RISTOCETIN COFACTOR ASSAY

Cat. No. 5370

Helena Laboratories

The Helena Ristocetin Cofactor Assay Kit is intended for the quantitation of an activity that reflects von Willebrand factor activity.

SUMMARY

The von Willebrand factor protein is the protein which corrects the bleeding time abnormality in von Willebrand's disease. Several variant forms of von Willebrand's disease have been identified. They are usually differentiated into qualitative and quantitative abnormalities of Factor VIII Related Antigen and Factor VIII von Willebrand factor. The ristocetin cofactor is a property of von Willebrand factor which promotes agglutination of platelets in the presence of the antibiotic ristocetin. The estimation of the ristocetin cofactor activity does allow quantitation of an activity thought to reflect von Willebrand factor activity.[1]

PRINCIPLE

The Helena Ristocetin Cofactor Assay Kit measures the ability of a patient's plasma to agglutinate formalin-fixed platelets in the presence of ristocetin. The rate of ristocetin induced agglutination is related to the concentration of von Willebrand factor and the percent normal activity can be obtained from the aggregometer tracing.

REAGENTS

1. **Lyophilized Platelets**
   Ingredients: This reagent contains washed formalin-fixed platelets in a tris-buffered saline.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE. Avoid ingestion.
   Preparation for Use: Reconstitute each vial with 5.0 mL of the Tris-Buffered Saline. Allow to stand approximately 20 minutes, then mix well. Vortexing for 1-5 minutes improves uniform suspension of platelets. An alternative method to vortexing is aspiration of the 5 mLs of platelet suspension into a 5 mL syringe with a 21 or 22G needle and forcibly returning the suspension to the vial 4 or 5 times.
   Storage and Stability: Helena Lyophilized Platelets are stable until the expiration date printed on the vial label when stored at 2 to 8°C. The reconstituted product is stable for 30 days when stored at 2 to 8°C. Before use, allow the reconstituted platelets to come to room temperature and resuspend platelets by mixing thoroughly.
   Signs of Deterioration: The reagent is a fine suspension of platelets. Large flaky particles may be indicative of product deterioration.
2. **Coagulation S.A.R.P. (Specialty Assayed Reference Plasma)**
   Ingredients: S.A.R.P. is prepared from a pool of fresh citrated plasma from healthy individuals. The pool is buffered and lyophilized to insure stability of plasma constituents.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE. S.A.R.P. has been found negative for Hepatitis B Antigen (HBsAg) and HIV antibody; however, it should be handled with the same precautions as with any human sample. Avoid ingestion.
   Preparation for Use: Reconstitute S.A.R.P. with 1.0 mL of deionized water. Swirl gently. Allow approximately 10 minutes for complete dissolution before use.
   Storage and Stability: Lyophilized S.A.R.P. is stable until the expiration date indicated on the vial when stored at 2 to 8°C. The ristocetin cofactor activity of the reconstituted product is stable for eight hours at 2 to 8°C or 30 days at -20°C.
   Signs of Deterioration: The lyophilized S.A.R.P. should appear as a light yellow, dry plug.
3. **Tris-Buffered Saline**
   Ingredients: Tris 0.05 M, Sodium chloride 0.15 M, Sodium azide 0.02%.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE. Avoid ingestion.
   Preparationfor Use: The reagent is ready for use as packaged.
   Storageand Stability: The reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.
   Signsof Deterioration: The reagent should be a clear colorless solution. Cloudiness may be indicative of contamination.
4. **Ristocetin Cofactor Abnormal Control Plasma**
   Ingredients: This plasma contains a human citrated plasma from an individual with von Willebrand's disease. The plasma is buffered and lyophilized to insure stability of the plasma constituents.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE. The plasma has been found to be negative for Hepatitis B Antigen (HBsAg) and HIV antibody; however, the plasma should be handled with the same precautions as those observed when handling patient plasma.
   Preparationfor Use: Reconstitute each vial of control with 0.5 mL of deionized water. Swirl gently and allow to stand for 10 minutes at room temperature to insure complete dissolution.
   Storageand Stability: The lyophilized product is stable until the expiration date on the label when stored at 2 to 8°C. The reconstituted control is stable for eight hours at 2 to 8°C or 30 days at -20°C.
   Signsof Deterioration: The lyophilized control should appear as a light yellow, dry plug.
5. **Helena Ristocetin**
   Ingredients: Contains lyophilized ristocetin obtained from Nocardia lurida. Stabilizers have been added to assure stability. The reconstituted concentration is 10 mg/mL.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE. Avoid ingestion.
   Preparation for Use: Reconstitute one vial of Ristocetin with 1.5 mL of deionized water. Swirl gently and allow to stand 10 minutes at room temperature for complete dissolution.
   Storageand Stability: The lyophilized product is stable until the expiration date on the label when stored at 2 to 8°C. The reconstituted product is stable eight hours at 2 to 8°C or 30 days at -20°C.
   Signs of Deterioration: The lyophilized product should appear as a dry, white plug.

INSTRUMENTS

Helena Ristocetin Cofactor Assay Reagents are suitable for use with any turbidimetric aggregation monitoring device. Recommended is the AggRAM Analyzer (Cat. No. 1484) or the PACKS-4® Platelet Aggregation System (Cat. No. 1471).

SPECIMEN COLLECTION AND HANDLING

Specimen: Plasma obtained from whole blood collected with 3.2% sodium citrate as an anticoagulant is the specimen of choice.

Specimen Collection: Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood specimens with hematocrits (HCT) of < 55% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant.[8] For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.[2]

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1 - .\text{HCT})} \times 9$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is determined abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

Specimen Preparation: Centrifuge the whole blood specimen at 1600-2000 x G for 10 minutes. Immediately separate the plasma from the red blood cells, and place it in a plastic test tube with cap.

Storage and Stability: Prior to testing, the plasma should be stored in the capped plastic tubes at 2 to 8°C. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C or colder for up to one month. Thaw quickly at 37°C prior to testing, but do not allow to stand at 37°C for more than 5 minutes.

PROCEDURE

Materials Provided:

Cat. No. 5370

Lyophilized Platelets
Coagulation S.A.R.P.
Tris-Buffered Saline
Ristocetin Cofactor Abnormal Control Plasma
Helena Ristocetin (10 mg/mL)
Ristocetin Cofactor Assay Report Form

Materials Required but not provided in the kit:

Platelet aggregometer (AggRAM Analyzer or PACKS-4 Analyzer recommended)
Plastic pipette tips
Plastic or siliconized test tubes
Pipettes to deliver 50 µL or 25 µL
Pipettes to deliver 400 µL or 200 µL

STEP BY-STEP METHOD

A. Recorder Preparation

Prepare aggregometer for use as recommended in the Operator's Manual.

B. Preparation of Reagents

1. Reconstitute Coagulation S.A.R.P. with 1.0 mL deionized water and swirl gently. Allow 10 minutes for complete dissolution.
2. Reconstitute one vial of Lyophilized Platelets with 5 mL of Tris-Buffered Saline. Allow to stand 20 minutes then mix well. Vortexing for 1-5 minutes improves uniform suspension of platelets. Inadequate platelet suspension may cause erroneous results.
3. Reconstitute one vial of Ristocetin Cofactor Abnormal Control Plasma with 0.5 mL of deionized water and swirl gently. Allow 10 minutes for complete dissolution.

4. Reconstitute one vial of Helena Ristocetin with 1.5 mL of deionized water to give a concentration of 10 mg/mL. (Final concentration 1 mg/mL in assay.) Swirl gently. Allow 10 minutes for complete dissolution.

C. Standard Curve Preparation

1. Prepare the following dilutions of Coagulation S.A.R.P. with Tris-Buffered Saline.

Tube	Dilution Ratio	mL S.A.R.P.	mL Buffer	Actual % Activity
1	1:2	0.1	0.1	50
2	1:4	0.1	0.3	25
3	1:8	0.1	0.7	12.5

D. Preparation of Test and Control Plasma

1. Prepare test and control dilutions with Tris Buffered Saline. Dilutions should be tested within 30 minutes.

Tube	Dilution Ratio	Control or Test Plasma	mL Buffer	Actual % Activity
4	1:2	0.1	0.1	50
5	1:4	0.1	0.3	25

E. Performance of Assay

NOTE: The AggRAM and the PACKS-4 can be run with the recommended (standard) volumes or with one-half (micro) volumes. Standard volume tests are done at 1000 rpm and micro volume tests are done at 600 rpm. Patient results should be compared to normal ranges run under the same conditions.
The following steps are for standard volume; for micro volume use one half of the standard volumes.

1. Pipette 250 µL of Helena's lyophilized platelet suspension and 250 µL of Tris-Buffered Saline into an aggregometer cuvette with a stir bar. Set 100% activity using the Aggregation Blank.
2. Place a stir bar into a cuvette for each sample to be tested. Add 400 µL of platelet suspension to each cuvette.
3. Place the cuvette into the cuvette well. Add 50 µL of ristocetin to the cuvette. This will automatically activate a 1-3 minute incubation period.
4. At the computer prompt, add 50 µL of each reference, patient or control plasma dilutions.
5. The aggregometer will record the aggregation for 5 minutes. It will automatically calculate the slope and produce a Standard Curve; or patient or control percent activity can be calculated from a stored Standard Curve. Printouts of the curve, control and patient data are available.

Quality Control: Quantitation of ristocetin cofactor should be monitored using the abnormal control, provided in the kit, and a normal control such as S.A.C.-1 (Cat. No. 5301). These controls should be used with each run and can verify all phases of the procedure. Refer to the package insert provided with the S.A.C.-1 for detailed information and assay values.

RESULTS

A. Preparation of Manual Standard Curve

1. Use the report form provided and the slope values obtained from the aggregometer, to plot the actual percent activity of the Coagulation S.A.R.P. plasma dilutions (50%, 25% and 12.5%) on the horizontal axis against the corresponding slope values on the vertical axis.
2. Draw a line of best fit through these points.

B. Determination of Test and Control Plasma Activity

1. Plot the slope value of the test plasma and control plasma dilutions on the vertical axis of the standard curve.
2. Read the corresponding percent ristocetin cofactor activity on the horizontal axis and multiply results by the dilution factor and the assay value of the reference plasma.
   Example:
   49% (% ristocetin cofactor activity from graph) x 2 (dilution factor) x 1.0 (assay value) = 98% (Ristocetin Cofactor Activity).
3. For activities greater than the highest point on the curve, dilute the plasma specimen appropriately in order to obtain slope values in the linear portion of the standard curve. Multiply the test results by the reciprocal of the plasma dilution.
4. For activities less than the lowest point on the curve, repeat the assay on undiluted test plasma and read the results directly off the standard curve.

REFERENCE RANGE

A random sampling of 22 normal plasma specimens was tested for ristocetin cofactor activity. An established range of 58-166% (0.58-1.66 units/mL) was obtained. Fifty (50) normal plasma specimens were tested with the AggRAM and a normal range of 56 -187% (0.56 - 1.87 units / mL) was obtained. It is recommended that each laboratory determine a reference range for its particular population and instrument/reagent system. Low values are an indication of von Willebrand's disease.

LIMITATIONS

The ristocetin cofactor activity fails to reflect accurately von Willebrand's disease in several situations such as pregnancy[3], infusion of commercial Factor VIII concentrates[4, 5] or administration of 1-deamino (8-d-arginine)-vasopression (DDAVP).[6] In such instances, VIIIR:RCo may be corrected, yet the bleeding time remains prolonged. In addition, VIIIR:RCo levels may be normal in Type II B von Willebrand's disease even though the bleeding time is prolonged.[7]

PERFORMANCE CHARACTERISTICS

Comparison Studies: Correlation studues were done using the Helena Ristocetin Cofactor Assay Method on the AggRAM and the PACKS-4. Forty samples were tested by both methods resulting in a linear regression equation of Y = 0.97X + 1.3 (where Y is the AggRAM and X is the PACKS-4) and a correlation coefficient of 0.974.

BIBLIOGRAPHY

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3. Ratnoff, O.D., Bennett, B., Clues to Pathogeneis of Bleeding in von Willebrand's Disease, N Eng J Med, 289:1182-1183,1973.
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6. Mannucci, P.M., Pareti, F.I., Holmberg, L., et al., Studies on the Prolonged Bleeding Time in von Willebrand's Disease, J Lab Clin Med, 88:662 671,1976.
7. Ruggeri, Z.M., Pareti, F.I., Mannucci, P.M., et al., Heightened Interaction Between Platelets and Factor VIII/von Willebrand Factor in a New Subtype of von Willebrand's Disease, N Eng J Med, 302:1047-1051,1980.
8. NCCLS, Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, 2nd ed. - H21-A2, 1991.

Ristocetin Cofactor Assay Kit

Cat. No. 5370

Lyophilized Platelets (4 x 5.0 mL)
Coagulation S.A.R.P. (2 x 1.0 mL)
Tris-Buffered Saline (1 x 35 mL)
Ristocetin Cofactor Abnormal Control Plasma (2 x 0.5 mL)
Helena Ristocetin 10 mg/mL (2 x 1.5 mL)
Ristocetin Cofactor Assay Report Forms

Components offered individually

	Cat. No.
Lyophilized Platelets (5 x 5.0 mL)	5371
Coagulation S.A.R.P. (10 x 1.0 mL)	5185
S.A.C.-1 (Specialty Assayed Control) (10 x 1 mL)	5301
Tris-Buffered Saline (1 x 250 mL)	5365
Helena Ristocetin 10 mg/mL (5 x 1.5 mL)	5372
Ristocetin Cofactor Abnormal Control Plasma (5 x 0.5 mL)	5373

Other Supplies and Equipment

-The following items, needed for the performance of the Helena Ristocetin Cofactor Assay Kit must be ordered individually.

	Cat. No.
AggRAM Analyzer	1484
AggRAM Stir Bars	1489
PACKS-4 Platelet Aggregation System	1471
AggRAM/PACKS-4 Cuvettes (200)	1473
PACKS-4 Stir Bars (30)	1474

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

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