STA® - STACLOT® APC-R

Assessment of Activated Protein C Resistance

- Kit Containing:

– 4 x 2-ml Vials of Reagent 1 (F. V Deficient Plasma)
– 4 x 2-ml Vials of Reagent 2 (Venom)
– 4 x 2-ml Vials of Reagent 3 (PCa)
– 4 x 1-ml Vials of Reagent 4 (Control N)
– 4 x 1-ml Vials of Reagent 5 (Control P)

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1/ INTENDED USE

The **STA® - Staclot® APC-R** kit is intended for use with analyzers of the STA® line suitable to these reagents for the assessment of the activated protein C resistance (APC-R) in plasma.

2/ SUMMARY AND EXPLANATION

As early as 1990, L. Amer suggested in a patient with thromboses the presence of a plasma constituent which inhibited the activated protein C (PCa) anticoagulant activity (1). Then, a mechanism characterized by a resistance to the activated protein C action was described by B. Dahlbäck (2, 3). In 1994, R.M. Bertina found that this anomaly was associated with a mutation located in exon 10 of the factor V gene (4). This mutation leads to the synthesis of the factor V Leiden in which arginine 506 is replaced by glutamine. This position corresponds to the first cleavage site of factor Va by PCa (8). It is more difficult for PCa to inactivate factor Va Leiden than normal factor Va (5). Other mutations responsible for activated protein C resistance have been reported (10, 12).
The factor V Leiden mutation is autosomal dominant (6) and it is frequently found in Caucasians (7). This anomaly is associated with a higher risk of thromboses, especially when the co-existence of other risk factors is present in the carrier (9).

3/ TEST PRINCIPLE

The principle of the assessment of activated protein C resistance (APC-R) is based on an unusually small prolongation of the clotting time of the tested plasma in the presence of PCa and in calcified medium. In the STA® - Staclot® APC-R system, coagulation of the diluted test plasma is achieved in the presence of factor V deficient plasma and of *Crotalus viridis helleri* venom. This venom acts as an activator of factor X and therefore triggers the coagulation cascade downstream from factor X, thus eliminating the influence of all coagulation factors acting upstream.
The prolongation of the clotting time of a normal plasma in the presence of PCa results from the capacity of the PCa derived from Reagent 3, to inactivate the factor Va of the tested plasma.

4/ KIT REAGENTS

A bar-code insert with three bar-codes, one for the Reagents 1, 2 and 3 and one for each control (Reagents 4 and 5), is provided in the box. Each bar-code contains the following information: lot number, kit code number, reagent code number(s), expiration date and for Reagents 4 and 5 clotting time values determined with analyzers of the STA® line for the relevant lot.

- **Reagent 1**: human plasma immuno-depleted in factor V and enriched with phospholipids, freeze-dried.
- **Reagent 2**: freeze-dried preparation containing venom from *Crotalus viridis helleri*.
- **Reagent 3**: vial containing human activated protein C in calcium medium, freeze-dried.
- **Reagent 4**: freeze-dried citrated normal human plasma, used as a negative control.
- **Reagent 5**: freeze-dried citrated human plasma, used as a positive control.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
Some reagents provided in this kit contain materials of human and/or animal origin. Whenever human plasma is required for the preparation of these reagents approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with regulatory safety precautions in the manipulation of these biological materials as if they were infectious.

5/ CAUTION

For *in vitro* diagnostic use only. Store at 2-8 °C. These reagents are to be used by certified medical laboratory personnel only. The disposal of waste materials must be carried out according to current local regulations.
The STA® - Staclot® APC-R kit is designed for use with analyzers of the STA® line suitable to these reagents. Read carefully the Operator's Manual of the analyzer model before starting. Exercise great care in the handling of these reagents and of patient samples; refer to the "Warnings" chapter of the Operator's Manual.

6/ SPECIMEN COLLECTION AND TREATMENT

- Collect blood (9 vol.) in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant solution (1 vol.).
- Centrifuge blood samples at 2,500 g for 15 minutes.
  Double centrifugation at 4 °C is recommended in order to remove platelets. Collect the plasma as soon as possible.
- Plasma samples are stable for: 8 hours at 20 ± 5 °C
  6 months at **–80 °C** (plasmas must be frozen within two hours after sample collection).
  Frozen plasmas must be thawed directly in a 37 °C water-bath for at least 15 minutes and thoroughly mixed by swirling before use.
  Plasmas can be frozen only once. **Do not re-freeze plasmas.**

7/ REAGENT PREPARATION AND STORAGE

The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.

- **Reagents 1, 2 and 3**
  Reconstitute each vial with 2 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Then, swirl the vial gently to mix before use.
  Once reconstituted, it remains stable for 8 hours on STA®, STA Compact® and STA-R®.
- **Reagents 4 and 5**
  Reconstitute each vial with 1 ml of distilled water. **Shake vigorously**. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Then, swirl the vial gently to mix before use.
  Once reconstituted, it remains stable for 8 hours on STA®, STA Compact® and STA-R®.

8/ REAGENT AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - Owren-Koller** (REF 00360).
- Analyzer of the STA® line suitable to these reagents.
- Common clinical laboratory equipment and materials (centrifuge, distilled water...).

9/ PROCEDURE

Refer to the appropiate chapters of the Operator's Manual, particularly those for loading and quality control.

9.1. Patients' Plasmas

Patients' plasmas are tested undiluted. They are loaded in the instrument (see the Operator's Manual of the analyzer model). Dilution with Owren-Koller buffer is automatically carried out by the instrument. Then select the test(s) to be performed.

9.2. Quality Control

The Reagents 4 and 5 (**these are lot-specific**) are necessary to ensure accuracy and reproducibility of the results. Prepare these controls and load them in the instrument according to the recommendations of the Operator's Manual of the analyzer model. The vial position in the instrument is the following:

– on STA® model, place the control vials in one of the positions 8, 9 or 10 in one of the two sample drawers
– on STA Compact® model, place the control vials in one of the positions 1 to 18 or 35 to 38 of the product drawer
– on STA-R® model, place the control vials in the R0 area of the product drawer.

These controls are used undiluted; the dilution with Owren-Koller is automatically prepared by the analyzer.

9.3. Test

Refer to the procedure booklet of the instrument for full details on how to proceed from this point.

The assessment of the APC-R of the plasmas to be tested starts automatically as soon as sample loading is completed.

10/ RESULTS

The clotting time (in seconds) of the plasmas being tested is displayed in the "Test Panel/Test Status" screen of the instrument (see Operator's Manual).

Ensure that the values obtained for the controls are within the ranges stated in the Assay Value insert provided in the box. If the control values are outside the stated ranges, check all components of the test system to ensure that all are functioning correctly, i.e., assay conditions, reagents, integrity of the plasmas being tested, etc. If necessary, repeat the tests.

11/ LIMITATIONS

- **It is essential** that the maintenance procedures of the analyzer as described in the Operator's Manual are carried out properly.
- Do not use suspect samples (presence of mircro clots, hemolyzed samples, etc.).
- A low level (< 50 %) of factor V in the plasma may lead to a false negative result (clotting time ≥ 120 seconds). However, a result found positive (clotting time < 120 seconds) even in presence of factor V below 50 % is still valid.
- The STA® - Staclot® APC-R procedure is insensitive to heparin (unfractionated and low molecular weight heparins) levels up to 1 IU/ml.
- Thrombin inhibitors (e.g., hirudin, argatroban...) present in the sample to be tested may lead to a false negative result for this sample.
- The presence of aprotinin in the plasma to be tested may lead to a false positive result.

12/ EXPECTED VALUES

Plasmas whose clotting times obtained with the STA® - Staclot® APC-R procedure, as measured by instruments of the STA® line suitable to these reagents, are equal to or greater than 120 seconds are considered to be APC-R negative. On the other hand, plasmas whose clotting times are less than 120 seconds are considered APC-R positive.

13/ PERFORMANCE CHARACTERISTICS

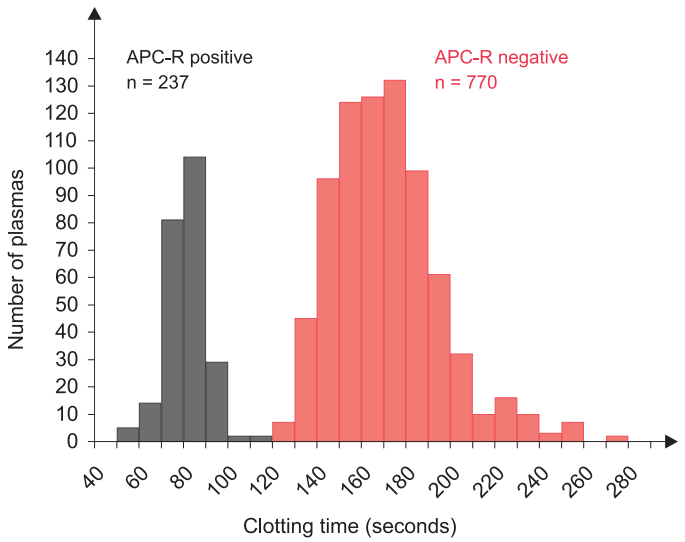
- Result discrepancies beetween genetic tests and activity tests may be observed in certain transplant patients (11).
- Plasmas whose factor V Leiden presence were documented by molecular biology and whose characteristics might interfere in the test were assayed with the STA® - Staclot® APC-R procedure. Results obtained were the following:
  – plasmas deficient in protein S (protein S level beetween 23 % and 55 %, n = 27 of which 7 were APC-R positive)
  – plasmas with lupus anticoagulants (n = 20 of which 2 were APC-R positive)
  – plasmas from patients receiving heparin therapy (UFH or LMWH, n = 47 of which 4 were APC-R positive)
  – plasmas from patients receiving oral anticoagulants (n = 53 of which 12 were APC-R positive).

  In all these cases no interference in the test was observed.
- One normal and one abnormal samples were used in intra-assay (n = 21) and inter-assay (n = 10) reproducibility studies with the STA® - Staclot® APC-R kit. In all cases, the normal sample has been found negative and the APC-R positive sample gave positive results.

14/ COMPARISON WITH MOLECULAR BIOLOGY

1,007 plasmas that had been characterized by molecular biology for the factor V Leiden (of which 236 were APC-R positive by molecular biology) were tested with the STA® - Staclot® APC-R procedure. Compared with molecular biology, the STA® - Staclot® APC-R procedure demonstrated the following performance characteristics:
– sensitivity: 99.6 %
– specificity: 99.7 %
– positive predictive value: 99.2 %
– negative predictive value: 99.9 %.

As an illustration, the distribution of the clotting times measured with the STA® - Staclot® APC-R reagents for these 1,007 plasmas is indicated in the diagram below:



REFERENCES

1. AMER L., KISIEL W., SEARLEST R.P., WILLIAMS Jr. R.C.:
"Impairment of the protein C anticoagulant pathway in a patient with systemic lupus erythematosus, anticardiolipin antibodies and thrombosis". Thromb. Res., **57**, 247-258, 1990.
2. DAHLBACK B., CARLSSON M.:
"Factor VIII defect associated with familial thrombophilia". Thromb. Haemostasis, **65**, 6, 658, abstract 39, 1991.
3. DAHLBACK B., CARLSSON M., SVENSSON P.J.:
"Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C". Proc. Natl. Acad. Sci. USA., **90**, 1004-1008, 1993.
4. BERTINA R.M., KOELEMAN B.P.C., KOSTER T., ROSENDAAL F.R., DIRVEN R.J., DERONDE H., VAN DER VELDEN P.A., REITSMA P.H.:
"Mutation in blood coagulation factor V associated with resistance to activated protein C". Nature, **369**, 64-67, 1994.
5. KALAFATIS M., RAND M.D., MANN K.G.:
"The mechanism of inactivation of human factor V and human factor Va by activated protein C". J. Biol. Chem., **269**, 50, 31869-31880, 1994.
6. SVENSSON P.J., DAHLBACK B.:
"Resistance to activated protein C as a basis for venous thrombosis". N. Engl. J. Med., **330**, 8, 517-522, 1994.
7. REES D.C., COX M., CLEGG J.B.:
"World distribution of factor V Leiden". Lancet, **346**, 1133-1134, 1995.
8. TROSSAERT M., CONARD J., HORELLOU M.H., ELALAMY I., SAMAMA M.M.:
"Le diagnostic biologique de la résistance à la protéine C activée". Rev. Fr. Lab., **279**, 19-23, 1995.
9. ZOLLER B., HILLARP A., DAHLBACK B.:
"Activated protein C resistance: clinical implications". Clin. Appl. Thromb. Haemost., **3**, 1, 25-32, 1997.
10. CHAN W.P., LEE C.K., KWONG Y.L., LAM C.K., LIANG R.:
"A novel mutation of Arg306 of factor V gene in Hong Kong Chinese". Blood, **91**, 4, 1135-1139, 1998.
11. CROOKSTON K.P., HENDERSON R., CHANDLER W.L.:
"False negative factor V Leiden assay following allogeneic stem cell transplant". Br. J. Haematology, **100**, 600-602, 1998.
12. WILLIAMSON D., BROWN K., LUDDINGTON R., BAGLIN C., BAGLIN T.:
"Factor V Cambridge: a new mutation ($Arg^{306} \rightarrow Thr$) associated with resistance to activated protein C". Blood, **91**, 4, 1140-1144, 1998.

DIAGNOSTICA STAGO
9, rue des Frères Chausson
92600 ASNIERES (FRANCE)
Tel: 33 1 46 88 20 20
e-mail: stago@stago.fr

English