APTT
Ellagic acid

Quantitative determination of Activated Partial Thromboplastin test

IVD

PACKAGING

Ref. 104-0020	Cont.: 5 x 4 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Activated partial thromboplastin time (APTT) test is one of the most common coagulation tests used in any coagulation laboratory. This test is used for diverse purposes of preoperative screening, screening for coagulation factor deficiency, screening for various types of coagulation inhibitors (e.g. lupus anticoagulants) and for controlling heparin therapy.

The APTT is a measure of the integrity of the intrinsic and final common pathways of the coagulation cascade. The APTT represents the time, in seconds, for patient plasma to clot after the addition of phospholipid, an intrinsic pathway activator (such as silica, celite, kaolin, ellagic acid), and calcium (to reverse the anticoagulant effect of the oxalate-citrate in the collection tube).

APTT reagent is called partial thromboplastin because tissue factor is not present in conjunction with the phospholipid as it is in the PT reagent. Thus, deficiencies or inhibitors of clotting factors within the intrinsic and final common pathways result in prolongation of the APTT.

PRINCIPLE OF THE METHOD

Citrated plasma, a contact activator, and procoagulant phospholipids (partial thromboplastin of animal origin) are mixed and incubated at 37ºC. The contact agent activates the contact system, including high molecular weight kininogen, prekalilkrein, Factor XI, and Factor XII. The phospholipid provides a surface for interaction of coagulation factors. After incubation, an appropriate concentration of calcium ions is added, and time to clot formation is measured. Calcium ions are required to assemble the complex for activation of the intrinsic coagulation cascade subsequent to Factor XIa.

REAGENTS

R 1 - Activator	Ellagic acid Buffers and preservatives
R 2 - Starter	Calcium chloride ($CaCl_2$) 0.02M

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

A yellow sediment may form after prolonged storage.

Do not use reagents over the expiration date. Do not freeze.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Quality control values outside established ranges.
- Product colour variations.

ADDITIONAL EQUIPMENT

- Coagulometer or stopwatch and bath at 37º C ± 0.5º C.
- General laboratory equipment[(Note 1)].

PREPARATION

All the reagents are ready to use.

R1: Stable for 1 month at 2-8º C after opening.

SAMPLES

Handle blood samples as potentially infectious. Label sample tubes correctly, respecting the patient's privacy, with unequivocal tracking to the test request form including full patient information. It is recommended that blood specimens be collected by venipuncture using a blood collection system that collects the specimen directly into glass or plastic evacuated tube containing sodium citrate as anticoagulant. Discard clotted samples and tubes that have been over or under filled: it is important that 9 parts of blood are mixed with 1 part of sodium citrate solution (0.11 mol/L).

NOTES

1. All labware must be clean and free of trace amounts of detergents.
2. Always follow instrument manufacturer's instructions; the results must be validated by the test laboratory.

PROCEDURE

The reagent can be used by manual method, mechanical, photo-optical or other means of clot detection[(Note 2)]. In case to be used in automatic analyzers, follow the analyzer's instructions.

Manual method

1. Incubate at 37º C the reagents and the sample:
2. Mix thoroughly the reagents.
3. Pipette into a clean and dry tube:

Citrated plasma (µL)	100
R 1 (µL)	100

4. Mix and incubate exactly for 5 min. at 37º C (activation time).
5. Pipette:

R 2 (µL)	100

6. Mix thoroughly.
7. On addition of R2 start stopwatch or timer on the coagulation analyzer and determine the coagulation time.

CALCULATIONS

It is possible to report the results as seconds or as APTT ratio, dividing the results of the sample (sec) by the results of plasma Control (sec).

$$APTT\ ratio = \frac{APTT\ of\ the\ patient\ in\ \text{sec}\ onds}{APTT\ of\ normal\ plasma\ (pool\ \%)\ in\ \text{sec}\ onds}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL NORMAL & PATHOLOGIC.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

A study has been run with 79 samples of healthy people, and as a result the following reference values have been established:

APTT (in seconds) 20 - 33 sec.

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Heparin Sensitivity:

Heparin conc. (units/mL)	APTT (seconds)
0.0	24.8
0.1	35.9
0.2	47.7
0.3	69.8
0.4	105.7
0.5	134.4

Factor Sensitivity:

FVIII		FIX		FXI		FXII	
%	APTT (s)	%	APTT (s)	%	APTT (s)	%	APTT (s)
88	25.5	105	25.27	89	25.6	109	25.43
30	30.37	75	36.2	50	33.67	85	31.67
20	33.3	50	45	30	38.83	75	33.8
15	35.2	30	50.17			65	36.37
						30	40.4

These values should only be used as guidelines. Each laboratory should establish his own sensitivity to individual factors.

INTERFERENCES

The effect of the most common interfering substances on the APTT results with Spinreact's reagent was tested by spiking different amounts of such substances into plasma samples. The results were compared against samples spiked with an equivalent volume of saline solution. There is no interference up to 200 mg/dL of hemoglobin, up to 500 mg/dL of lipids and up to 15 mg/dL of bilirubin.

A list of drugs and other interfering substances with the determination has been reported[4,5].

BIBLIOGRAPHY

1. Clinical and Laboratory Standards Institute (CLSI) H47: One-stage Prothrombin Time test and Activated partial Thromboplastin Time test; approved guideline.
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3. Clinical and Laboratory Standards Institute (CLSI) EP5-A: Evaluation of Precision Performance of Quantitative Measurement Methods; approved guideline.
4.Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
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