













SYMBOL KEYS

	Temperature limitation		Manufacturer		Batch Number/ Lot Number		Activated Partial thromboplastin time test
	Use by		Consult Instructions for use		In vitro Diagnostic Medical Device		Description of reagent
	Date of Manufacture		Catalogue Number		This side up		Authorised Representative in the European Community



REGD. OFFICE: GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

MANUFACTURING UNIT: PLOT NOS. 92/96, PHASE II C, VERNA IND. EST., VERNA, GOA-403 722, INDIA.

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain



CEPHALOPLASTIN REAGENT FOR PARTIAL THROMBOPLASTIN TIME (APTT) DETERMINATION USING ELLAGIC ACID, AS AN ACTIVATOR

SUMMARY

The arrest of bleeding depends upon primary platelet plug formed along with the formation of a stable fibrin clot. Formation of this clot involves the sequential interaction of a 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, IX, 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.

PRESENTATION

REF	*10631025	10630003	10630123
LIQUICELIN-E®	2.5 ml	3 ml	12 X 3 ml
3.2% Tri-Sodium Citrate	12. 5 ml	-	-
Pack insert	1	1	1

* REF 10631025 represents Precision combi pack which contains LIQUICELIN-E® reagent along with 3.2% Tri-Sodium Citrate solution used for blood collection.

REAGENT

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

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

REAGENT STORAGE AND STABILITY

a) Store the reagent at 2-8°C. **DO NOT FREEZE.** b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened the reagent is stable for **3 months at 2-8°C, 1 week at 18-25°C, 2 days at 37°C provided it is not contaminated and capped tightly when not in use.**

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.

NOTE

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 temperature and contamination. **Immediately** replace cap after use and store at recommended temperatures **only**.
6. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The veinpuncture must be a 'clean' one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into tubes, after detaching the needle from the syringe.

Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11 mol/l, 3.2%). Centrifuge immediately for 15 minutes at 1500 g and transfer the plasma into a clean test tube. **Plasma must be tested within three hours of blood collection.** For heparin determination, platelet deficient plasma should be used, hence higher centrifugation time is required.

FNP COLLECTION

Prepare a plasma pool (FNP) of freshly collected blood from atleast five normal healthy donors and process as above. Plasma must be tested within three hours of blood collection.

ADDITIONAL MATERIAL REQUIRED

(a) 12 x 75 mm glass test tubes. (b) precision pipettes. (c) Stop watch. (d) Water bath or heating block at 37°C. (e) FRESH NORMAL POOLED PLASMA. (f) CaCl₂ (~0.025mol/l)*.

*AVAILABLE FROM TULIP DIAGNOSTICS; CAT. NO: 10633010,10633100

TEST PROCEDURE

Manual Method

1. Before use, the reagent should be mixed well by gentle swirling. Do not shake.
2. Aspirate from the reagent vial enough reagent for the immediate testing requirement in a thoroughly clean and dry test tube. Bring this reagent to room temperature before prewarming at 37°C for testing purposes.
3. Separate test tubes containing LIQUICELIN-E® and TULIP Calcium Chloride Solution should be brought to 37°C. (Depending on volume, approximately 5 to 10 minutes required). Do not incubate the test plasma.
4. To a 12 x 75 mm test tube, add 100µl test plasma and 100µl LIQUICELIN-E®. Shake tube briefly to mix the reagent and plasma; place tube at 37°C for 3 to 5 minutes.
5. Following incubation period, add forcibly 100µl of prewarmed calcium chloride into the plasma and LIQUICELIN-E® mixture; simultaneously start a stopwatch. Shake tube briefly to mix contents, keep at 37°C for 15 seconds.
6. Following 15 seconds incubation, remove the tube; gently tilt back and forth until a gel clot forms; stop the watch; record time.
7. Repeat steps 4-6 for a duplicate test using the same test plasma.
8. Find the average from the duplicate test values. This is the Activated Partial Thromboplastin Time (APTT of patient plasma).
9. Similarly repeat steps 4-6 twice, and record duplicate values using FNP in place of test plasma (APTT of FNP).

If a coagulation instrument is being used to perform the tests, the instrument manufacturers instructions must be strictly adhered to.

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 200µl of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1 U/ml concentration.
3. Dilute the heparin standard as prepared above (1U/ml) with FNP as follows:

Test tube no.	1	2	3	4	5	6	7
Heparin standard (1 U/ml) in µl	500	400	300	200	100	100	-
FNP in µl	-	100	200	300	400	900	500
Heparin Concentration (U/ml)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 100µl each of the seven heparin dilutions into clean test tubes.
5. Add 100µl LIQUICELIN-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 100µl calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stop watch.
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 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 AND REPORTING OF RESULTS

Manual Method

- a) The results may be reported directly in terms of the mean of the double determination of the APTT of the test plasma clotting time. It is suggested that the results be reported to the clinicians in conjunction with the normal range.
OR
- b) As a ratio as follows:
$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$$
- c) 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 heparin concentration in U/ml.

EXPECTED VALUES

Reference values for healthy individuals may vary from laboratory to laboratory depending on techniques and instrumentation used.

In a study of 96 apparently healthy individuals using LIQUICELIN-E® reagent on an Opto-mechanical instrument, a reference range of 22-37 seconds was obtained. Each laboratory must establish the reference range for a reagent with instrument, specimen collection and testing techniques used in that laboratory.

REMARKS

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 induce prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and also 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 manufacturers methodology be strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the *in-vivo* 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-neutralising 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.

PERFORMANCE CHARACTERISTICS

The intra and inter assay were performed with normal and abnormal plasma controls .

Intra assay n=20	Normal plasma control	Abnormal plasma control
Mean (sec)	28.8	68.9
S.D(sec)	0.56	1.71
C.V %	1.94	2.48

Inter assay n=20	Normal plasma control	Abnormal plasma control
Mean (sec)	29.2	68.8
S.D(sec)	0.85	2.26
C.V %	2.91	3.28

Comparison with commercially available reagent of same method.

50 plasma samples were tested on semiautomated analyser : $y = 0.9247 X + 2.3640$, $r = 0.9548$.

WARRANTY

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

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3. CRC, Handbook Series in Clinical Laboratory, Science, Section 1: Haematology, Volume III, 1980. CRC Press, Inc. Boca Raton, Florida.
4. NCCLS guideline H21-A3, Vol. 18, No. 20.
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