

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

APTT Reagent: vials containing cephalin with diatomaceous earth as a particulate activator.

Calcium Chloride: 0.025 mol/l calcium chloride solution.

NON-PROVIDED REAGENTS

Bidistilled or deionized water.

INSTRUCTIONS FOR USE

Calcium Chloride: ready to use.

Preparation of APTT Reagent:

- Remove the metal seal and open one vial. Take the rubber stopper out carefully to prevent any loss of the contents.
- Add the volume of bidistilled or deionized water indicated on the bottle label.
- Check that the water temperature; does not exceed 37°C.
- Cap and gently shake until a homogeneous suspension is obtained. Homogenize every time before using.

WARNINGS

Reagents are for "in vitro" diagnostic use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10°C) until the expiration date shown on box.

APTT Reagent: once reconstituted it is stable 14 days in refrigerator (2-10°C) or 30 days frozen (-20°C).

Freezing and thawing should only be performed once. Therefore, fractioning is recommended, according to work requirements.

SAMPLE

Plasma

a) Collection: obtain blood carefully using plastic syringes (avoid 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 anticoagulant. Mix gently. Centrifuge and separate plasma before 30 minutes.

b) Additives: use 130 mmol/l 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.

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

Incubate the Calcium Chloride reagent in a water bath at 37°C before performing the test.

In a hemolysis tube place:

Sample (unknown plasma or control)	100 ul
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APTT Reagent (homogenized)	100 ul
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Mix and incubate for 3 minutes at 37°C. Then add:

Calcium Chloride (at 37°C)	100 ul
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Start the stopwatch at the same time. Shake briefly to ho-

mogenize contents. Keep in water bath about 25 seconds. Then remove tube from the water bath; tilt gently once every second and stop the stopwatch when a clot is formed. Record the coagulation time.

Level	S.D.	C.V.
45 seconds	± 1.1	2.5%
62 seconds	± 1.8	3.0%

KIT SIZE

Kits for 150 tests (6 x 2.5 ml) (Cat. 41003).

REFERENCES

- Bell, W.N.; Alton, H. G. - Nature 174:880 (1954).
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- Wintrobe, M. M. - Hematología Clínica, 3º Edición, Inter-médica (1969).
- Bragos, I.; Rodríguez Pécora, S.: Lorenzo, L.; Capriotti, G. - "Evaluación de un nuevo reactivo de Tiempo Parcial de Tromboplastina Activada" - 53º Triduo Bioquímico Científico Anual; Bahía Blanca (1988).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

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

Control Plasma normal - pathologic.

REFERENCE VALUES

The interval of values observed in normal individuals ranges between 33-48 seconds.

The 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.

CALIBRATION CURVE

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

The technique used is the following:

- 1) Prepare a heparin Working Solution in saline solution (concentration: 10 Units/ml). Use the same heparin administered to the patient.
- 2) Dilute this Working Solution using a pool of fresh normal plasma as diluent; 0.8; 0.6; 0.4; 0.2 and 0.1 Units/ml dilutions should be obtained in this way.
- 3) 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

a) Reproducibility: when replicates of the same samples were assayed on the same day the following results were obtained:



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