

## BioMed-Liquicellin-E for APTT determination

### Activated Partial Thromboplastin Time



REF: PTT202180 (6x3 ml)

#### INTENDED FOR USE:

For quantitative determination of Partial Thromboplastin activity in plasma

#### PRINCIPLE:

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.

APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

#### SPECIMEN COLLECTION:

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Activated Partial Thromboplastin Time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, VIII, X, V, II and Fibrinogen.

Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy

#### REAGENT COMPOSITIONS :

**LIQUELIN-E** is liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

#### PACKAGE: Collection and storage.

- Store the reagent at 2-8°C. DO NOT FREEZE.

- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The reagent is stable at 2-8°C up to the expiration date.

#### PRECAUTIONS & WARNING :

Avoid pipetting with mouth.

The preparation, according to current regulation, is classified as not dangerous.

The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.

Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

#### REAGENT PREPARATION & STABILITY :

- Use freshly collected blood taken into 0.11 mol/L trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.

- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.

- Plasma should be tested within 3 hours (keep refrigerated).

#### REQUIRED MATERIALS NOT PROVIDED:

(a) 12 X 75 mm glass test tubes. (b) 0.1 ml. (c) Stop watch. (d) Water bath or heating block at 37°C. (e) Fresh Normal Pooled Plasma. (f) CaCl<sub>2</sub> (0.02 mol/l).

#### PROCEDURE:

##### Manual method

1. Pre-incubate the Calcium Chloride Reagent to 37°C for at least 10 minutes. Pipette 100µl of test or control plasma into a test cuvette.
2. Incubate the plasma at 37°C for 1 to 2 minutes.
3. Pipette 100µl of the APTT reagent, into reagent cuvette containing the plasma. Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate at 37°C for 3 minutes.
5. Add 100µl preincubated Calcium Chloride solution and simultaneously start the timer.
6. Record the clotting time in seconds.

##### Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.
2. Mix 0.2 ml of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1U/ml concentration.
3. Dilute the heparin standard as prepared above (1U/ml) with FNP as follow

Test tube no.	1	2	3	4	5	6	7
Heparin standard (1U/ml) in 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 concentration (U/ml)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 ml each of the seven heparin dilutions into clean test tubes.
5. Add 0.1 ml LIQUELIN-E reagent to each test tube.
6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
7. Forcibly add 0.1 ml calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.
8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds.
9. Repeat steps 4-8 for each dilution for duplicate test, and find the average of the duplicate test values.
10. Plot the mean of the double determination in seconds, against each heparin concentration using BioMed-LIQUICELIN-E graph paper.
11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/ml.

#### CALCULATION:

##### Manual Method

- (a) The results may be reported directly in terms of the mean of the double determination of APTT of the test plasma.

APTT of patient plasma (in seconds)

(b) Or as a ratio 'R':  $R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$

#### Calibration Curve Method

Heparin concentration in the test sample can be directly obtained from the LIQUICELIN-E calibration curve by interpolating the test plasma clotting time against the heparin concentration in U/ml.

#### EXPECTED VALUE:

Normal values using LIQUICELIN-E reagent are between 28-40 seconds at 3 minutes activation time. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish its own normal range.

#### WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

#### REFERENCES:



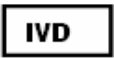


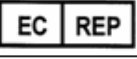

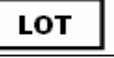

1. Biggs R. and R.G. McFarlane: Human Blood Coagulation and its Disorders, Blackwell Scientific Publications, Oxford, 1962.
2. Hoffmann, J.J.M.L. and Neulendijk P.N.: Thrombos. Haemosta. (Stuttgart) 39, 640 (1978).
3. CRC, Handbook Series in Clinical Laboratory, Science, Section 1: Haematology, Volume III, 1980. CRC Press, Inc. Boca Raton, Florida

#### PERFORMANCE:

1. Due to inter and intra laboratory variations users must establish their own normal population range as well as normal and abnormal range.
2. It is recommended that controls with known factor activity should be run simultaneously with each test series routinely.
3. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
4. Incorrect dilution of heparin is also a potential source of error.
5. Oxalated plasma may include prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depends upon the time lag between the specimen collected and the last dose.
7. Abnormalities of coagulation factor VII, factor XIII and platelets are not detected by this test procedure.
8. For automated equipment it is strongly recommended that the equipment manufacturer methodology be strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the in-vitro half-life of heparin is approximately 1.5 hours. When it is administered intravenously it has an immediate anti coagulant effect but its efficacy decreases rapidly with time.
10. Platelet factor IV, a heparin-neutralizing factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in-vitro the specimen should be collected with a minimum of trauma.
11. Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

#### LIMITATIONS :

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. LIQUICELIN-E reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded.
3. Reagent contains 0.01% Thimerosal as preservative.
4. It is very important that clean and dry micropipette tips be used to dispense the reagent.
5. Avoid exposure of the reagent to elevated temperatures, contamination. Immediately replace cap after use and store at recommended temperatures only.

	Consult Instructions for Use
	Caution, consult accompanying Documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by

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