

## RISTOCETIN COFACTOR ASSAY

(von Willebrand Factor Assay)

Part #299 (15 determinations)

### I. INTENDED USE

The Chrono-Log Ristocetin Cofactor Assay is for use in the quantitative determination of Factor VIII Ristocetin Cofactor activity in citrated plasma.

### II. SUMMARY

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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3,4</sup> Levels of Ristocetin Cofactor activity are determined by the ability of the test plasma and Ristocetin to induce aggregation in a standardized platelet suspension.<sup>5-7</sup>

### III. PRINCIPLE

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 is determined and the Ristocetin Cofactor activity is interpolated from the standard curve.

### IV. REAGENTS

**Ristocetin (7.5 mg/vial).** Store at 2°– 8° C. Reconstitute with 0.75 mL distilled water at room temperature for a final concentration of 10 mg/mL. Allow to stand at room temperature (21° C ± 4° C) with occasional swirling for 30 minutes. Never shake this reagent. Be sure that all particulate matter is well dissolved. Keep on ice during use. Any unused portion may be stored frozen at –20° C or below for up to 30 days.

**Lyophilized Human Platelets, 6 mL.** Reconstitute a vial of lyophilized human platelets with 6 mL of Tris Buffered Saline. Allow to stand 30 minutes prior to use. Prior to each use, mix by gentle inversion. Store at 2°– 8° C. Stable for 30 days after reconstitution at 2°– 8° C or may be frozen up to 30 days.

**von Willebrand Reference Plasma, Normal.** Human plasma standardized for Ristocetin Cofactor Activity, 1.0 mL, dried. Reconstitute with 1.0 mL distilled water. Allow to stand for 20 minutes at room temperature with occasional swirling. Make certain all particulate matter is well dissolved. 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.** Derived from human congenital von Willebrand syndrome patients and contains less than 30% of normal Ristocetin Cofactor activity, 0.5 mL, dried. Reconstitute with 0.5 mL distilled water. Allow to stand 20 minutes at room temperature with occasional swirling. Make certain all particulate matter is well dissolved. Stable for 4 hours at 2°– 8° C after reconstitution or may be stored frozen at –20° C for up to 30 days.

**Tris Buffered Saline (TBS), 12 mL.** Use for the reconstitution of lyophilized Platelets. Store at 2°– 8° C. Do not use after expiration date on label.

For in vitro diagnostic use.

### V. SPECIMEN PREPARATION

- A. Anticoagulant. Use buffered sodium citrate, 3.8% or 3.2%.
- B. Specimen Collection. (See, for example, Ref. 8)
  1. Obtain venous blood by clean venipuncture.
  2. Immediately mix 9 parts blood with 1 part anticoagulant, mix well by inversion of tube against the stopper.
  3. Centrifuge the specimen at 1000–2400 g for 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 2 hours, otherwise store frozen at –20° C for up to two weeks or –70° C for up to six months and thaw just prior to use.

### VI. PROCEDURE

- A. Materials Provided:
  - Ristocetin
  - Lyophilized Platelets
  - von Willebrand Reference Plasma, Normal
  - von Willebrand Reference Plasma, Deficient
  - Tris Buffered Saline
- B. Materials Required:
  1. Method of measuring platelet aggregation with chart recorder or AGGRO/LINK System.
  2. Aggregometer cuvettes.
  3. Stir-bars.
  4. Precision pipettors for 1 mL, 0.5 mL, 0.4 mL, and 0.05 mL.
  5. Plastic transfer pipettes.
  6. Distilled water.
- C. Performance of Test
  1. Preparation of Standard Curve (Must be prepared for each set of assays).
    - a. Prepare dilutions of Reference Plasma, Normal for a Standard Curve.
      - i. Label 3 plastic test tubes (12 x 75 mm.) as 100%, 50%, 25%.
      - ii. Add 0.2 mL TBS to each tube.

- iii. Add 0.2 mL Reference Plasma, Normal to tube labeled as 100%. Mix well.
  - iv. Transfer 0.2 mL from 100% tube to 50% tube. Mix well.
  - v. Transfer 0.2 mL from 50% tube to 25% tube. Mix well.
  - b. Prepare Control and Patient Test Plasma dilution(s) by adding 0.1 mL Test Plasma to 0.1 mL TBS in a plastic tube. Mix well.
2. Preparation of Blank  
Add 0.25 mL TBS and 0.25 mL reconstituted Platelets to Aggregometer cuvette. Mix well.  
The Blank will be used to set the baseline for each sample cuvette.
3. Assay Procedure
- a. Add 0.4 mL reconstituted Platelets to a pre-warmed aggregometer cuvette containing a stir bar.
  - b. Add 0.05 mL Ristocetin to the sample cuvette. Mix well.
  - c. Place cuvette in Aggregometer.
  - d. Incubate in test well for two (2) minutes.
  - e. Set the 0% and 100% baselines according to instrument instructions.
  - f. After mixing well, begin Aggregation by adding 0.05 mL of the 100% Reference Plasma, Normal to the sample cuvette containing the Platelet-Ristocetin mixture. Observe and record Aggregation pattern until complete. When using the AGGRO/LINK, the system will automatically record the Aggregation curve and determine its slope.
  - g. Repeat steps a.– e. for the 50% and 25% dilutions of Reference Plasma, Normal.
  - h. Repeat steps a. – e. for the test plasma (previously prepared 1:2 dilution).

## VII. RESULTS

- A. Determination of Slope.
- 1. Most commercially available Aggregometers provide a direct read-out of Slope.
- B. Prepare Standard Curve from Slope Values.
- 1. Using the log-log graph paper provided, plot the % of Ristocetin Cofactor in the dilution of Reference Plasma, Normal (100%, 50%, 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.  
(Chrono-log Ristocetin Cofactor Assay software plots the points and calculates the best fit standard line automatically.)
- C. Determine Ristocetin Cofactor Activity 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. (See Example on graph paper.)  
(Chrono-log Cofactor Assay software calculates the Ristocetin Cofactor activity in the test plasma automatically.)
- NOTE: The test plasma Slope must fall within the linear portion of the standard curve. It may be necessary to vary the test plasma dilution to achieve a value in this range.**

## VIII. EXPECTED VALUE

Results of less than 40% Ristocetin Cofactor activity (von Willebrand) Factor) are generally considered abnormal and are indicative of von Willebrand disease.<sup>1-4</sup> A normal range should

be determined by the individual laboratory utilizing its particular reagent/instrument combination.

## IX. QUALITY CONTROL

von Willebrand Reference Plasma, Deficient is 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 Activity.

If the 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 Chrono-Log.

## X. 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, VIII:C activity, bleeding time, and family history.

## XI. REFERENCES

1. Weiss, H.J., Hoyer, L.W., Rickles, F.R., Varma, A. and Rogers, J.; J. Clin. Invest. 52:2708, 1973.
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4. George, J.N., Nurden, A.T. and Phillips, D.R.; N. Eng. J. Med. 311:1084, 1984.
5. Allain, J.P., Cooper, H.A., Wagner, R.H., et al; J. Lab. Clin. Med. 85:318, 1975.
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7. Ramsey, R. and Evatt, B.L.; Am. J.Clin. Path. 72:996, 1979.
8. National Committee for Clinical Laboratory Standards: Guidelines for the Standardized Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays.

## CAUTION

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 (HB<sub>s</sub>Ag) as well as for Hepatitis C (HCV) and found negative (not repeatedly reactive). Donors have been screened for Creutzfeldt-Jakob Disease (CJD) and new variant Creutzfeldt-Jakob Disease (nvCJD) and found acceptable. 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.

This product is warranted to perform in accordance with its labeling and literature. Chrono-Log disclaims any implied warranty of merchantability or fitness for any other purpose and in no event will Chrono-Log be liable for any consequential damages arising out of aforesaid express warranty.

## CHRONO-LOG RISTOCETIN COFACTOR ASSAY

**Example:**

Reference Plasma

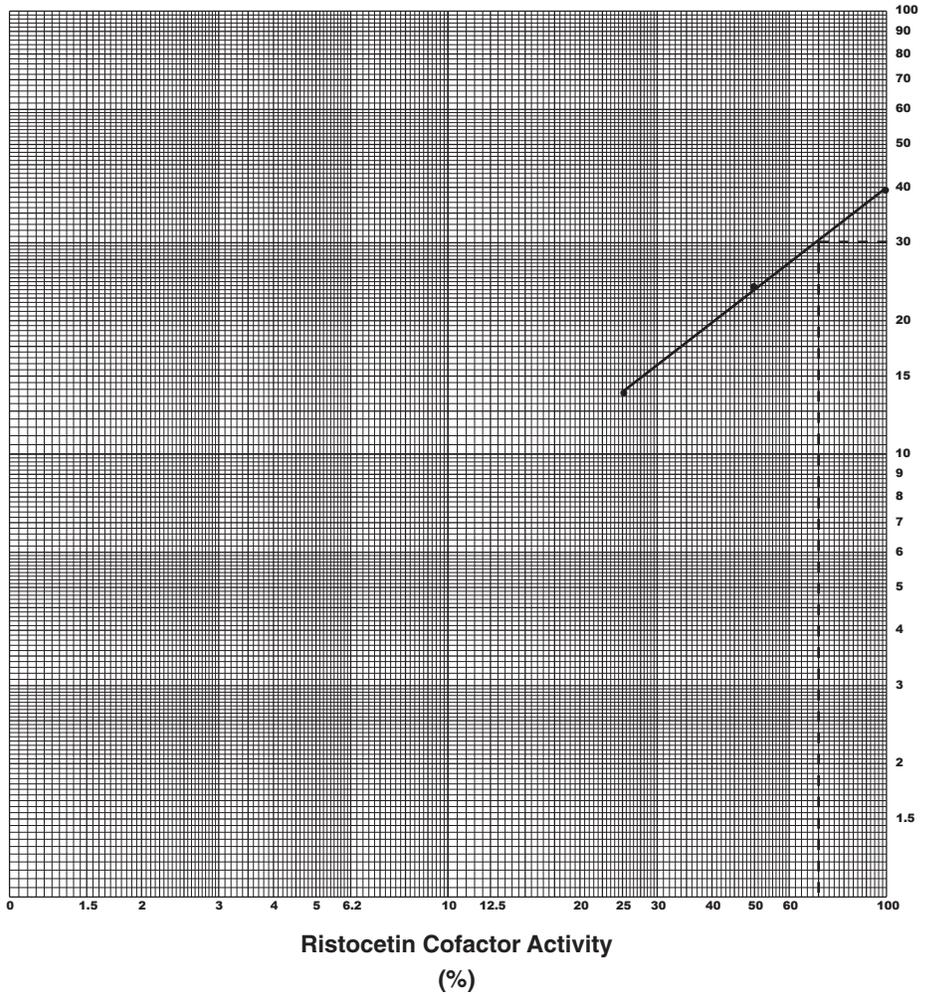
Normal	Slope
100%	37
50%	24
25%	13

**Test Sample:**

Slope: 30

Ristocetin Cofactor Activity: 70%  
(Interpolated)

S  
L  
O  
P  
E



## REPORT FORM

**Standard Curve**

	Slope
100%	_____
50%	_____
25%	_____

**Sample ID**

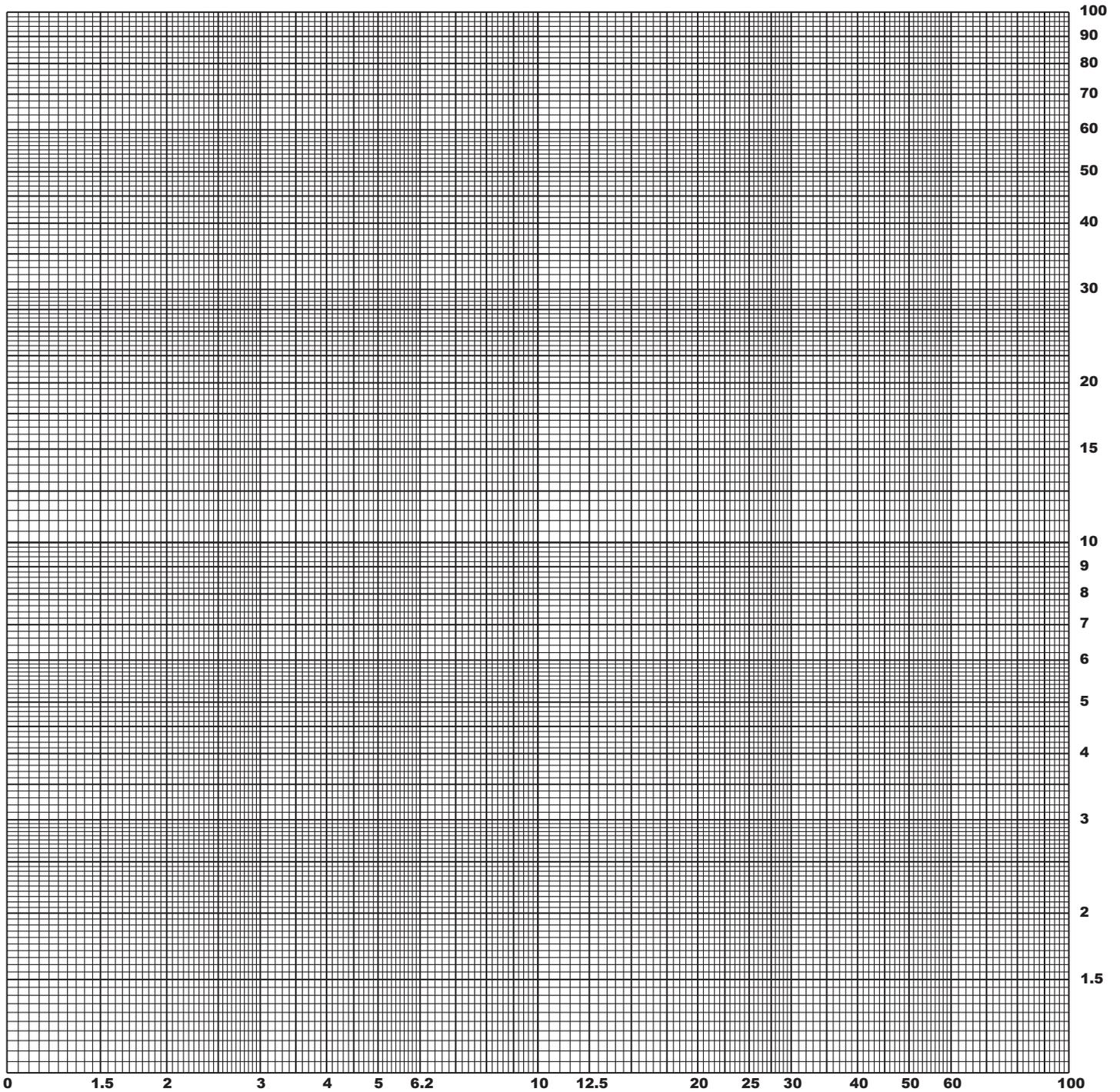
	Slope	Activity
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____

Technologist: \_\_\_\_\_

Date: \_\_\_\_\_

# CHRONO-LOG RISTOCETIN COFACTOR ASSAY

(See reverse for sample calculations)



RISTOCETIN COFACTOR ACTIVITY  
(%)