CHRONOLAB

CE

IVD

APTT
Ellagic acid

Quantitative determination of Activated Partial Thromboplastin test

PACKAGING

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

Store at 2-8ºC

CLINICAL SIGNIFICANCE

The time measurement of APTT is the most common coagulation procedure performed in routine laboratories, apart from the PT.
The APTT is particularly sensitive to defects of the intrinsic coagulation pathway (Factors VIII, IX, XI, XII).
It is commonly used for monitoring heparin anticoagulant therapy[1,2].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

When phospholipids complex and calcium chloride ($CaCl_2$) are added to citrated plasma, the factors of intrinsic coagulation system are activated; the time to formation of a fibrin clot is then measured[3,4].

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

Plasma from venous puncture diluted 1/10 in trisodium citrate solution 3.8 %.
Mixing immediately the blood with anticoagulant. Avoid foaming the specimen.
Centrifuge the sample at 2500 x g for 15 min and transfer the plasma into a siliconized glass or plastic containers.
Turbid, icteric, lipemic or hemolyzed samples may generate erroneous results.

The sample is stable for 2 hours at room temperature (15-25º C) or 28 days if immediately frozen at below -20º C.

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 BIOBAS1000 analyzer, 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

An exhaustive study has been run with 250 samples of healthy people, and as a result it has been established the following reference values:

APTT (in seconds) 24 - 36 sec.

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

PERFORMANCE CHARACTERISTICS

Heparin Sensitivity:

Heparin conc. (units/mL)	APTT (sec)
0.0	28.8
0.1	38.3
0.2	50.1
0.3	63.1
0.4	80.9
0.5	98.0

Factor Sensitivity:

Adequate sensitivity should demonstrate < 30-40% factor activity.

Factor	% actividad	APTT (sec)
VIII	<1%	82.0
VIII	20%	44.8
IX	<1%	83.5
IX	20%	40.9
XI	<1%	134.2
XI	20%	47.8
XII	<1%	>200
XII	20%	36.2
Prekallikrein	<1%	69.5

% Factor VIII	APTT (sec)
100%	32.5
70%	34.0
50%	36.9
40%	38.9
30%	40.8
20%	44.4
10%	50.6
5%	56.1
1%	68.1
<1%	83.6

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

INTERFERENCES

Do not use sodium oxalate, EDTA or heparin as anticoagulant.
Oral contraceptives, estrogens or pregnancy interfere in the assay.
A list of drugs and other interfering substances with the determination has been reported[5,6].

BIBLIOGRAPHY

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