APTT-EA

KIT FOR DETERMINATION OF APTT (ELLAGIC ACID)

Kit name	Kit size	Cat. No
APTT-EA-4	10 x 4 ml	K-420
APTT-EA-10	10 x 10 ml	K-450

INTRODUCTION

From its origins through the work of Langdell and coworkers, and later modified by others, the Activated Partial Thromboplastin Time test (APTT, kaolin-kephalin time) has been widely used for a number of years as a pre-surgical screen for assessing certain coagulation factors and in monitoring Heparin therapy. All Factors of the Intrinsic Pathway are necessary for normal results when performing the APTT test.

The APTT test is also used to monitor Heparin therapy, showing prolonged test results at approximately 0.1 units and above.

The test is also used in the quantitative determination (Factor Assays) of Factors VIII, IX, XI, XII and Fletcher Factor.

METHOD PRINCIPLE

APTT-EA-REAGENT contains phospholipid and ellagic acid as the activator and is sensitive Factor deficiencies and to heparin.

The APTT-EA test measures the clotting time of test plasma after the addition of APTT-EA-REAGENT and followed by the addition of calcium chloride.

Deficiencies of approximately 40% and lower of Factors VIII, IX, XI and XII will result in a prolonged APTT. Heparin also results in a prolonged APTT.

REAGENTS

Package

	APTT-EA-4	APTT-EA-10
APTT-EA-REAGENT	10 x 4 ml	10 x 10 ml

Working reagent preparation and stability

The reagents are ready to use.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. Do not freeze.

Opened vials are stable for 30 days when stored at 2-8°C

Concentrations in the test

ellagic acid	0.003%
BSA	0.005%
phenol	0.30%
buffers	1.9%

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- APTT-EA-REAGENTS are designed to work at 37°C ± 0.5°C. Frequently check the temperature of all heating elements.
- Mix gently before use.
- Always follow instrument manufacturer's instructions for proper maintenance.

ADDITIONAL EQUIPMENT

- CALCIUM CHLORIDE 0.020M reagent (Cat. No K-455);
- a manual, mechanical or photo-optical means of clot detection;
- timer;
- control plasmas;
- general laboratory equipment.

SPECIMEN

A. Anticoagulant- sodium citrate - 3.2% (0.105M).

B. Specimen Collection:

1. Obtain venous blood.
2. Immediately mix 9 parts blood with 1 part anticoagulant, mix well by inversion of tube.
3. Centrifuge the specimen at 1000 rcf for 15 min.
4. Remove plasma from the tube within 60 min using a plastic pipette and store in a plastic tube.
5. Test plasma sample within 2 hours, otherwise store frozen and thaw just prior to use.

Plasma pH will increase if exposed to air. Store samples stoppered. Do not delay mixing the blood with anticoagulant. Avoid foaming the specimen. Use only plastic containers.

PROCEDURE

Kit is suitable for use with manual, mechanical or automated instrument for clot detection. See instrument manufacturers instructions for full details.

Manual assay

1. Prewarm CALCIUM CHLORIDE 0,020M to 37°C. Mix gently.
2. Add 0.1 mL test plasma to cuvette and prewarm to 37°C.
3. Add 0.1 mL APTT-EA-REAGENT to the test plasma. Mix and start timer.
4. Incubate the plasma-reagent mixture at 37°C for 3-5 minutes (activation time). For consistent results, test all plasmas with the same activation time.
5. Forcibly add 0.1 ml prewarmed CALCIUM CHLORIDE 0,020M.
6. Note time for clot formation.
7. Perform duplicate determinations.

Calculation

Report clotting times for each plasma to the nearest 0.1 second. Report result as APTT time, secs. A Normal Reference Range can also be reported for comparison. Do not report patient values relative to commercial control plasma clotting times. Controls are intended only for quality assurance of the test system, such as: temperature, reagents, pipettes, instrument etc.

REFERENCE VALUES

Method	Mean	Range (± SD)
mechanical	29.9	24.0 – 35.2
photo-optical	29.8	24.2 – 36.3

These values should only be used as a guideline. Each laboratory should establish a Normal Reference Range (NRR) using instrumentation, blood collection methods, and testing techniques used in that laboratory.

A new NRR should be established with any change in instrumentation, blood collection, techniques, anticoagulant and when changing to new lots of reagents.

QUALITY CONTROL

CONTROL PLASMA NORMAL (Cat. No K-100) CONTROL PLASMA-ABNORMAL LEVEL 1 and LEVEL 2 (Cat. No K-101 and K-102) should be tested in conjunction with patient plasmas. It is recommended that Controls be run at least each shift and a minimum of once per 20 patient samples.

A Control Range should be established by the laboratory to determine the allowable variation in day to day performance of each Control Plasma.

Each laboratory should establish a control range for each control.

LIMITATIONS

A. Plasma samples with hematocrits outside the range of 20-55% may be improperly anticoagulated and should be adjusted appropriately.

B. Turbid, icteric, lipemic, or hemolyzed specimens may generate erroneous results.

C. Freezing and thawing plasma can affect results.

D. Acute inflammatory reactions can shorten APTT results because of elevated fibrinogen.

E. Sodium oxalate, EDTA, and heparin are not suitable anticoagulants. Oral contraceptives, estrogen, pregnancy, coumarin type drugs, heparin, asparaginase, and naloxone have been reported to influence APTT results.

PERFORMANCE CHARACTERISTICS

1. Heparin sensitivity:

For example, the following results were obtained on a photo-optical instrument with one lot of Cormay reagent:

Heparin conc. (U/ml)	APTT (s)
0.0	28.8
0.1	38.3
0.2	50.1
0.3	63.1
0.4	80.9
0.5	98.0

Each laboratory should establish its own heparin sensitivity ranges.

2. Factor sensitivity:

Cormay reagents was evaluated on mildly and severely deficient plasmas with the following results:

Factor	% activity	APTT (s)
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

Furthermore, the sensitivity of APTT-EA to Factor VIII has been determined as follows:

% Factor VIII	APTT (s)
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 sensitivity to individual factors using instruments, reagents, and techniques used in their laboratory.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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Date of issue: 04. 2011.

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04/11/04/11