results observed. There are fluctuations in the levels of the immunologically detectable protein as well as in the functional activity in the Factor VIII molecule. Each laboratory should establish its own reference range for this procedure.

**BIBLIOGRAPHY**


**Figure 2:** Standard Curve prepared with Coagulation S.A.R.P. on 2 cycle semi-logarithmic paper. The rocket lengths of the standards are as follows:

- 100% activity = 26 mm
- 50% activity = 21 mm
- 25% activity = 16 mm
- 12.5% activity = 11 mm

From the curve, a patient with a rocket length of 17 mm for 1:2 dilution would have 30% activity. See Step G.4. of the Step-By-Step Method for complete patient calculations.

**TROUBLESHOOTING**

1. When applying the samples to the plate wells, do not allow the pipette tip to touch the sides of the wells.
2. Do not overdry plate as it will cause the plate to peel.
3. Store unused Rocket Plates in the protective bag. The plate must be maintained in a moist environment.
4. For best results, a constant current power supply should be used.
5. Insure proper placement of plate on the Sponge Wicks; always have the wells on the negative side of the chamber, never side down.

**PERFORMANCE CHARACTERISTICS**

**A. Precision Studies**

Precision of the Helena von Willebrand Factor Antigen Rocket EIA Method was determined by reconstituting seven vials of Coagulation S.A.R.P. on eight different days and performing the test in duplicate each day. The S.A.R.P. was run undiluted, and in dilutions of 1:2, 1:4 and 1:8. The mean coefficient of variation was 7.8%.

**B. Sensitivity**

The sensitivity limits are defined by the von Willebrand Factor Antigen Reference Standards (S.A.R.P.), Patient values greater than the highest value on the standard curve must be diluted as follows:

- 12.5% activity = 11 mm
- 25% activity = 16 mm
- 100% activity = 26 mm

The FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Ingredients: Rocket Stain is a preparation of Coomassie Brilliant Blue stain.

**STORAGE AND STABILITY**: The stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. Discard diluted buffer if the material shows signs of dampness or discoloration. Discard diluted buffer if it becomes turbid.

**SPECIMEN COLLECTION AND HANDLING**

**Specimen Preparation:** Collect the blood specimen in either 3.2% (0.109 M) or 3.8% (0.129 M) sodium citrate. Add nine parts whole blood to 1 part citrate solution. Centrifuge the blood sample immediately after collection and store at 2 to 8°C.
8°C until testing is performed. Plasma stored at 2 to 8°C must be tested within one hour after sample collection. Plasma von Willebrand Factor Antigen is stable at -70°C for one month.

PROCEDURE

Materials Provided: The following materials are needed for the procedure.

- Cat. No. 5301 Rocket Plates (5 plates/box)
- Cat. No. 5016 Electra B1 Buffer (100 pkg/box)
- Cat. No. 5185 Coagulation S.A.R.P. (10 x 1.0 mL)
- Cat. No. 5300 Rocket Stain
- Cat. No. 5037 Titan Blotter Pads (100/pkg)
- Cat. No. 9015 Sponge Wicks (2/pkg)
- Cat. No. 5301 Coagulation S.A.C.-T (10 x 1 mL)
- Cat. No. 4063 TITAN GEL Chamber
- Cat. No. 6210, 6211 Microdispenser and Tubes (10 µL)
- Cat. No. 5014 Development Weight
- Cat. No. 4061 Staining Dish

Materials Needed But Not Provided:
- Power supply with constant current
- Lint-free tissues
- Destaining solution: Prepared with 350 mL deionized water
- Additional materials: Plastic tubes must be used for storage and testing.
- Willebrand Factor Antigen is stable at -70°C for one month.

Preparation of Electra B1 Buffer

- 400 µL of dissolved buffer into each of the outer sections of the TITAN GEL chamber (requires a total of 400 µL buffer).
- Place one sponge wick in the buffer along with each inner wall of the chamber.

Application of Samples

1. Place the Rocket Plate from refrigerator. Remove the plastic lid and allow approximately 5 to 20 minutes to let the plate reach room temperature and for excess buffer to be absorbed. Remove any moisture from wells, if necessary. Excess moisture on the plate can result in poor rockets.

2. Apply 10 µL of each patient sample or dilution to the designated wells taking care not to damage the wells. Standard curve samples must be run on each plate. Duplicate applications of patient samples are advisable.

3. Allow five (5) minutes for specimens to diffuse into the buffer.

E. Electrophoresis

1. Place the plate, agarose side down, in the chamber on the agarose side up. Place the sample point (wells) toward the cathode (−).

2. Place the cover on the chamber. Electrophorese the plates at 16 mA per plate (2 plates = 32 mA) for four (4) hours.

3. At the end of 4 hours, remove the plates from the chamber. The buffer chamber after each run.

F. Staining Procedure

1. Rinse the Rocket Plate briefly with deionized water and wash in 0.85% saline, agrose side up, overnight.

2. A laboratory rotator should not be used during the rinsing process.

3. After the overnight wash, again rinse the plate with deionized water and then wash in deionized water for 15 minutes. Drain or shake the excess water from the plate. Place the plate on a flat surface, agarose side up.

4. Place 4 to 5 Titan Blotter Pads and then a Development Weight on the plate for five (5) minutes.

5. Remove the weight and the top 2 to 3 Blotter Pads. Replace with 2 fresh Blotter Pads and then a weight for an additional ten (10) minutes.

6. Remove the Development Weight, blotters, and tissue.

7. Score the edges of the plate with a sharp instrument to prevent peeling.

G. Measurements and Calculations

1. Place the plate on a light box or white paper and mark the length of each rocket peak. A laboratory rotator should not be used during the rinsing process.

2. Using the Helena Rocket Ruler, measure the length of each peak in millimeters. The peak is measured from the top of each well to the apex of the rocket.

3. Plot the percent activity of the reference curve versus each rocket height on the Helena Factor VIII Related Antigen Report Form or on 2 cycle semi-logarithmic paper. Draw the “best fit” line for the four points. Refer to Figures 1 and 2 for an example of a completed Rocket Plate and a standard curve drawn on 2 cycle semi-logarithmic paper.

4. Read the patient or control values from the standard curve and multiply each by the appropriate dilution factor. Use a pre-diluted standard to treat the same with no change in the dilution factors used. The only difference would be the final reference value used. If Coagulation S.A.R.P. is used to prepare the standard curve, the pre-diluted value read from that curve must be multiplied by the assigned or pre-diluted von Willebrand Factor Antigen value of the appropriate lot of Coagulation S.A.R.P. as well as the dilution factor.

Ex: Patient value from curve = 30%

V/WF, P, and Johnson

5. W/F Related Antigen = 30 x 2 x 0.95 = 57.0%

6. Unknown patient samples with von Willebrand Factor Antigen levels greater than the range of the standard curve must be reassayed using the appropriate dilutions.

7. Dry the plate in the laboratory drying oven at 60 to 70°C for 10 to 20 minutes. Periodically inspect the agarose and look for it to become more transparent. The plate will be transparent when completely dry. Do not over-dry plates. If the agarose starts to peel at the edges before the entire plate is dry, reduce the temperature of the drying oven. If a dryer/oven is not available, the plates may be covered with the wet lint-free tissue and allowed to dry at room temperature overnight or under a fan for 3 hours at room temperature.

8. When completely dry, pour the stain over the entire surface of the plate, agrose side up, for 5 minutes.

9. Prepare the destaining solution by mixing together:

- 350 mL deionized water
- 20 mL methanol
- 30 mL glacial acetic acid

10. Destain the plate by placing it in destain solution until the rockets can be distinguished easily. The background will still be bluish purple. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in destain overnight. If over destaining does occur, repeat Step F8. and stain the rockets again.

11. Pour the stain from the destain solution and rinse briefly in deionized water.

12. Dry the plates at 37°C for 5 minutes or at room temperature until dry. Tape the edges of the agarose to the plastic backing with clear tape. This will prevent peeling of the agarose and prolong the life of the plate.

Reference Values

The expected normal range for the Helena von Willebrand Factor Antigen Rocket EIA Method is 50% to 150%. This range was established by testing 50 normal individuals, 25 males and 25 females. Testing on male donors versus female donors showed no significant difference in mean values. This normal range compared favorably with normal range values reported by Zimmerman and Johnson. Severe von Willebrand’s patients often show no detectable rocket precipitation by this method. Other patients with von Willebrand’s disease have been reported with normal levels of von Willebrand Factor Antigen.3 The diagnosis of von Willebrand’s syndrome should not be made on the basis of this test alone. Factor VIII coagulant activity, ristocetin aggregation, template bleeding times and a thorough history and physical are essential for an accurate diagnosis of this disease. On the basis of the results from various tests, von Willebrand’s patients may be classified into several categories. Suspected von Willebrand’s patients should be tested on several different occasions due to the variability in the test.
von Willebrand Factor Antigen Rocket Plate
Electra B1 diluted to 1000 mL
10 µL

B. Preparation of Standards and Test Plasmas

Development Weight 5014
Coagulation S.A.R.P.-1 (10 x 1 mL) 5301
Rocket Stain 5360
Microdispenser and Tubes (10 µL) 6210, 6211
Development Weight 5014
Staining Dish 4061

Materials Needed But Not Provided:
Lint-free tissues
Materials Needed But Not Provided:
Rocket Plates (5 plates/box) 5361

1. Dilute each patient sample and control with 0.85% saline. Prepare a 1:2 dilution (1 part patient plasma and 1 part saline) and a 1:4 dilution (1 part patient plasma and 3 parts saline). Additional dilutions may be necessary depending on the history. Samples from von Willebrand’s patients may need to be tested undiluted.

C. Preparation of TITAN GEL Chamber

1. Pour 200 mL of distilled buffer into each of the outer sections of the chamber (requires a total of 400 mL buffer).
2. Place one sponge wick in the buffer along with each inner wall of the chamber.

D. Application of Samples

1. Remove the Rocket Plate from refrigerator.
2. Place the plastic lid and allow approximately 5 to 20 minutes to the plate to reach room temperature and for excess buffer to be absorbed. Remove any moisture from wells, if necessary. Excess moisture on the plate can result in poor rockets.
3. Apply 10 µL of each patient sample or dilution to the designated wells taking care not to damage the wells. Standard curve samples must be run on each plate. Duplicate applications of patient samples are advisable.
4. Allow five (5) minutes for samples to diffuse into the buffer.

E. Electrophoresis

1. Place the plate, agarose side down, in the chamber. Place the application points (wells) toward the cathode (negative).
2. Place the cover on the chamber. Electrophorese the plates at 16 mA per plate (2 plates = 32 mA) for 4 (four) hours.
3. At the end of 4 hours, remove the plates from the chamber. Discard the buffer after each run.

F. Staining Procedure

1. Rinse the Rocket Plate briefly with deionized water and wash in 0.85% saline, agarose side up, overnight.
2. After the overnight wash, again rinse the plate with deionized water and then wash in deionized water for 15 minutes. Drain or shake the excess water from the plate.
3. Place the plate on a flat surface, agarose side up.
4. Cover the agarose with a single, lint-free tissue.
5. Place 4 to 5 Titan Blotter Plates and then a Development Weight on the plate for five (5) minutes. Remove the weight and the top 2 to 3 Blotter Plates. Replace with 2 fresh Blotter Pads and then a weight for an additional ten (10) minutes.
6. Remove the Development Weight, blotters, and tissue.
7. Score the edges of the plate with a sharp instrument to prevent peeling.

G. Measurements and Calculations

1. Place the plate on a light box or white paper and mark the endpoints of each peak with a marker.
2. Using the Helena Rocket Ruler, measure the length of each peak in millimeters. The peak is measured from the top of each well to the apex of the rocket.
3. Plot the percent activity of the reference curve versus each rocket height on the Helena Factor VIII Related Antigen Report Form or on 2 cycle semi-logarithmic paper. Draw the “line of best fit” for the four points. Refer to Figures 1 and 2 for an example of a completed Rocket Plate and a standard curve drawn on 2 cycle semi-logarithmic paper.
4. Read the patient or control values from the standard curve and multiply each by the appropriate dilution factor. Use of a pre-diluted standard would be treated the same with no change in the dilution factors used. The only difference would be the final reference value used. If Coagulation S.A.R.P. is used to prepare the standard curve, each value read from that curve must be multiplied by the assigned or pre-diluted von Willebrand Factor Antigen value of the appropriate lot of Coagulation S.A.R.P. as well as the dilution factor.

Ex:
Patient value from curve = 30% von Willebrand Factor Antigen
[from assay sheet] = 95%
Dilution factor = 2
Actual Patient Factor VIII = 30 x 2 x 0.95 = 57.0%

5. Unknown patient samples with von Willebrand Factor Antigen levels greater than the range of the standard curve must be reassayed using the appropriate dilutions.
6. Dry the plate in the laboratory drying oven at 60 to 70°C for 10 to 20 minutes. Periodically inspect the agarose and look for it to become more transparent. The plate will be transparent when completely dry. Do not over-dry plates. If the agarose starts to peel at the edges before the entire plate is dry, reduce the temperature of the drying oven. If a dryer/oven is not available, the plates may be covered with the wet lint-free tissue and allowed to dry at room temperature overnight or under a fan for 3 hours at room temperature.
7. When completely dry, pour the stain over the entire surface of the plate, agarose side up, for 5 minutes.
8. Prepare the destaining solution by mixing together:
350 mL deionized water
20 mL methanol
30 mL glacial acetic acid
9. Destain the plate by placing it in destain solution until the rockets can be distinguished easily. The background will still be bluish purple. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in destain overnight. If over destaining does occur, repeat Step F.8 and stain the rockets again.
10. Place the plate from the destain solution and rinse briefly in deionized water.
11. Dry the plates at 37°C for 5 minutes or at room temperature until dry. Tape the edges of the agarose to the plastic backing with clear tape. This will prevent peeling of the agarose and prolong the life of the plate.

Figure 1: Rocket patterns on a von Willebrand Factor Antigen Rocket Plate. The lengths of the rockets (in millimeters) of the Coagulation S.A.R.P. (the standard) dilutions are used to prepare the standard curve. Patient results are read from the curve. In this illustration, Patient #3 has no rocket formation indicating a von Willebrand Factor Antigen deficiency.

Quality Control
A control material of known concentration, such as S.A.C.-1 (Cat. No. 5301) should be used on each run to verify all phases of the procedure and should be used on each gel. Refer to the package insert provided with the control for reconstitution instructions and assay values. When using S.A.C.-1 with asterisked values on the package insert, dilute the reconstituted vial (or portion of the vial) one part control plus one part 0.85% saline. Then from this diluted sample, the appropriate dilutions can now be made and should give values within the asterisked ranges. If the control does not perform as expected, the patient results should be considered invalid or suspect.

REFERENCE VALUES
von Willebrand Factor Antigen values are generally expressed in relative percents as compared to a standard or pooled normal plasma. The expected normal range for the Helena von Willebrand Factor Antigen Rocket EIA Method is 50% to 150%. This range was established by testing 50 individuals, 25 males and 25 females. Testing on male donors versus female donors showed no significant difference in mean values. This normal range compared favorably with normal range values reported by Zimmerman and Johnson.

Severe von Willebrand’s patients often show no detectable rocket precipitation by this method. Other patients with von Willebrand’s disease have been reported with normal levels of von Willebrand Factor Antigen. The diagnosis of von Willebrand’s syndrome should not be made on the basis of this test alone. Factor VIII coagulant activity, ristocetin aggregation, template bleeding times and a thorough history and physical are essential for an accurate diagnosis of this disease. On the basis of the results from various tests, von Willebrand’s patients may be classified into several categories. Suspected von Willebrand’s patients should be tested on several different occasions due to the variability in the test
Results observed. There are fluctuations in the levels of the immunologically detectable protein as well as in the functional activity in the Factor VIII molecule. Each laboratory should establish its own reference range for this procedure.

**Bibliography**


**Performance Characteristics**

**Precision Studies**

Precision of the Helena von Willebrand Factor Antigen Rocket EIA Method was determined by reconstituting seven vials of Coagulation S.A.R.P. on eight different days and performing the test in duplicate each day. The S.A.R.P. was run undiluted, and in dilutions of 1:2, 1:4 and 1:8. The mean coefficient of variation was 7.8%. The sensitivity limits are defined by the von Willebrand Factor Antigen Reference Standards (S.A.R.P.). Patient values greater than the highest value on the standard curve must be diluted and reassayed. Patient values less than the lowest value on the curve must be reported as such but no absolute value may be extrapolated.

**Troubleshooting**

1. When applying the samples to the plate wells, do not allow the pipette tip to touch the sides of the wells. Do not dry plate as it will cause the plate to peel.
2. Store unused Rocket Plates in the protective bag. The plate must be maintained in a moist environment.
3. For best results, a constant current power supply should be used.
4. Insure proper placement of plate on the Sponge Wicks; always have the wells on the negative side of the chamber.

**Additional Equipment and Supplies**

**For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.**

Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement of any product found to be defective. In no case will Helena Laboratories be liable for consequential damages. Although each Helena product has been tested under laboratory conditions, no warranty is given as to which shall be made. These alternatives shall be buyer’s exclusive remedies. In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised of the possibility of such damages.

**Warning: For In-Vitro Diagnostic Use Only. Do Not Ingest.**

**Preparation for Use:** Collect the blood specimen in either EDTA or Heparin. Complete the venomant and store at 2°C to 8°C. The reconstituted product is stable for four hours after reconstitution. Discard the plate if dry in appearance or if the wells are not round. A crystalline appearance indicates the agarose has been frozen and should be discarded.

**Specimen Collection and Handling**

Specimens: Plasma from whole blood collected in sodium citrate as an anticoagulant.

**Preparation for Use:** Collect the blood specimen in either 3.2% (0.109 M) or 3.8% (0.129 M) sodium citrate. Add nine parts whole blood to 1 part sodium citrate solution. Centrifuge the blood sample immediately after collection and store at 2°C to 8°C.