ROBONIK®

CE

prieclot™...Coagulation Reagents

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) TEST KIT

(Activated with Ellagic Acid)

INTENDED USE :

- Activated Partial Thromboplastin Time is the main test for screening intrinsic clotting defects, including haemophilia and laboratory monitoring of heparin administration.
- In vitro diagnostic test kit, for laboratory and professional use.
- This manual contains instructions for operation by qualified personnel only.

ORDERING INFORMATION :	**Pack Size**	**Cat No.**
	1 X 3 ml	APTT 01 03
	6 X 3 ml	APTT 06 03

CLINICAL SIGNIFICANCE : Prolongation of the APTT is encountered in the following conditions.

a. Congenital Deficiencies

1. If PT is normal, the following factors may be deficient factors VIII, IX, XI, XII.
2. If all these factors are normal, a deficiency of PK or HMWK be considered.

b. Acquired Deficiencies and Abnormal conditions

1. Liver Diseases.
2. Circulating Anticoagulants (Lupus Anticoagulant and other inhibitors).
3. During heparin and oral anticoagulant therapy.
4. Oral contraceptives, estrogen, pregnancy and coumarin type drugs have been reported to influence APTT results.

PRINCIPLE : The test system includes an incomplete thromboplastin phospholipid extract and an activator. Activator accelerates the conversion of factor XII to XI via and further reaction proceeds to ultimately form as fibrin clot in presence of phospholipids and Ca^{++}. The time required to form VIII, IX, XI, XII, the common pathway factor X, V, II, I, the inhibitory effect of heparin and the LA (Lupus Anticoagulant).

REAGENT COMPOSITION :

1. Ellagic Acid
2. Rabbit Brain Phospholipids
3. Buffer
4. Stabilizers & Antimicrobial

REAGENT STORAGE : Strictly at 2 to 8°C.

REAGENT STABILITY : Till expiry date Stated on the vial label when stored at 2 to 8°C.

PRECAUTION : Venous blood should be directly transferred into the tube containing the anticoagulant. The absence of micro clots should be checked. Separate plasma immediately by centrifugation after collection of blood. Plasma must be stored in siliconized glass tubes or plastic containers. Avoid turbid, lipemic or hemolyzed samples. Use clean, dry micropipette tips and glassware to dispense the reagent. Close reagent vial and transfer immediately at 2 to 8°C after dispensing.

WASTE MANAGEMENT : For disposal of these biomedical waste refer local biosafety regulations.

MATERIAL REQUIRED BUT NOT PROVIDED : Calcium Chloride ($CaCl_2$), Centrifuge, Pipettes, Stop Clock / Timer or Coagulometer.

SAMPLE COLLECTION & PREPARATION : Mix gently, 9 parts of blood in a plastic tube or siliconized glass tube containing 1 part of 3.2 % trisodium citrate solution (0.109M). Centrifuge immediately for 15 minutes at 3000 RPM (2000 to 2500 G) to obtain platelet poor plasma. Transfer supernatant plasma in a silicon glass tube or plastic tube immediately, do not disturb buffy coat while collecting supernatant plasma. The test should be performed within a time limit not exceeding 2 hours after blood collection.

I) TEST PROCEDURE FOR APTT :

The Activated Partial Thromboplastin Time test for each sample should be determined in duplicate.

REAGENT PREPARATION FOR MANUAL AS WELL AS INSTRUMENT METHOD :

1. Bring contents of the vial to room temperature and gently swirl the vial before use. Do not shake.
2. Dispense from the vial enough APTT Reagent for immediate use, in a thoroughly clean and dry test tube.
3. Pre warm the dispensed APTT Reagent to 37°C for 10 minuets.
4. Dispense from the vial enough $CaCl_2$ reagent for immediate use, in a thoroughly clean and dry test tube.
5. Pre warm the dispensed $CaCl_2$ Reagent to 37°C for 10 minuets.

A) MANUAL METHOD :

1. Pipette 100 µl of patient or control plasma in to a test cuvette and incubate at 37°C for 1 minute.
2. Pipette 100 µl APTT Reagent into respective test cuvette.
3. Mix well and incubate at 37°C for 3 minutes.
4. Add forcibly 100 µl of pre-warmed $CaCl_2$ into the test cuvette.
5. Start a timer simultaneously and record the clotting time in seconds.

B) INSTRUMENT METHOD (for *eazy CLOT* & *FOURCLOT*) :

1. For setting up the instrument, please refer to the instrument user manual.
2. Select Clot time mode in instrument follow the procedure as prompted on display.
3. Place the magnetic bead in to cuvette as shown.
4. Pipette 50 µl of plasma in cuvette.
5. Add to the above cuvette, 50 µl of APTT reagent mix well and incubate at 37°C for exactly 3 minutes.
6. After 3 minutes incubation place the test cuvette in the measurement chamber of the instrument
7. Instrument will sense the insertion of cuvette in the measurement chamber and the display will prompt "Dispense Reagent".
8. Dispense 50 µl of 25 mM Calcium Chloride reagent. The instrument display the time taken for the clot formation.

* Note : Refer to the instrument manual for instrument specific details.

PRECAUTION : Reagent left over in the cuvette after the test procedure should not be put back into the reagent bottle. Doing so can lead to contamination.

II) MANUAL METHOD FOR ESTIMATION OF HEPARIN :

CALIBRATION CURVE FOR DETERMINATION OF HEPARIN CONCENTRATION :

- Dilute Heparin (as used for treatment) with physiological saline to concentration of 1 0 IU/ml.
- Mix 0.2 ml of 10 IU/ml diluted heparin with 1.8 ml of FNP (Fresh Normal Plasma) to yield heparin standard of 1 IU/ml concentration.
- Dilute the Heparin standard as prepared above (1IU/ml) with FNP as follows :

Test Tube	1	2	3	4	5	6	7
Heparin Std. (1 IU/ml)	0.5	0.4	0.3	0.2	0.1	0.1	—
FNP in ml	—	0.1	0.2	0.3	0.4	0.9	0.5
Heparin Conc. (IU/ml)	1	0.8	0.6	0.4	0.2	0.1	0.0

PROCEDURE :

Manual method to estimate Heparin concetration in the plasma / sample.

- Pipette 0.1 ml of each Heparin dilution into clean test tubes.
- Add 0.1 ml APTT Reagent (pre-warmed at 37°C). Mix and incubate exactly 3 minutes at 37°C.
- Add 0.1 ml Calcium Chloride (25 mM) pre warmed at 37°C and simultaneously start stopwatch.
- Observe clot formation carefully and note the time at the appearance of first fibrin web.
- Plot mean of double determination in second in correlation to dilution of heparin on graph paper.
- Connect points in straight line.

* Clotting time (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in IU/ml.

EXPECTED VALUE : Normal Range 23 to 38 Sec.

THERAPEUTIC RANGES : Therapeutic Ranges depends on Risk factor.

WARRANTY : This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIOGRAPHY :

1. J. V. Dacie and S.M. Lewis : Practical Hematology. 1984 R Biggs and R G McFarlane: Human blood coagulation and its disorders 1962
2. B. F. Rodak, Hematology, clinical principles and applications- II Edition.
3. John Bernard Henry: Clinical Diagnosis and Management by Laboratory Methods XX Edition.
4. Tomenson J A and Thomson J M Standardization of Prothrombin Time. Blood coagulation and Hemostasis : a practical guide, Edinburgh Churchill Livinigstone : 1985 page 370-409.
5. Williams: Hematology VII edition.
6. WHO expert committee on biological standardization 1984. 34th report.
7. Data on file.

ROBONIK®

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IVD For in vitro diagnostic use | Store at | Consult Instructions for use | REF Catalogue Number | Exp. Date | LOT Lot No. | Manufacturer's Address | Date of Manufacture

Ver.: APTT/1018/01

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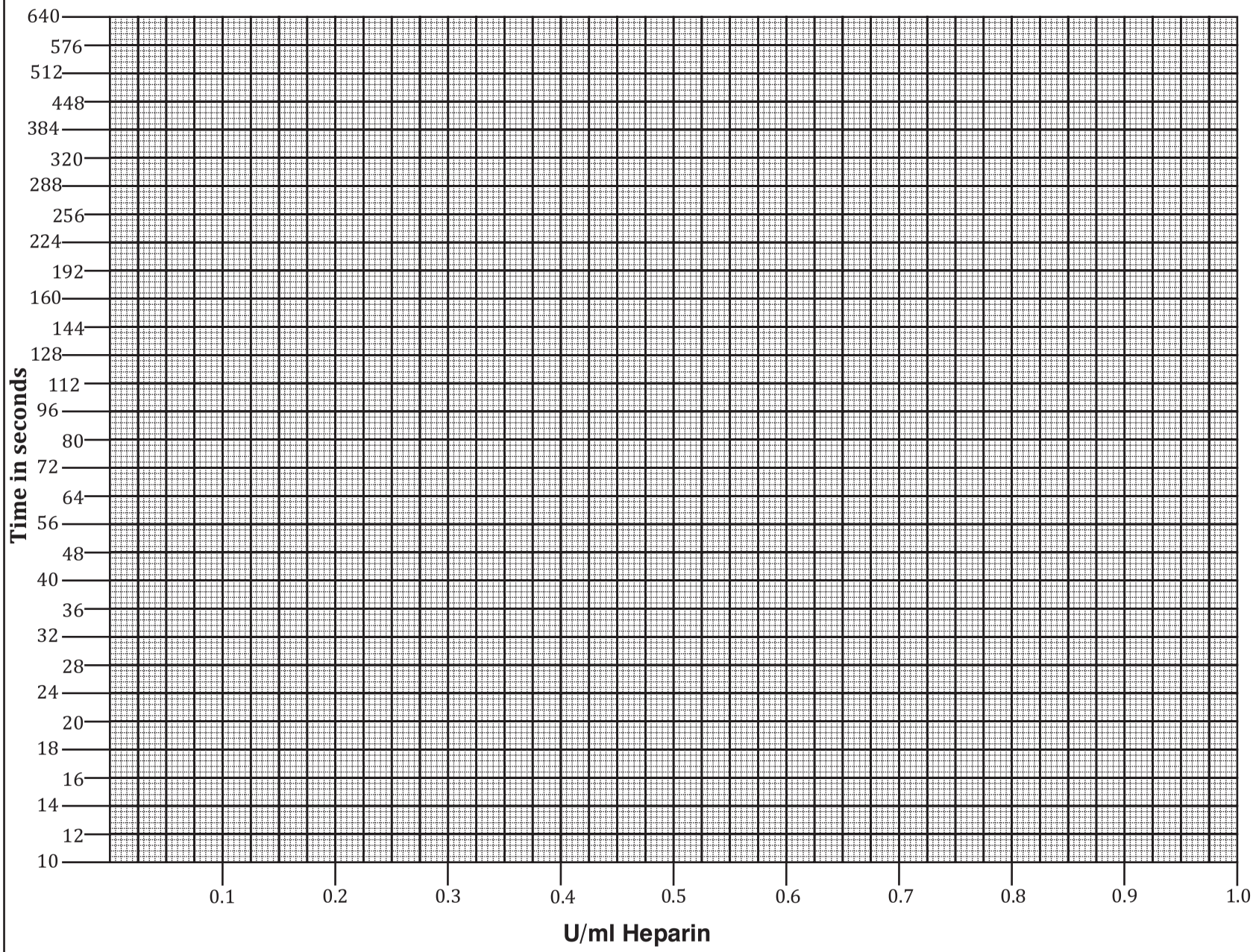
Heparin Curve

Heparin	APTT
U/ml	Sec.
0	
0.1	
0.2	
0.4	
0.6	
0.8	
1.0	

Lot. No.: _____

Date : _____

Test Performed By : _____



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