

Ristocetin Cofactor Activity/von Willebrand Factor Activity (VWF:RCo or VWF:Act)

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INTRODUCTION

Ristocetin cofactor measurement is essential for the diagnosis of von Willebrand disease (VWD). While ristocetin-induced platelet aggregation (in platelet-rich plasma) can be carried out when platelet aggregation studies are being done, the test is not sufficiently sensitive, and impaired aggregation may be encountered in disorders other than VWD. The VWF:RCo assay is particularly useful in detection of type 2A, 2B, and 2M VWD, where the VWF:Ag may be normal or near normal whereas the VWF:RCo is markedly reduced.

RISTOCETIN COFACTOR ASSAY

The method described here combines a macroscopic assay method and the platelet fixation technique of Evans and Austen.

REAGENTS

- Reference plasma
- Fixed washed platelets
Formaldehyde-fixed normal human platelets are prepared from platelet concentrates such as those used for treatment of patients with platelet disorders, by the method of Evans and Austen (1977). See "Fixed platelet preparation", on page 85.
- Ristocetin (Ristocetin A SO₄ Macrofarm Ltd., Third Floor, 27 Cockspur Street, Trafalgar Square, London SW1Y 5BN)
100 mg powder is diluted in 3.3 ml saline and dispensed into 0.1 ml amounts in capped plastic tubes and stored at -70°C. This stock solution therefore has a concentration of 30 mg/ml. The final concentration required in the assay tube is 1.0 mg/ml. Since there is a 1 in 4 dilution in the assay, this requires a solution of 4.0 mg/ml. For 4.0 mg/ml, add 0.65 ml saline to 0.1 ml of the 30 mg/ml stock solution. (This should give a blank time, i.e. 0.2 platelets + 0.1 ml ristocetin + 0.1 buffer of > 60 seconds).
- 6 g% albumin citrate-saline buffer
Use citrate/saline (one part 0.11M trisodium citrate:5 parts normal saline) with 1.2 g of bovine serum albumen added.

METHOD

- 1 Using albumin citrate-saline buffer, dilute the normal and test plasmas as follows:
 - Standard plasma: 1/2, 1/4, 1/8
 - Test plasma: 1/2, 1/4, 1/8
- 2 Test each dilution at room temperature as follows:
 - i. In a glass clotting tube, place:
 - 0.2 ml fixed washed platelets (at a count of $800 \times 10^9/1$ in platelet suspending solution)
 - 0.1 ml diluted plasma
 - ii. Mix well, avoiding formation of bubbles as much as possible. Add 0.1 ml ristocetin (4.0 mg/ml, giving a final concentration of 1.0 mg/ml).
 - iii. Start stopwatch. Tilt the tube to and fro briskly over a dark background with a lamp shining on the tube.
 - iv. Record the time taken for large visible aggregate to form.

Test each dilution in duplicate, and in triplicate if the difference between the duplicates is >10%.

CALCULATION

Plot the time taken for agglutination of the normal plasma dilutions against dilution/concentration on 2-cycle log-log paper. Plot the times obtained with the dilutions of test plasma. The graphs should be parallel straight lines.

The concentration of ristocetin cofactor in the test plasma is read from the standard and corrected for dilution and value of the standard in a similar way to that described in the section describing one-stage FVIII assays (Section 23).

The normal range should be established locally, but it is typically close to 50–150 IU/dl.


29.1 Fixed Platelet Preparation

Figure 29.1. Reagents for fixed platelet preparation

0.2% EDTA solution	2 g disodium-EDTA, 8.5 g NaCl, distilled water to 1 litre, pH6.4
Fixing solution	20 ml × 40% formaldehyde solution (or 22.2 ml 36% formaldehyde). 0.2 g disodium-EDTA, 8.5 g NaCl, 0.4 disodium hydrogen phosphate, 1.1 g sodium dihydrogen phosphate dihydrate, distilled water to 1 litre, pH 6.4.
Washing solution	1 part trisodium citrate solution (3.8%) 5 parts saline solution, pH 6.4
Suspending solution	As washing solution, but pH 7.4
Storage solution	0.2 g disodium-EDTA, 8.5 g NaCl, 0.10g sodium azide, distilled water to 1 litre, pH 6.4.

METHOD

- 1 Obtain platelets as fresh as possible in citrate phosphate dextrose (as used for blood product collection) or take blood into 0.109M citrate and prepare platelet-rich plasma (PRP).
- 2 Leave PRP in a capped plastic container at room temperature for one hour, then at 37°C for one hour. Mix nine parts of PRP with one part of EDTA solution. Let stand for two minutes at room temperature.
- 3 Add an equal volume of fixing solution at 4°C and leave at 4°C overnight.
- 4 Centrifuge at 280 g for 20 minutes. Discard supernatant and drain platelet pellet.
- 5 Add 2% of the starting PRP volume of washing solution and resuspend the platelets.
- 6 Further dilute to 25% of the starting PRP volume and leave at 4°C for one hour.
- 7 Centrifuge at 280 g for 20 minutes, drain, and resuspend in suspending solution for immediate use or in storage solution for storage at 4°C and usage later.
- 8 Resuspend to a concentration of approximately $800 \times 10^9/l$.



The platelets will settle on storage to form a loose sediment. Before commencing an assay, remove the storage solution from above the platelets and replace with an equal volume of suspending solution. Platelets are then stable for at least two months.

REFERENCE

Evans RJ, Austen DE. Assay of ristocetin cofactor using fixed platelets and a platelet counting technique. *Brit J Haemato* 1997; 37: 289-94.