BIO/DATA CORPORATION

vW Factor Assay® Kit
(Ristocetin Cofactor Assay)

PRODUCT DESCRIPTION
The vW Factor Assay® Kit is a system of reagents and control plasmas for the evaluation of von Willebrand Syndrome.

INTENDED USE
The Ristocetin Cofactor Assay Kit causes lyophilized platelets to aggultinate in the presence of patient Platelet Poor Plasma and Ristocetin.

PRINCIPLE
Ristocetin cofactor is the in vitro activity of the von Willebrand factor which is responsible for the agglutination of platelets in the presence of Ristocetin.[12-14] Decreased von Willebrand factor is associated with von Willebrand syndrome, thus making quantitation of ristocetin cofactor activity most valuable in the diagnosis and evaluation of this coagulopathy.[13-15] Levels of ristocetin cofactor activity are determined by the ability of a test plasma and ristocetin to induce agglutination of a standardized platelet suspension. [16]

PRECAUTIONS
vW Factor Assay is for *PROFESSIONAL LABORATORY USE ONLY AND IN-VITRO* DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION. The plasma and platelets have been tested at the source and found to be negative for HIV-1Ag, anti-HIV-1/2, Hepatitis B surface antigen, Hepatitis C antibody, Human T-Lymph tropic Type I and II (anti-HTLV I/II) and negative by a serological test for Syphilis. However, all plasma and platelets of human origin should be handled as being potentially hazardous.

MATERIALS PROVIDED
1. Lyophilized Platelets, 4.0mL. Store at 2° - 8° C prior to reconstitution.
2. Ristocetin Reagent, 0.5mL. Store at 2° - 8° C prior to reconstitution.
3. Normal Reference Plasma (von Willebrand Factor), 0.5mL standardized to 90-110% von Willebrand Factor activity with a World Health Organization traceable reference material. Store at 2° - 8° C prior to reconstitution.
4. Abnormal Control Plasma (von Willebrand Factor Deficient), 0.5mL. Store at 2° - 8° C prior to reconstitution.
5. Tris Buffered Saline, pH 7.5, 10.0mL. Store at 2° - 8° C.
6. Graph Paper, 2 cycle log-log.

Note: For the 10 assay test kit, 1 vial of each product listed above are provided; for the 20 assay test kit, 2 vials of each product are provided. For larger test volumes, reagents from 2 or more kits (same lot numbers) may be pooled. The standard curve should be derived from the pooled reagents. Supplemental assay components are also available individually. (Refer to PRODUCT AVAILABILITY)

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified water (distilled, deionized or reagent grade), pH 5.3 - 7.2
3. Pipettors (4.0mL, 1.0mL, 0.5mL, 0.45mL, 0.05mL volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes
6. Rocker (Mechanical Rotation Device)

INSTRUMENTATION
vW Factor Assay will perform as described when used on most optical platelet aggregometers.[1] Follow the manufacturer's instructions for operating the aggregometer in use.

SPECIMEN COLLECTION AND PREPARATION OF TEST SAMPLE

Refer to the current NCCLS Approved Guideline H18 A2 for detailed specimen collection and sample preparation instructions.[6]

1. PATIENT PREPARATION
   Patients should refrain from taking aspirin or medications containing aspirin, other medications, and dietary supplements known to affect platelet function for 7-10 days prior to specimen collection. Patients should fast and avoid fatty foods and dairy products for 12 hours prior to specimen collection.[6]

2. SPECIMEN COLLECTION:
   Blood collection should be performed with care to avoid stasis, hemolysis, contamination by tissue fluids, or exposure to glass. Keep specimens at room temperature. [8]

   Each of the following can cause test results to be inaccurate; and affected specimens should be rejected: hemolysis, RBC contamination, lipemia, chylous, icterus, thrombocytopenia (<75,000/mm^3) clots in specimen, and hypofibrinogenemia. Reuse of disposable items may result in inaccurate test results.

   Observe standard precautions throughout the specimen collection, sample preparation and analytical processes.[2,3] Dispose of sharps and biological waste in accordance with laboratory policy.

   NOTE: When the patient's hematocrit is <30% or >55%, the blood to anti-coagulant volumes must be adjusted.[4]

   Evacuated Collection Tube Technique
   1. Use a butterfly needle for the venipuncture
   2. Draw blood using (plastic) tubes containing 0.11M Sodium Citrate anti-coagulant
   3. Gently invert 4-5 times to mix.

NOTE: When using plastic vacuum collection tubes, make sure the citrate anti-coagulant is 0.11M by checking the label. Colored tops do not vary with differing citrate concentrations. Follow the manufacturer's instructions for specimen collection.

PREPARATION OF PLATELET POOR PLASMA
1. Centrifuge blood at 2500 x g for 20 minutes.
2. Remove plasma from cells, being careful not to disturb the buffy coat. Plasma should be free of red cells and platelets.
3. If testing is delayed, refrigerate the plasma at 2° - 8° C for a maximum of 2 hours.

RECONSTITUTION
NOTE: Reagents must be at room temperature (15° - 28°C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.
1. Resuspension of Lyophilized Platelets: To a vial of Lyophilized Platelets, add 4.0mL of Tris Buffered Saline that is provided, allow to rock for at least 30 minutes. Reconstituted platelets are stable for 30 days when stored at 2°- 8°C. After refrigeration and prior to use it is also necessary to mechanically mix for at least 30 minutes at ambient temperature to allow the reagent to equilibrate and de-gas.
2. Ristocetin Reagent: Reconstitute with 0.5mL purified water for working concentration of 10mg/mL. Invert gently to mix and allow to rehydrate for 30 minutes at room temperature. Reconstituted Ristocetin is stable for 7 days when stored in the original, closed container at 2°- 8°C.
3. Normal Reference Plasma: Reconstitute with 0.5mL purified water. Let stand for 20 minutes at room temperature. Invert to mix. Reconstituted plasma is stable for 8 hours when stored in the original, closed container at 2°- 8°C. When diluted, stability of Reference Plasma is 45 minutes at room temperature.
4. Abnormal Control Plasma: Reconstitute with 0.5mL purified water. Let stand for 20 minutes at room temperature. Invert to mix. Reconstituted plasma is stable for 8 hours when stored in the original, closed container at 2°- 8°C. When diluted, stability of Control Plasma is 45 minutes at room temperature.

TEST PROCEDURE
IT IS ESSENTIAL THAT A STANDARD CURVE BE PREPARED WITH EACH SET OF ASSAYS.

A. Preparation of Normal Reference and Test Plasma Dilutions
   1. Prepare Normal Reference Plasma dilutions for standard curve as follows:
      a. Label 3 tubes: 100%, 50%, and 25%.
      b. Pipette 0.2mL Tris Buffered Saline into each tube.
      c. Pipette 0.2mL Normal Reference Plasma into tube labeled 100%. Mix thoroughly.
      d. Transfer 0.2mL from tube labeled 100% to tube labeled 50%. Mix thoroughly.
      e. Transfer 0.2mL from tube labeled 50% to tube labeled 25%. Mix thoroughly.
   2. Prepare Test Plasma Dilution as follows:
      a. Label a tube (sample identification) for each plasma to be tested.
      b. Prepare a 1:2 dilution of each test plasma by pipetting 0.1mL Tris Buffered Saline and 0.1mL test plasma into the tube. Mix thoroughly.

B. Preparation of the Aggregometer Blank: Pipette 0.25mL reconstituted platelets and 0.25mL Tris Buffered Saline into an aggregometer cuvette and mix thoroughly. This blank is to be used to set 100% baselines for each dilution throughout the assay. Mix prior to setting blank each time.

C. Performing the Assay:
   1. Pipette 0.4mL reconstituted platelets into an aggregometer cuvette.
   2. Add 0.05mL Ristocetin to the cuvette, being careful to avoid the introduction of air bubbles.
   3. Add new stir bar making sure stir bar is on its side. Be careful not to cause any droplets on the side of the cuvette.
   4. Incubate the cuvette at 37°C for one minute without stirring.
   5. Set blank according to manufacturer's instructions for the aggregometer in use.
   6. Place the cuvette in the test well and allow incubation to continue for an additional 2 minutes while stirring.
   7. Start channel and add 0.05mL of the 100% dilution of the normal reference plasma. Do not allow plasma dilution to run down the side of the cuvette. Also, be careful not to spike the platelet suspension with pipetting technique.
   8. Observe agglutination on the chart recorder until the reaction is complete. (No further increase in light transmission is observed.)
   9. For the 50% and 25% Normal Reference Plasma dilutions repeat steps 1-8, substituting each of these dilutions for the 100% Normal Reference Plasma dilution in Step 7 above.
   10. For the test plasma dilution repeat steps 1-8, substituting test plasma for the 100%Normal Reference Plasma dilution in step 7.

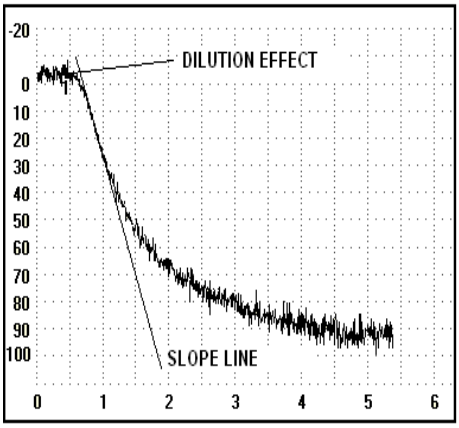

FIGURE 1
Draw a slope line along the steepest linear portion of the agglutination curve.

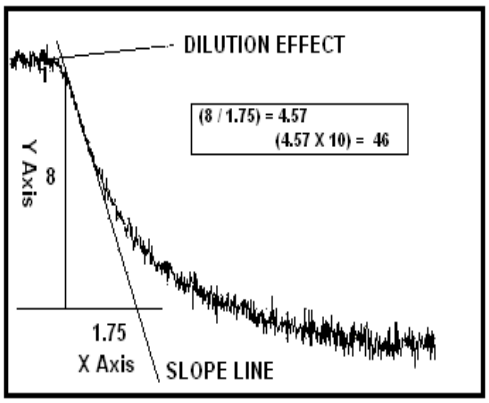

FIGURE 2
Select two points on the line. Measure the Y (vertical) axis and the X (horizontal) axis (see Figure 1). Calculate the Slope by dividing the Y axis by the X axis. Multiplying the sum by 10 then round off to the closest whole number.

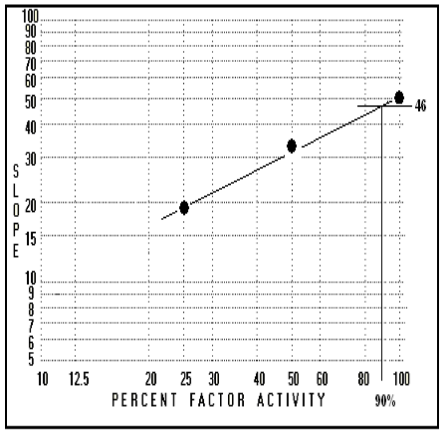

FIGURE 3
TEST PLASMA ACTIVITY
Slope Value =46
von Willebrand Activity =90%

QUALITY CONTROL

A von Willebrand factor deficient plasma is included as an abnormal control and should be assayed as a test plasma with an expected result of ≤ 45% activity. This control ensures that the assay system is specific for the von Willebrand factor and that agglutination will not be influenced by other normal plasma proteins. Additionally, it is suggested that Normal and Abnormal Assayed Reference Plasma controls be run to validate standard curves. (See Product Availability.)

RESULTS

A. Determine Slope Value:
1. Draw a line along the steepest linear portion of each agglutination curve (see Fig. 1). This is where the reaction rate is the greatest and occurs immediately after the onset of the agglutination. Additionally, there is a lag phase (delay time) from the addition of the plasma dilution to the onset of agglutination. That lag phase increases as the slope and the extent of agglutination decreases and is helpful in differentiating actual agglutination from artifactual changes in optical density.
2. Select two points on the line. Measure the Y (vertical) axis and the X (horizontal) axis (see Figure 1).
3. Calculate the Slope by dividing the Y axis by the X axis, multiplying the sum by 10, then round off to the nearest whole number.

SLOPE=(Y/X)10

example (see figure 2): (8/1.75) = 4.57
4.57 x 10 = 45.7
45.7 rounded = 46

B. Preparation of Standard Curve[2] (See Figure 3):
1. Using graph paper provided plot the percent von Willebrand factor activity for the 100%, 50% and 25% Normal Reference Plasma dilutions on the horizontal axis against their corresponding slope value on the vertical axis.
2. Draw a "best-fit" line through these points.

C. Determination of von Willebrand factor activity of the Test Plasma or the Abnormal Control Plasma:
1. Plot the slope value for the test plasma on the vertical axis of the standard curve
2. Read the corresponding percent von Willebrand factor activity level on the horizontal axis

D. Values Outside the Range of the Standard Curve:
1. Values greater than 100% factor activity may be verified by preparing a 1:4 dilution of the test plasma (3 parts Tris Buffered Saline, 1 part test plasma), repeating the assay procedure and multiplying the test results by two (2).
2. Values less than 25% factor activity may be tested by repeating the assay procedure on undiluted test plasma and dividing the test results by 2.

EXPECTED VALUES

A result of less than 40% von Willebrand factor is considered abnormal and suggestive of von Willebrand Syndrome.[7] However, other properties of the von Willebrand molecule must be considered for diagnosis of the variant forms of von Willebrand Syndrome. Since reference ranges for von Willebrand factor are dependent on blood type, each laboratory should establish blood type specific reference ranges for its patient population.[17]

Abnormal Control Plasma will yield von Willebrand factor assay results of ≤ 45%. The ability to generate a quantitative value in this range is dependent upon the sensitivity of the assay system in use.

LIMITATIONS

The quantitation of von Willebrand factor is considered by some to be the single most important assay for the diagnosis of von Willebrand Syndrome. However, diagnosis of the variant forms of this coagulopathy necessitates a series of clinical and laboratory evaluations including patient and family history, bleeding time, factor VIII related antigen, factor VIII coagulant activity[3,4,9,10] and multimeric studies.[9,10] Serial assays may be required to confirm diagnosis.

PERFORMANCE CHARACTERISTICS

The components of the vW Factor Assay were tested on the plasmas of diagnosed von Willebrand Syndrome patients as well as normal patients. Studies have shown that the accuracy and sensitivity of these components were such that varying levels of von Willebrand factor were detected.

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