**RISTOCETIN COFACTOR ASSAY**

**(von Willebrand Factor Assay)**

**INTENDED USE**

The Ristocetin Cofactor Assay is used for the quantitative determination of Factor VIII Ristocetin Cofactor activity in plasma.

**SUMMARY AND PRINCIPLE**

Ristocetin Cofactor activity in plasma may be determined by the agglutination of a standardized suspension of platelets in the presence of von Willebrand factor using the antibiotic, Ristocetin.1 The Ristocetin Cofactor activity is the in vitro activity of the von Willebrand factor which is responsible for the agglutination of platelets in the presence of Ristocetin. Although the platelets play a passive role in such agglutination, there is an absolute requirement that the Ristocetin-dependent receptor be intact.1 von Willebrand disease is associated with a decrease in von Willebrand factor or Ristocetin Cofactor activity and it is generally accepted that the Ristocetin Cofactor activity is the most useful in vitro assay for the diagnosis of von Willebrand disease.2,3

Levels of Ristocetin Cofactor activity are determined by the ability of the test plasma and Ristocetin to induce aggregation in a standardized platelet suspension.2,5

Following reconstitution, the lyophilized platelets are treated with Ristocetin in the presence of dilutions of a normal standardized human plasma with known amounts of Ristocetin Cofactor activity. A standard curve is prepared. A patient plasma is then used as a source of Ristocetin Cofactor activity in the presence of Ristocetin and reconstituted platelets from which an aggregation pattern (slope) is determined. The Ristocetin Cofactor activity is interpolated from the standard curve.

**REAGENTS**

**REAGENT DESCRIPTION**

- **Ristocetin**: 1 x 7.5 mg, 50750A
- **Lyophilized Human Platelets**: 1 x 6 ml, 50750B
- **von Willebrand Reference Plasma, Normal**: 1 x 10 ml, 50750C
- **Human plasma standardized for Ristocetin Cofactor activity, dried**: 50750 D
- **Tris Buffered Saline (TBS)**: 1 x 12 ml, 50750E
- **Ristocetin, 1 x 7.5 mg, 50750A**
- **Plastic transfer pipettes**
- **Precision pipettors for 4ml, 1ml, 0.5ml, 0.4ml, and 0.05ml**
- **Stir bars**
- **Anticoagulant**

**REAGENT PREPARATION**

- **Ristocetin**: Reconstitute with 0.75 ml reagent grade water for a final concentration of approximately 10 mg/ml. Allow to stand at room temperature (18-25°C) with occasional swirling for 10-15 minutes. Be sure that all particulate matter is well-dissolved.
- **Lyophilized Human Platelets**: Reconstitute a vial of lyophilized human platelets with the quantity of Tris Buffered Saline indicated on the vial label. Allow to stand at least 10 minutes prior to use. Prior to each use, mix by gentle inversion.
- **von Willebrand Reference Plasma, Normal**: Reconstitute with 1.0 ml reagent grade water. Allow to stand 10 minutes at room temperature (18-25°C) with occasional swirling. Make certain all particulate matter is well-dissolved.
- **von Willebrand Reference Plasma, Deficient**: Reconstitute with 0.5 ml reagent grade water. Allow to stand 10 minutes at room temperature (18-25°C) with occasional swirling. Make certain all particulate matter is well-dissolved.
- **Tris Buffered Saline (TBS)**: Use for the reconstitution of lyophilized platelets.

**ADDITIONAL MATERIAL REQUIRED**

- Method of measuring platelet aggregation with chart recorder
- Aggregometer cuvettes
- Stir bars
- Precision pipettes for 4ml, 1ml, 0.5ml, 0.4ml, and 0.05ml
- Plastic transfer pipettes

**STORAGE AND STABILITY**

- **Ristocetin**: Store at 2-8°C. After reconstitution any unused portion may be stored frozen at -20°C or below.
- **Lyophilized Human Platelets**: Store at 2-4°C. Stable for 30 days after reconstitution at 2-8°C, or may be frozen up to 30 days.
- **von Willebrand Reference Plasma, normal**: Stable for 4 hours at 2-8°C after reconstitution or may be stored frozen at -20°C for up to 30 days.
- **von Willebrand Reference Plasma, deficient**: Stable for 4 hours at 2-8°C after reconstitution or may be stored frozen at -20°C for up to 30 days.

**STORAGE AND STABILITY**

- **Ristocetin**: Store at 2-8°C. Do not use after expiration date on label.

**SPECIMEN COLLECTION AND PREPARATION**

**Anticoagulant**

Use buffered sodium citrate 3.2%.

**Specimen Collection**

1. Obtain versus blood by clean venipuncture.
2. Immediately mix nine parts blood with one part anticoagulant, mix well by inversion of tube against the stopper.
3. Centrifuge the specimen at 1500 g for no less than 15 minutes.
4. Remove plasma from the tube within 60 minutes using a plastic pipette and store in a plastic tube.
5. Test plasma sample within 4 hours; otherwise, store frozen at -20°C for up to two weeks or -70°C for up to six months and thaw once just prior to use.

**Determination of Slope**

1. Most commercially available Aggregometers provide a direct readout of Slope. See instrument manufacturer’s instructions.

**Prepare Standard Curve from Slope Values**

Using log-log graph paper, plot the % of Ristocetin Cofactor in the dilution of Reference Plasma Normal (100%, 50%, and 25%) on the indicated axis versus their corresponding slope values on the other axis.

2. Draw a line of best fit through the 3 points.

**Determine Ristocetin Cofactor Value in Test Plasma**

1. From the slope value of the test plasma, intersect the line of best fit, extending the line to the Ristocetin Cofactor axis, interpolating the level of Ristocetin Cofactor activity in the test sample.

**INTERPRETATION OF RESULTS**

1. The Reference Plasma is assigned a Ristocetin activity value. The value interpolated from the graph, for the level of Ristocetin Cofactor Activity in the test sample assumes that the Reference Plasma has 100% activity. The Ristocetin Cofactor Activity in the test sample must be corrected to account for the assigned Ristocetin value of the Reference Plasma. The correction factor used is the assigned value of the Reference Plasma.

**WARNINGS AND PRECAUTIONS**

Each unit of source plasma used in the preparation of this product has been tested by FDA approved methods for the presence of antibody to Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HBsAg) as well as for Hepatitis C (HCV) and found negative (not repeatedly reactive). However, no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious material. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

**Assay Calibration**

**1. Preparation of Standard Curve (must be prepared for each set of assays).**


   i. Label three plastic test tubes (12 x 75mm) as 100%, 50%, and 25%.

   ii. Add 0.2ml TBS to each tube.

   iii. Add 0.2ml Reference Plasma, Normal to tube labelled as 100%. Mix well.

   iv. Transfer 0.2ml from 100% tube to 50% tube. Mix well.

   v. Transfer 0.2ml from 50% tube to 25% tube. Mix well.

b. Prepare Test Plasma dilution by adding 0.1ml Test Plasma to 0.1ml TBS in a plastic tube. Mix well.

2. Preparation of Blank.

   a. Add 0.25ml TBS and 0.25ml reconstituted Platelets to Aggregometer cuvette. Mix well.

   b. The Blank will be used to set the 100% baseline for each sample cuvette.

**TEST PROCEDURE**

**1. Add 0.4ml reconstituted Platelets to an aggregometer cuvette.**

2. Add 0.05ml Ristocetin to the sample cuvette. Mix well. Add a stir bar to the cuvette.

3. Place cuvette in Aggregometer.

4. Set the 0% and 100% baselines according to instrument manufacturer’s instructions. Generally, the Blank (prepared in Assay Calibration section) is used for the 100% baseline and TBS alone as the 0% baseline.

5. Begin Aggregation by adding 0.05ml of the 100% Reference Plasma, Normal to the sample cuvette containing the Platelet-Ristocetin mixture. Observe and record aggregation pattern until complete.

6. Repeat steps 1-5 for the 50% and 25% dilutions of Reference Plasma, Normal.

7. Repeat steps 1-5 for the test plasma (previously prepared 1:2 dilution).

**QUALITY CONTROL**

- **von Willebrand Reference Plasma, Deficient**: Included in the test kit to function as an abnormal control. The Ristocetin Cofactor value of this control is less than 30%, and when used in the place of test plasma, should result in reduced aggregation, reduced slope, and an interpolated value of less than 30% Ristocetin Cofactor.

- **von Willebrand Reference Plasma, Deficient**: Does not assay at less than 30%, repeat the standard curve and control. If the abnormal control is again out, this is indicative of reagent deterioration or technical error. Contact Technical Services at Tcoag.
2. Calculation
Ristocetin Cofactor Activity (%) = Interpolated value for Ristocetin Cofactor Activity of Reference Plasma.

Example
Ristocetin Cofactor Activity

<table>
<thead>
<tr>
<th>Test</th>
<th>101%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Plasma</td>
<td>90%</td>
</tr>
</tbody>
</table>

Ristocetin Cofactor Activity = \[
\frac{101 \times 90}{100} = 90.9\%
\]

EXPECTED RESULTS
Results of less than 40% Ristocetin Cofactor (von Willebrand Factor) are generally considered abnormal and are indicative of von Willebrand disease. A normal range should be determined by the individual laboratory utilizing its particular reagent/instrument combination.

LIMITATIONS
The Ristocetin Cofactor Assay is considered by many investigators to be the single most important assay for von Willebrand disease. However, a complete diagnosis and determination of the variant forms of this coagulopathy requires an evaluation of other factors, such as Factor VIII-Rag, Factor VIII: C activity, bleeding time, and family history.

REFERENCES

ORDERING INFORMATION
Kit                        Ristocetin Co-factor Assay
Catalogue No.               50750

Additional Reagents Available

<table>
<thead>
<tr>
<th>Kit No.</th>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>50705</td>
<td>Ristocetin 7.5mg/vial</td>
<td>10 x 0.5 ml</td>
</tr>
<tr>
<td>8851</td>
<td>Collagen</td>
<td>3 x 1 ml</td>
</tr>
<tr>
<td>50704</td>
<td>ADP</td>
<td>3 x 0.5 ml</td>
</tr>
<tr>
<td>50710</td>
<td>Platelets</td>
<td>3 x 6 ml</td>
</tr>
</tbody>
</table>