 and 1/16 dilutions of the reconstituted 200% standard).

Cryoprecipitate and Cryosupernatant controls: Reconstitute the cryoprecipitate and Cryosupernatant controls with 2ml of wash buffer solution. Allow to stand at room temperature for at least 15 minutes before use.

Conjugate: Reconstitute conjugate with 11ml of wash buffer solution at least 5 minutes before it is required for use. It is important that the conjugate is protected from light exposure. Substrate: Ready for use.

Storage and stability

All components are stable until the expiry date shown on the vial when stored unopened at 2-8°C.

Reconstituted standard and control plasmas may be stored in aliquots at -20°C for 1 month or -80°C for 3 months. Do not freeze-thaw more than once.

Do not freeze standards after further dilution with wash buffer solution. Samples should be thawed at 37°C then equilibrated to room temperature before use.

Unused conjugate is stable for 3 months at -20°C or 6 months at -80°C.

Wash buffer solution may be stored for 1 month at 2-8°C.

Replace unused microwell strips in foil pouch provided and seal with tape until use.

Reagents should not be interchanged between different kit lots.

Indications of instability/deterioration

If there is no evidence of vacuum when the vials are opened and/or the reagent does not appear dry, or if the liquid substrate solution appears blue before use, the kit should be returned to the manufacturer or local distributor.

SPECIMEN COLLECTION AND PREPARATION

Sample collection

Caution: Treat all plasmas as potentially infectious.

Collect and process blood in accordance with NCCLS Standard H21-A3: Collection, Transport and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays; Approved Guideline -Third Edition (1998).

Mix 9 parts of freshly collected blood with I part 3.2% (0.109M) trisodium citrate. Centrifuge as soon as possible after collection at ≥ 1500g for 15 minutes. Sample filtration is not recommended for VWF assays.

Sample preparation

Dilute all samples to 1 in 40 with wash buffer solution.
Cryoprecipitate and Cryosupernatant do not require further dilution.

Sample stability

Separated plasma should be stored at 2-8°C and tested within 4 hours of collection, or may be frozen at < - 20°C for up to 2 weeks. Samples should be thawed at 37'C then equilibrated to room temperature before use.

Materials provided
Each pack of the Collagen Binding assay - Order code CBAE-1 contains
1 x 96 well collagen coated microwell plate (6x16 well strips)
2 x 2ml VWF antigen standard
4 x 2ml VWF antigen controls
1 x 11 ml anti-VWF:HRP Conjugate
2 x 11 ml Wash Buffer concentrate
1 x 11 ml Substrate solution
1 x 11 ml Stop solution

Materials required but not provided
Micropipettes
Pipettes: 1ml and 10ml
Purified water, USP or equivalent
Microwell plate reader capable of reading at 450nm (optional dual measurement at 450nm & 650 ± 50nm)
Test tubes or blank microwell plate for sample dilution.

PROCEDURE

1. Remove required number of microwell strips from foil pouch.
2. Add l00µl of diluted standards and controls to duplicate wells.
3. Add l00µl of diluted samples to duplicate wells.
4. Add l00µl of wash buffer solution to duplicate wells for use as a zero point on the standard curve.
5. Ensure that all samples are added within 5 minutes to minimize variation in incubation times. Mix by tapping gently on all 4 sides or using a mechanical mixer.
6. Cover plate (or place in moist chamber) and incubate for 60 minutes at 20-25°C.
7. Reconstitute Conjugate at least 5 minutes prior to use (see reagent preparation).
8. Thoroughly aspirate contents of all wells.
9. Wash plate 3 times by filling all wells with 300ul of wash buffer solution, then aspirating. Tap upside down on blotting paper after final aspiration.
10. Add l00µl of conjugate to each well. Mix by tapping gently on all 4 sides or using a mechanical mixer. It is important that sequential reagents are added quickly and that the period of sample incubation is consistent.
11. Cover (or place in moisture chamber) and incubate for 60 minutes at 20-25°C.
12. Wash plate 4 times (see step 9).
13. Add 100µl/ well of substrate solution.
14. Incubate uncovered for 5 minutes at 20-25°C.
15. Add 100µl stop solution to each well, adding at the same rate and in the same sequence as the substrate.
16. Within 15 minutes, read the absorbance at 450nm, or at 450nm with a 650± 50nm reference if dual wavelength plate reader available.

QUALITY CONTROL
The results for the cryosupernatant and cryoprecipitate plasmas supplied in the kit should be within the limits stated on the enclosed lot specific data sheet.

RESULTS
After calculating the mean absorbance of duplicate values for the standards, prepare the standard curve by plotting VWF concentration (X-axis) against absorbance (Y-axis) for each dilution of normal standard. Draw a smooth curve of best fit. Patient sample result can be read from the standard curve.

LIMITATIONS AND INTERFERENCES
Avoid using lipemic, hemolyzed or icteric plasmas.
Repeated freeze-thawing of patient samples is not recommended.

EXPECTED VALUES

Normal Range; 50-400%. Note that each laboratory should establish its own normal range for this assay.

SPECIFIC PERFORMANCE CHARACTERISTICS
Intra assay precision: 11%

BIBLIOGRAPHY

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3. Brown JE, Bosak JO. An ELISA test for the binding of von Willebrand antigen to collagen. Thrombosis Research. 43, 303-311, 1986
4. Gill JC, Endres-Brooks J, Bauerm PJ et al. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood. 69; 1691-1695, 1987

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