CEPHALOPLASTIN REAGENT FOR PARTIAL THROMBOPLASTIN TIME (APTT) DETERMINATION ON CoaLAB 6000

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.

REAGENT
LIQUICELIN-E® system pack reagent 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.

c) The reagent is stable for: 1 year at 2-8°C, 1 week at 18-25°C, 2 days at 37°C.

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® system pack 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 important to ensure that the reagent cup is clean and dry before dispensing the reagent into the reagent cup.

5. It is very important that clean and dry micropipette tips be used to dispense the reagent in the reagent cups.

6. Do not transfer the reagent from the reagent cup into the reagent vial at the end of day's work. Discard the left over reagent.

7. Do not mix reagents of different lots in the reagent cups.

8. It is necessary to use Teflon stirrers in reagent cups for homogenising the reagent to obtain accurate and consistent results.

9. Avoid exposure of the reagent to elevated temperatures and contamination. Immediately replace cap after use and store at recommended temperatures only.

10. The test procedure in this package insert has been designed for application on CoaLAB 6000 only. However the reagents can be programmed on other automated coagulometers also, provided the reagents have been standardised on them.

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 vein puncture 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%) or PROFACT available from Tulip; Cat No. 10660020. Centrifuge immediately for 15 minutes at 3000 rpm (approximately 2000 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 at least five normal healthy donors and process as above. Plasma must be tested within three hours of blood collection.

**ADDITIONAL MATERIAL REQUIRED**
1. Fresh normal pooled plasma.
2. CaCl₂ (0.025 mol/l) available from Tulip; Cat. No: 10633010, 10633110.
3. Physiological saline.

**TEST PROCEDURE**
Bring all the reagents and samples to room temperature.
The test procedure for LIQUICELIN-E® system pack has been preprogrammed on CoaLAB 6000. The clotting time of FNP for every lot of LIQUICELIN-E® system pack reagent must be determined and edited in the CoaLAB 6000 PTT programme as mentioned below.

**Determination of the clotting time for FNP**
- Enter the **Routine Menu**.
- Load the prepared fresh normal pool plasma into the sample cup and assign the appropriate identity no.
- Create Job list of PTT test for the FNP.
- Load the required amount of Liquicelin-E® system pack reagent and calcium chloride in the respective reagent positions.
- Measure the partial thromboplastin time for the FNP using the existing PTT programme.
- Edit the test parameters of the existing PTT test programme with the obtained partial thromboplastin time value as mentioned below:
  1. Enter the **Test menu** in CoaLAB 6000
  2. Modify test: Enter
  3. Select test: PTT: Enter
  4. PTT parameter: 2ⁿ conversion: Enter
  5. Ratio: Enter the obtained partial thromboplastin time in seconds for 100% activity.

**The PTT time for the FNP is applicable only for the same Lot of LIQUICELIN-E® system pack.**

**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 1 U/ml concentration.
3. Dilute the heparin standard as prepared above (1 U/ml) with FNP as follows

<table>
<thead>
<tr>
<th>Test tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin Standard (1U/ml) in ml</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>FNP in ml</td>
<td>-</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Heparin Concentration (U/ml)</td>
<td>1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- Enter the **Routine Menu**.
- Load each of the prepared dilutions of heparinised plasma into the separate sample cups and assign them appropriate Identity Nos.
- Create Job list of PTT test for the heparinised plasmas.
- Load the required amount of Liquicelin-E® system pack reagent and calcium chloride in the respective reagent positions.
- Measure the partial thromboplastin time for the heparinised plasma using the existing PTT programme.
• Edit the test parameters of the existing PTT test programme with the obtained partial thromboplastin time value as mentioned below:
  1. Enter the **Test menu** in CoaLAB 6000
  2. Modify test: Enter
  3. Select test: PTT: Enter
  4. 1st conversion: Enter
  5. Curve interpol: Enter
  6. Enter the PTT clotting time in seconds with the obtained clotting time in seconds for the respective heparin concentrations.

**Test procedure for the samples**
• Enter the **Routine Menu**.
• Load the required nos. of sample plasmas individually into the sample cup with appropriate identity nos.
• Create Job list of PTT test for the sample plasmas.
• Load the required amount of Liquicelin-E® system pack reagent and calcium chloride in the respective reagent positions.
• Measure the partial thromboplastin time for the sample plasmas using the PTT programme.

**EXPECTED VALUES**
Normal values using LIQUICELIN-E® System Pack reagent are between 22-34 seconds.

**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.

**WARRANTY**
The product is guaranteed 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.

**BIBLIOGRAPHY**
4. Data on file: Tulip Diagnostics (P) Ltd.