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, two indicating platelet dysfunction.

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

O.D. Initial/O.D. Maximum x 100 = 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.11 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 institutions, 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) idopathic thrombocytopenia.11 An important consideration is that von Willebrand platelets correct with normal platelet-poor plasma while other disease states do not.

PREPARATION OF PLASMA
1. Collect and prepare blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING section.
2. Prepare platelet rich plasma (PRP) by centrifuging both the anticoagulated samples at 150-1500 x g 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.
3. 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.
4. A platelet count should be performed on the patient PPP and a normal control PPP. The platelet count should be standardized (usually 250,000/mm³) by adjusting the PRP with autologous platelet poor plasma. Platelet counts below 100,000/mm³ may give variable results.

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: Ristocetin

BIBLIOGRAPHY
11. Walsh, R.T. The Platelet in von Willebrand’s Disease: Interactions with Factor VIII von Willebrand factor. The ristocetin cofactor is a property of von Willebrand factor which promotes aggregation 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.

Cat. No. 5199

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. In 1971, Howard and Firkin found that platelets from 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 product of von Willebrand factor which promotes aggregation 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.

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 yellow to pale yellow fluid.

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 unconstituted 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, syringes, 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 < 50% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant.2 For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.

Parts whole blood to PPP = (0.6 x HCT) + 9

One part anticoagulant is added to one part PPP for 600 rpm. Two parts anticoagulant is added to one part PPP for 1000 rpm.

For specimens being studied according to directions in SPECIMEN COLLECTION AND HANDLING section.

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

O.D. Initial-O.D. Maximum x 100 = % Aggregation
O.D. Initial

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

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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 institutions’ laboratories and local populations, it is recommended that each laboratory establish its own range of expected values.

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Parts whole blood to = 0.6 x 9
1 part anticoagulant

3.2% sodium citrate as an anticoagulant is the specimen of choice. Hemolyzed samples may cause erroneous aggregation results.

PROCEDURE
Materials Provided:
Ristocetin
Cat. No. 5199

Materials Required but not provided in the kit:
Platelet Aggrometer (Aggam 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 Aggam and the PACKS-4 can be run with the recommended (standard) volumes or with one-half (micro) vol- umes.Standard volume tests are done at 1000 rpm and micro volume tests are done at 600 rpm. Patient results should be compared only with normal ranges for 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 specimens according to directions in SPECIMEN COLLECTION AND HANDLING section.