



APTT_{Test} *ellágico*

Reagents for the determination of Activated Partial Thromboplastin Time

SUMMARY

The Activated Partial Thromboplastin Time (APTT) is a test sensitive to the deficiency of the plasma coagulation promoting factors, as well as to the presence of certain coagulation inhibitors.

It detects disorders in the coagulation intrinsic pathway, such as the necessary factors for the formation of the intrinsic prothrombin activator - i.e. factors VIII, IX, XI and XII.

It also detects serious deficiencies in the factors II, V, X, and fibrinogen, but not the platelet disorders, the deficiencies in the factors VII and XIII nor vascular problems. The test's speed, simplicity and reproducibility make it adequate for monitoring heparin anticoagulant therapy. It also allows the quick identification of potential hemophiliacs so that they can be subjected to pre-surgical preventive treatment to avoid bleeding problems.

PRINCIPLE

The assay is based on measuring the coagulation time of a decalcified plasma placed on a water bath at 37°C and in the presence of an excess of cephalin, activator and calcium.

PROVIDED REAGENTS

A. Reagent A: vials containing cephalin with ellagic acid as a soluble activator.

B. Reagent B: 0.025 mol/l stable calcium chloride solution.

INSTRUCTIONS FOR USE

Reagent A: ready to use. Homogenize before use.

Reagent B: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze.

SAMPLE

Plasma

a) Collection: obtain blood carefully (avoiding stasis or trauma) and place into a tube with anticoagulant in an exact 9+1 proportion (e.g.: 4.5 ml blood + 0.5 ml Wiener lab.'s **Anticoagulante TP**). Mix gently. Centrifuge and separate

the plasma before 30 minutes. It is recommended to use plastic syringes for withdrawal.

b) Additives: use Wiener lab.'s **Anticoagulante TP** or 130 mmol/l (3,8%) or 109 mmol/l (3,2%) sodium citrate to obtain plasma.

c) Known interfering substances:

- Contaminations whether visible or not lead to falsely prolonged times.

- Do not use EDTA or heparin to obtain plasma.

- Visible hemolysis makes difficult the photo-optic measurement of the results.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: the plasma should be kept in the refrigerator (2-10°C) until the test is performed. This period should not be extended for more than 4 hours. If the assay cannot be performed within this period, freeze the plasma at -20°C. It should be quickly frozen and thawed (immersing the sample tube in a 37°C water bath) just before testing.

Kept the sample in plastic tubes until assayed to minimize the contact activation that may occur with glass tubes.

REQUIRED MATERIAL (non-provided)

- Hemolysis tubes

- Pipettes and micropipettes for measuring the stated volumes

- Water bath at 37°C

- Stopwatch

- Light source, for clot observation

PROCEDURE

Warm the Reagent B in water bath at 37°C before performing the test. In a hemolysis tube place:

Sample (unknown plasma or control)	100 ul
---	--------

Reagent A	100 ul
------------------	--------

Mix and incubate 3 minutes at 37°C. Then add:

Reagent B (at 37°C)	100 ul
----------------------------	--------

Start stopwatch simultaneously. Shake briefly to homogenize content and keep in water bath for 25 seconds. Then remove the tube from the water bath, tilt gently once every second and stop the stopwatch when a clot is formed. Automatic or semi-automatic instruments, capable of detecting fibrin clot formation by photo-optic or mechanic methods, can be used to read the results. Record the coagulation time.

INTERPRETATION OF RESULTS

The results may be expressed as:

- 1) The Activated Partial Thromboplastin Time in seconds
- 2) The ratio between the time obtained with the unknown and the time obtained with a control plasma

QUALITY CONTROL METHOD

Wiener lab.'s Plasma Control normal - patológico.

REFERENCE VALUES

The reference value range observed in normal individuals, with the mentioned manual technique, vary between 30-43 seconds.

Values which differ in more than 6 seconds with a normal control plasma are not considered within the normal range. It is recommended that each laboratory processes a pool of normal plasma with each lot of reagents used and to correlate the patients' values with that of the control plasma, recording the results in the report.

Each laboratory should establish its own reference values based on the techniques and devices used, as the APTT values of healthy individuals vary with each laboratory depending on the technique used.

CALIBRATION CURVE

This method is useful to control the patients' response to heparin when treated with this anticoagulant.

The technique used is the following:

Prepare heparin Working Solution in saline solution with a concentration of 10 units/ml. Use the same heparin administered to the patient. Prepare dilutions of this Working Solution using a pool of fresh normal plasmas or Wiener lab.'s Plasma Control normal as diluent. Dilutions of 0.8; 0.6; 0.4; 0.2 and 0.1 units/ml, should be obtained.

Determine the partial thromboplastin time for each of these solutions as well as for the plasma pool. Plot APTT vs. Heparin concentration on semilog graph paper. The value obtained for the patient should be correlated with the values on the graph to obtain current concentration of circulating heparin.

PROCEDURE LIMITATIONS

See Known interfering substances and Stability and storage instructions under SAMPLE.

The coagulation process involves a series of enzymatic reactions, which might be influenced by any condition affecting enzymatic systems in general, that is why methodological cautions should be taken.

Consider that variations in the ratio anticoagulant/sample or in the citrate concentration used, affect activated partial thromboplastin times, thus, it is recommended to check the anticoagulant dose used for sample collection.

PERFORMANCE

Reproducibility: precision studies were performed following a modification of the guidelines contained in NCCLS (National Committee on Clinical Laboratory Standards) EP5-A document. These results were obtained:

Within Run

Sample	Mean	S.D.	C.V.
Plasma Control normal	34.1 sec	± 0.49 sec	1.44%
Plasma Control patológico	87.9 sec	± 0.49 sec	0.56%
Normal plasma pool	29.3 sec	± 0.35 sec	1.18%

Total Run

Sample	Mean	S.D.	C.V.
Plasma Control normal	34.1 sec	± 1.00 sec	2.93%
Plasma Control patológico	87.9 sec	± 3.66 sec	4.16%
Normal plasma pool	29.3 sec	± 0.51 sec	1.75%

WIENER LAB PROVIDES

- 150 tests (6 x 2.5 ml) (Cat. N° 1705004).

REFERENCES

- Bell, W.N.; Alton, H.G. - Nature 174:880 (1954).
- Dacie, J.B.; Lewis, S.M. - Hematología Práctica - Ediciones Toray, 2ª Edición (1970).
- Wintrobe, M.M. - Hematología Clínica, 3a. Edición Inter-médica (1969).
- Bragos, I.; Rodríguez Pécora, S.; Lorenzo, L.; Capriotti, G. - 53° Triduo Bioquímico Científico Anual, Bahía Blanca. (1988).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 3rd ed., 1990.

Symbols

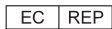
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Do not freeze



Calibrator



Biological risks



Control



Volume after reconstitution



Positive Control



Contents




Negative Control



Batch code



Catalog number

 Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
<http://www.wiener-lab.com.ar>
Dir. Téc.: Viviana E. Cétola
Bioquímica
A.N.M.A.T. Registered product
Cert. N°: 1225/95



Wiener lab.

2000 Rosario - Argentina