CEPHALOPLASTIN REAGENT FOR PARTIAL THROMBOPLASTIN TIME (APTT) DETERMINATION ON AUTOMATED COAGULATION ANALYZERS

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 10632063
LIQUICELIN-E 6 x 3 ml Pack insert 1

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) Once opened the reagent is stable as per the labeled shelf life 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.
d) Once opened the onboard stability for LIQUICELIN-E system pack reagent is 2 days at 18-25°C. It is advisable to cap the reagent vial 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 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 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%) 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, 10633100.
3. Physiological saline.

TEST PROCEDURE
- Applications suitable for Hemostar Auto and coaLAB 6000 are available on request.
- A defined application for the LIQUICELIN-E® system pack must be installed in accordance with the general instrument settings given below. For instructions refer the respective instrument manual.

General instrument settings

<table>
<thead>
<tr>
<th>Sample</th>
<th>50µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquicel E reagent</td>
<td>50µl</td>
</tr>
<tr>
<td>Incubation</td>
<td>180 seconds</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>50µl</td>
</tr>
</tbody>
</table>

Determine heparin concentration (not applicable for LMWH)
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</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 (U/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>0.1</td>
</tr>
<tr>
<td>FNP in ml</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Heparin Concentration (U/ml)</td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Determine the APTT time for each of the above samples (tube 1 to 7). Plot a curve of heparin clotting time vs heparin concentration.

Quality control:
The performance of LIQUICELIN-E® system pack must be validated using Plasmatrol HI/HII/HIII controls (Cat Nos).

Test procedure for specimen
- When the values obtained with plasmatrol are within expected range (provided in the assay value sheet) specimens can be measured as per instrument protocol. Ensure that sufficient amount of sample and reagents are present as per the requirement of the instrument protocol.

CALCULATIONS
Results may be reported in seconds in comparison to the FNP time.
The heparin concentration can be estimated from the heparin calibration curve.

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® system pack reagent on an Opto-mechanical instrument, a reference range of 20.1-30.5 seconds (mean ± 2SD) was obtained. Each laboratory must establish the reference range for a reagent with instrument, specimen collection and testing techniques used in that laboratory.

The above values should only be used as guidelines. Each laboratory should establish sensitivity to individual factors using techniques, reagents and instruments used in their laboratory.

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.