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. Tissue Thromboplastin, in the presence of calcium, is an activator, which initiates the extrinsic pathway of coagulation, which includes plasma coagulation factors VII, X, V, Prothrombin and Fibrinogen. During oral anticoagulant therapy most of these factors are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired. Prothrombin Time determination is the preferred method for presurgical screening, determination of congenital deficiency of factors II, V, VII and X and for monitoring of anticoagulant therapy and as a liver function test.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. 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 in the reagent cups.
5. 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.
6. Do not mix reagents of different lots in the reagent cups.
7. It is necessary to use Teflon stirrers in reagent cups for homogenising the reagent to obtain accurate and consistent results.
8. Avoid exposure of the reagent to elevated temperatures and contamination. Immediately replace cap after use and store at recommended temperatures only.
9. The test procedure in this package insert has been designed for application on CoaLAB 6000 only.
10. The reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

REAGENT
LIQUIPLASTIN® system pack: reagent is a liquid ready to use Calcium Thromboplastin Reagent, which is derived from rabbit brain. Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PRINCIPLE
Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When LIQUIPLASTIN® system pack reagent is added to normal anticoagulated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is a deficiency of factors / factor activity in the extrinsic pathway of the coagulation mechanism.

REAGENT STORAGE AND STABILITY
1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable for: 1 year at 2-8°C, 1 week at 18-25°C, 2 days at 37°C.
3. Once opened the onboard stability for LIQUIPLASTIN® system pack reagent is 3 days at 18-25°C. It is advisable to cap the reagent vial when not in use.

PRESENTATION

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Pack size</th>
</tr>
</thead>
<tbody>
<tr>
<td>10612065</td>
<td>LIQUIPLASTIN® system pack</td>
<td>6 X 5 ml</td>
</tr>
<tr>
<td></td>
<td>Pack insert</td>
<td>1</td>
</tr>
</tbody>
</table>

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9. 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 OF PPP
Though no special preparation of the patient is required prior to sample collection by approved techniques, it is preferable that patients are not heavily exercised before blood collection. Fasting or only light non-fatty meals prior to blood collection provide samples with a desirable opacity. 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 anticoagulated tubes, after detaching the needle from the syringe. Do not delay mixing blood with anticoagulant. Avoid foam formation during mixing. Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11mol/l, 3.2%) or PROFACT available from Tulip; Cat No. 10660020. For occasional patients with hematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid results. Centrifuge immediately for 15 minutes at 1500-3000 rpm (approximately 1500 g) on a laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PPP). Cap the test tube to prevent deterioration of the samples. Plasma must be tested preferably immediately. However if the specimen are held at 22-24°C then they may be tested within 2 hours and if the specimen is held at 2-4°C then they may be tested within 3 hours.

ADDITIONAL MATERIAL REQUIRED FOR CALIBRATION CURVE METHODS
Micropipettes and disposable tips for transferring reagents into the reagent cups. Fresh normal plasmas for establishing MNPT / calibrating % activity, isotonic saline.

TEST PROCEDURE
Programmer values for MNPT

1. Enter the Routine Menu.
2. Load the required numbers of normal plasmas into separate sample cups and assign them appropriate identity nos.
3. Enter the required amount of LIQUIPLASTIN® system pack reagent in the reagent cup.
4. Measure the prothrombin time for all the loaded samples using the existing PT programme.
5. Enter the MNPT value in seconds for 100% activity.
6. Enter the MNPT value in seconds for 50% activity.
7. Enter the MNPT value in seconds for 25% activity.
8. Enter the MNPT value in seconds for 12.5% activity.
9. Program the isotherm into the correct postion.
10. Enter the MNPT value in seconds for the respective % activity.

Test procedure for the samples

1. Enter the Routine Menu.
2. Enter the isotherm into the correct position.
3. Load the neat and each of the diluted FNP in separate sample cups and assign them appropriate identity nos.
4. Load the required amount of LIQUIPLASTIN® system pack reagent in the reagent cup as per the PT programme
5. Measure the prothrombin time for each of the neat & diluted FNP using the existing PT programme.

The ISI is applicable only for the same Lot of LIQUIPLASTIN® system pack reagents.

Determination of percentage Activity
Prepare a pool of fresh normal plasmas (at least five plasma samples). Dilute the pool plasma with isotonic saline as mentioned below:

<table>
<thead>
<tr>
<th>Activity</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>12.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic saline</td>
<td>-</td>
<td>300 ml</td>
<td>450 ml</td>
<td>700 ml</td>
</tr>
<tr>
<td>FNP</td>
<td>600 ml</td>
<td>300 ml</td>
<td>150 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

1. Enter the Test menu in CoaLAB 6000
2. Modify test: Enter
3. Select test: PT, Enter
4. PT parameter: 1st conversion: Enter
5. Curve Interpol: Enter
6. Enter the obtained PT time in seconds for the respective % activity.
7. These test parameters are applicable only for the same lot of LIQUIPLASTIN® system pack reagent.
2. Load the required no. of sample plasmas in separate sample cups giving appropriate identify nos. and create job list of 4 PT test for the sample plasmas.
3. Load the required amount of LIQUIPLASTIN® system pack reagent in the reagent cup.
4. Measure the prothrombin time for the samples.

**EXPECTED VALUES**

Normal values using LIQUIPLASTIN® system pack are between 10-14 seconds. The normal values may vary depending upon laboratory to laboratory on procedures used for sample collection, plasma storage and instrument used for clot detection. It is recommended that each laboratory must establish their own normal range based on procedure and instruments in use. It is mandatory that each laboratory must establish its own MNPT for each lot of LIQUIPLASTIN® system pack. Oral Anticoagulant Therapeutic Range: INR = 2.0-3.5.

**REMARKS**

1. It is recommended that controls (PLASMATROL H-VII Available from Tulip Cat. No: 11040061, 11041061) with known factor activity should be run simultaneously with each test series to validate test run.
2. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
3. Oxalated plasma may induce prolonged clotting times.
4. Since the PT test functions correctly only at 37 ± 0.5°C, temperature of all equipment must be calibrated daily.
5. 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.
6. Turbid, icteric, lipemic or grossly hemolyzed samples may generate erroneous PT results.
7. Sample cups and reagent cups used in the test must be scrupulously clean and free from even traces of acids / alkalies or detergents.
8. Plasma samples held at 4-8°C may undergo ‘cold activation’ leading to a marked shortening of the PT.
9. The PT may be shortened during acute inflammatory conditions, which are accompanied by increase in Fibrinogen levels and also by agents such as antihistamines, butobarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate, ethanol, tetracycline, aspirin, and anticoagulants such as heparin and warfarin.
10. It is important that each laboratory express the results in terms of INR for patients on oral anticoagulant therapy for the clinician to adjust the dosage based on INR.
11. Since the test uses platelet poor plasma, each laboratory must calibrate the necessary force and time required during centrifugation to yield the PPP. Contamination of plasma with excess platelets could falsely elevate levels of some of the factors.

**PERFORMANCE CHARACTERISTICS**

- **LIQUIPLASTIN®** is useful for measuring the deficiencies of factors of the extrinsic pathway. The factor sensitivity of LIQUIPLASTIN® was performed on Hemostar-XF (coagulometer based on turbodensitometric principle of clot detection) by diluting pool normal plasma with factor deficient plasmas in the range corresponding to 3.12-100 % activities.

<table>
<thead>
<tr>
<th>Activity of Factor (%)</th>
<th>Clotting time with LIQUIPLASTIN® with factor deficient plasmas (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factor VII</td>
</tr>
<tr>
<td>100</td>
<td>20.3</td>
</tr>
<tr>
<td>50</td>
<td>26.0</td>
</tr>
<tr>
<td>25</td>
<td>32.7</td>
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<tr>
<td>12.5</td>
<td>42.5</td>
</tr>
<tr>
<td>6.25</td>
<td>62.0</td>
</tr>
<tr>
<td>3.12</td>
<td>85.9</td>
</tr>
</tbody>
</table>

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

**SYMBOL KEYS**

- **LOT** Batch Number / Lot Number
- **REF** Date of Manufacture
- **PT** Prothrombin Time
- **EC** Authorized Representative in the European Community
- **EC** Product in the European Community
- **REP** Production Site
- **PS** Production Site

**BIBLIOGRAPHY**

Contents: UNIPLASTIN: 6 x 5 ml, ISI: Liquid rabbit brain thromboplastin reagent for PT determination.
Preservative: 0.01% Thimerosal
Store at 2°C to 8°C
Do Not Freeze
M. L. No.: 356

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

(Do not accept if the seal is broken)
Cat. No.: 106120065

6 x 5 ml

LIQUIPLASTIN
SYSTEM PACK

PT

Size: 48 x 23 mm

Cat. No.: 106120065

6 x 5 ml

LIQUIPLASTIN
SYSTEM PACK

PT

Size: 48 x 23 mm

Manufacturer: PLOT NO. 92/96, PHASE II C, VERNER INDUSTRIAL ESTATE, VERNER, GOA - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santa Cruz, Bambolim Complex P.O., GOA - 403 202, INDIA.

PS TULIP DIAGNOSTICS (P) LTD.