Helena Ristocetin

Cat. No. 5199

Helena Laboratories

The Helena Ristocetin is intended for use in platelet aggregation studies to aid in the diagnosis of von Willebrand's disease.

SUMMARY

Ristocetin is an antibiotic which was withdrawn from pharmaceutical use as a result of a high occurrence of thrombocytopenia in patients taking the drug.[1] In 1971, Howard and Firkin found that platelets of von Willebrand's patients had impaired aggregation response when exposed to ristocetin.[2] 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. Defective, ristocetin-induced aggregation has also been found in conjunction with other disease states, including the Bernard-Soulier Syndrome, as well as aspirin ingestion.[3]

PRINCIPLE

Ristocetin-platelet aggregation studies are performed utilizing platelet-rich plasma (PRP). The rate of ristocetin induced agglutination is related to the concentration of von Willebrand factor.

REAGENT

1. RISTOCETIN

Reactive Ingredients: Helena Ristocetin is a product of Nocardia which has been stabilized with buffers and lyophilized to assure stability. The concentration post-reconstitution is 15 mg/mL; the concentration after addition to plasma (1:10) is 1.5 mg/mL.

WARNING: FOR IN-VITRO DIAGNOSTIC USE AVOID INGESTION.

Preparation for Use: Reconstitute Helena Ristocetin with 0.5 mL of deionized water. Swirl gently and allow product to stand about 5 minutes at room temperature for complete dissolution. After reconstitution, the ristocetin should appear as a clear liquid with a slightly yellow tint.

Storage and Stability: The lyophilized ristocetin is stable until the expiration date on the label when stored at 2-8°C. The reconstituted product is stable for 4 hours at 15-30°C, or for one month at -20°C.

Signs of Deterioration: The unreconstituted Helena Ristocetin must appear as a white plug or pieces of that plug.

INSTRUMENTS

Helena Ristocetin Reagent is suitable for use with any turbidimetric aggregation monitoring device. Recommended is the Helena AggRAM (Cat. No. 1484) or the PACKS-4 (Platelet Aggregation Chromogenic Kinetic 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. Hemolyzed samples may cause erroneous aggregation results.

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.[4] For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.[5]

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

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. During all phases of collection and post collection of a specimen, only plastic or siliconized glass labware should be used.[6]

Specimen Preparation:

1. Prepare platelet rich plasma (PRP) by centrifuging both of the anticoagulated samples at 100 x g 10-15 minutes at room temperature. DO NOT BRAKE THE CENTRIFUGE. Remove the PRP from the cells with a plastic pipette and place in a plastic tube labeled PRP. Cap the tube and maintain at room temperature. Wait 30 minutes after PRP is removed before testing.
2. Prepare platelet poor plasma (PPP) by recentrifuging the remaining blood samples at 1600-2000 x g (or an equivalent centrifugation force) for 10-15 minutes at room temperature. DO NOT BRAKE THE CENTRIFUGE. Remove PPP, place in a plastic tube labeled PPP and cover. Maintain at room temperature.
3. A platelet count should be performed on the patient PRP and a normal control PRP. The platelet count should be standardized (usually 250,000/mm^3) by adjusting the PRP with autologous platelet poor plasma. Platelet counts below 100,000/mm^3 may give variable results.[7]

Storage and Stability: Plasma as well as whole blood should always be stored at room temperature (15-30°(C). Cover samples to maintain the pH. Tests should be performed within three hours after sample collection.

PROCEDURE

Materials Provided:

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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 450 µL or 225 µL

STEP BY STEP METHOD

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. Collect and prepare blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING section.

2. Reconstitute the Ristocetin according to the directions in the REAGENT section.
3. Prepare the AggRAM or the PACKS-4 for use as recommended in the Operator's Manual.
4. Pipette 450 μL of platelet poor plasma (PPP) into a cuvette and leave at room temperature. Pipette 450 μL of platelet rich plasma (PRP) into a different cuvette and incubate the specimen at 37°C for one to three minutes.
5. Insert the PPP specimen into the appropriate channel and set the instrument to 100% aggregation.
6. Add a stir bar to the warmed PRP specimen, and insert the tube into the channel.
7. Add 50 μL of Ristocetin to the PRP specimen and record the aggregation. Analyzer sets 0% when test is started.

Quality Control: Known normal specimens should be used to establish typical aggregation patterns. Normal values for these patterns are then compared with the results from samples with marked variation from the normal range, thus indicating platelet dysfunction.[8]

RESULTS

One of the several methods used to quantitate platelet aggregation is the Weiss formula.. It measures the initial and maximum O.D. to give a result in percent aggregation.[9]

$$\frac{\text{O.D. Initial-O.D. Maximum}}{\text{O.D. Initial}} \times 100 = \%\ \text{Aggregation}$$

Follow the Operator's Reference Manual for the aggregation monitoring instrument being used.

LIMITATIONS

A patient medical history of all prescriptions and non-prescription drugs should be taken before testing. Medication, especially aspirin, may interfere with aggregation. For the effects of various drugs on platelet aggregation activity, refer to Young, et al.[10] Prior to testing, patients should refrain from smoking or drinking, and if possible, from taking medication.

REFERENCE VALUES

Aggregation of 70-100% is a normal response when platelets are exposed to ristocetin. These values should serve as guidelines for expected values. Because differences may exist among instruments, laboratories and local populations, it is recommended that each laboratory establish its own range of expected values.

INTERPRETATION OF RESULTS

Impaired ristocetin-induced aggregation may be indicative of many things. Although most von Willebrand's patients exhibit abnormal ristocetin-induced aggregation, some do not. In addition, defective ristocetin-induced aggregation may be present in the following disease states: (a) acute myeloblastic or monocytic leukemia, (b) infectious mononucleosis, (c) platelet storage pool disease, (d) the Bernard-Soulier syndrome, and (e) idiopathic thrombocytopenia.[11] An important consideration is von Willebrand platelets correct with normal platelet-poor plasma while other disease states do not.

BIBLIOGRAPHY

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11. Walsh, R.T. The Platelet in von Willebrand's Disease: Interactions with Ristocetin and Factor VIII. Thrombosis and Hemostasis 2,105-115, 1975.

Supplies and Equipment

	Cat. No.
Ristocetin	5199
AggRAM Analyzer	1484
AggRAM Stir Bars	1489
Packs- 4 Platelet Aggregation System	1471
AggRAM/Packs- 4 Cuvettes	1473
Packs- 4 Stir Bars	1474
Packs- 4 Replacement Tips	
(50-100 μL)	1475
(100-1000 μL)	1476

Shading indicates that text has been modified, added or deleted.