RISTOCETIN 25 mg

Kit for evaluation of platelet aggregation on whole blood and on PRP, induced by Ristocetin

I. INTENDED USE
Ristocetin is for use in the platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation like von Willebrand Syndrome, the quantitation of von Willebrand factor and the identification of Bernard-Soulier Syndrome.

II. PRINCIPLE
The ristocetin, a concentration of 1.0-1.5 mg/ml, normal platelet aggregates in rich plasma or citrated whole blood through a mechanism in which the release of endogenous ADP plays only a small role. Ristocetin-induced platelet aggregation at concentrations of 1.2 mg/ml is absent or significantly lower in patients with von Willebrand’s syndrome. At concentrations of 1.5 mg/ml there is a lower degree of abnormality. The majority of patients with von Willebrand’s syndrome shows a negative response, as well as patients with Bernard-Soulier syndrome.

Aggregation study in whole blood is based on the evaluation of the electric resistance changes. Two electrodes while immersed in the sample are rapidly covered with platelet masses at the very first contact, appear in shape of monolayer. When the aggregating agent is added, additional platelets tend to attach the monolayer previously formed, determining an impedance increase between the two electrodes.

III. REAGENTS AND MATERIALS
Each kit contains:
1. Ristocetin: Ristocetin A sulphate lyophilised form. Antibiotic isolated from Nocardia lurida, containing in excess of 90% Ristocetin A.
2. Diluent A: dilution buffer containing TRIS, pH 7.3.

MATERIAL REQUIRED BUT NOT SUPPLIED
- Blood collection tubes, centrifuge tubes, tubes and pipettes for drawing up the blood and the PRP, all in siliconized glass or plastic. Trisodium citrate 3.8%. Cuvettes ad stirrers for aggregometer.
- Aggregometer.

IV. STORAGE
Store diluent and ristocetin tightly closed in refrigerator (2-8°C). The kit is stable until expiration date printed on the package label.

V. SAMPLE COLLECTION
Collect the sample from an antecubital vein without stasis using siliconized needle 18 or 20 G. Immediately transfer blood into a plastic tube containing the anticoagulant (trisodium citrate 3.8% in volume ratio of 1:9). The blood can be used as it is for platelet aggregation within 4 hours.

To obtain PRP centrifuge the blood at 160 g for 10 minutes, carefully draw off the supernatant (PRP); centrifuging time and speed depend on the kind of sample; inspect visually the supernatant. In case of red cells presence, centrifuge again. Collect the supernatant (PRP) with the plastic pipette and store in a plastic test tube, identified with the proper label, until the analysis. Dilute PRP with PPP to obtain a plasma with about 300,000 platelets/mm³. Maintain the PRP at room temperature and carry out the test within 4 hours.

VI. PRP TEST PROCEDURE

Before use, reconstitute a vial of Ristocetin with 0.5 ml of Diluent A. Concentration of work solution: 50 mg/ml. This solution is stable one month at -20°C. To avoid repeated thawing and freezing it is advised to subdivide the solution into aliquots of 0.1 ml and freeze. With the work scheme proposed here, the reagent is sufficient to carryout 33 aggregation test/vial.

1. Prepare PRP and PPP as described in section V.
2. Add 500 µl (250 µl) of PRP to an aggregation cuvette containing stirring bar and incubate at 37°C for 3 minutes.
3. Add 500 µl (250 µl) of PPP to an aggregation cuvette without stirrer.
4. Place PRP and PPP cuvettes in corresponding instrument sample wells and follow manufacturer’s instruction for setting base lines.
5. Add 15 µl (7.5 µl) reconstituted ristocetin to PRP cuvette to obtain a final concentration of 1.5 mg/ml.
6. Record platelet aggregation response for a minimum of 5 minutes.

The figures in parentheses are half volumes that a lot of aggregometers can now handle; using the proper rubber adhesive spacers.

If an aggregation is not obtained or is markedly reduced, (von Willebrand’s disease and Bernard-Soulier’s syndrome) repeat the test, in order to have a confirmation of the diagnosis, operating as follows:

To 0.4 ml of PRP add 0.1 ml of normal pooled plasma;
add 15 µl of Ristocetin to obtain a final concentration of 1.5 mg/ml;
If an increase in aggregation is recorded, the diagnosis of von Willebrand’s disease is confirmed.

VII. WHOLE BLOOD PROCEDURE
Ristocetin induces optimal platelet aggregation on whole blood at the concentration of 1 mg/ml.

1. Add 500 µl of saline solution and 500 µl of whole blood with anticoagulant in a 1 ml plastic cuvette containing stirring bar and incubate at 37°C for 5 minutes.
2. After connecting the electrode to the socket, put incubated at 37°C for 5 minutes.
3. After incubation, place it in the vial containing the diluted blood. (Place the filaments to the back of aggregometer).
4. Place the cuvette into the reaction well and incubate 2 minutes, holding the door closed. Open the door and pipette 20 µl of ristocetin reconstituted (Warning: avoid formation of air bubbles).
5. Record platelet aggregation.

VIII. INTERPRETING THE RESULTS

As the normal absolutes values are not available yet, for whole blood aggregation, it is recommended for each laboratory to establish their own normal ranges in order to compare with aggregation curves taken from pathological subjects.

NOTE: The following Normal Ranges were obtained from various laboratories and publications. They should be used as a guideline only.

<table>
<thead>
<tr>
<th>Normal values</th>
<th>Ristocetin in PRP - Concentration 1.5 mg/ml: % max aggregation 82 - 96%, Concentration 1.0 mg/ml: % max aggregation (ohm) &gt; 5.0 &lt;70 sec Lag time</th>
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</table>

IX. LIMITATION OF THE TEST
Carry out the test in subjects on an empty stomach, 8 hours no smoking, not assuming any medical remedies containing Acetylsalicylic Acid for one week or other drugs interfering the platelet aggregation.

X. PERFORMANCES

This product will perform as described prior to its expiration date when procedural and storage directions are followed.

Linearity, accuracy, precision.

Platelet aggregation induced by common agonist (like Ristocetin) is a nonlinear test system for some parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by parameters such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reagents or their concentration. In platelet aggregation, accuracy is a relative parameter and is dependent on the test system.

The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges.

XI. NOTE
To test at the same time optical test on PRP and the release of ATP with bioluminescent technique should work on a lumic-aggregometer. (Example 700-2). Refer to the Technical Manual and the instructions in User Manual of instrument.

XII. REFERENCES
Refer to the Technical Manual 3115xxx BE-07/11

<table>
<thead>
<tr>
<th>CONTENT</th>
<th>REF. 311500D</th>
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<tbody>
<tr>
<td>Ristocetin</td>
<td>6 x 0.5 ml</td>
<td>2 x 0.5 ml</td>
</tr>
<tr>
<td>Diluent A</td>
<td>1 x 5.0 ml</td>
<td>1 x 5.0 ml</td>
</tr>
<tr>
<td>Instruction for use</td>
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