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 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 VIII, IX, X, Prothrombin and Fibrinogen. During oral anticoagulant therapy most of the Vitamin K dependent factors such as IV, VII, IX, Protein C and Protein S are depressed, as well as the deficiencies of clotting factor activity which may be hereditary or acquired.

Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors II, VII, VIII and X and for monitoring of patients on oral anticoagulant therapy.

REAGENT PREPARATION
Bring the LYOPLASTIN® reagent to room temperature (23-30°C) prior to reconstitution. LYOPLASTIN® reagent is reconstituted with stated amount of {0.1 ml of distilled water (PPD)}. Immediately replace reagent cap after use and store at recommended temperatures only.

ADDITIONAL MATERIAL REQUIRED
12 x 75 mm test tubes (plastic tubes are preferred), 0.1 ml and 0.2 ml precision pipettes, 1 ml precision pipette, distilled water, Stop watch, Water bath or heating block at 37°C, Fresh normal plasmas for establishing MNPT.

REAGENT PREPARATION
Bring the LYOPLASTIN® reagent to room temperature (23-30°C) prior to reconstitution. LYOPLASTIN® reagent is reconstituted with stated amount of distilled water as mentioned on the label.

ADD A PRECISELY DETERMINED QUANTITY OF LYOPLASTIN® REAGENT TO THE TEST TUBE. GENTLY MIX TO AVOID AIR BUBBLES. IMMEDIATELY REPLACE REAGENT CAP AFTER USE AND STORE AT RECOMMENDED TEMPERATURES ONLY.

PREPARATION

Lyophilized Calcified Thromboplastin Reagent, which is derived from rabbit brain.

REAGENT STORAGE AND STABILITY
(a) Store the reagent at 2-8°C. (b) Do not freeze. (c) The shelf life of the reagent is 12 months. (d) The reconstituted LYOPLASTIN® reagent can be used for 10 days when stored at 2-8°C provided it is not contaminated. (e) It is strongly recommended that the reconstituted reagent should be placed in a refrigerator and replaced daily. (f) The reconstituted reagent should be immediately replaced at 2-8°C.

PRINCIPLE
Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When LYOPLASTIN® reagent is added to normal citrated 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 an acquired or congenital deficiency of factors factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy.

NOTE
1. In vitro diagnostic reagents for laboratory and professional use only not for medicinal use. 2. LYOPLASTIN® reagent is not from human sources hence contamination due to HBsAg and HIV is practically excluded. 3. Reagent contains 0.9% Thimerosal as a preservative. 4. It is very important that scrupulously clean and dry micropipette tips be used to aspirate / dispense the reagent. 5. Avoid exposure of the reconstituted LYOPLASTIN® reagent to elevated temperatures, contamination and undue stress due to high and low temperature exposure cycles. Immediately replace reagent cap after use and store at recommended temperatures only.

TEST PROCEDURE
1. Aspirate from the vein a sufficient amount of blood for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred). 2. Bring the reagent to room temperature before prewarming at 37°C for testing purpose. 3. Recap the reagent vial and replace immediately to 2-8°C. 4. To a 12 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a waterbath for 3 to 5 minutes at 37°C. 5. To the tube add 0.02 ml of LYOPLASTIN® reagent (preequillated at 37°C for at least 3 minutes) and simultaneously start a stopwatch. Shake the tube very gently to mix contents. 6. Gently fill the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel clot formation begins. Record the time in ‘seconds’. 7. Repeat steps 4-6 for a duplicate test on the same sample.

CALCULATION OF RESULTS
The results may be reported directly in terms of the mean of the double determination of PT of the test plasma in seconds. Or as a ratio ‘R’ = MNPT for the reagent

Or as International Normalized Ratio (INR), INR = [R]/[R0], where INR = International Sensitivity index of the reagent (Referrant reagent vial label).

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

EXPECTED VALUES
Normal values using LYOPLASTIN® are between 11-15 seconds. 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. It is mandatory that each laboratory must establish its own MNPT for each lot of LYOPLASTIN®.

Oral Anticoagulant Therapeutic range: INR 2.0-3.5

REMARKS
1. It is recommended that controls with known factor activity should be run simultaneously with each test series to validate test run.
2. Incorrect mixture of blood and trisodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
3. Osmolality plasma may induce prolonged clotting times.
4. Since the PT test functions correctly only at ± 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. Tubal idiosyncratic or gradually hemolyzed samples may generate erroneous PT results.
7. Glasswares and cuvettes used in the test must be scrupulously clean and free from even traces of acids / alkalies or detergents.

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 lower opacity. Withdraw blood without undue venous stasis or 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 disturbing needle from the syringe. Do not delay mixing blood with anticoagulant. Avoid air formation during mixing.

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 TULIC Cat. No. 10660020. For occasional patients with haematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid results. Centrifuge immediately for 15 minutes at 1500-2000 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 tubes to prevent deterioration of samples.

However if the specimen are held 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, as a liver function test, determination of congenital deficiency of factors II, VII, VIII and X and for monitoring of patients on oral anticoagulant therapy.

PRESENTATION

<table>
<thead>
<tr>
<th>REF</th>
<th>100.23123</th>
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<tbody>
<tr>
<td>LYOPLASTIN®</td>
<td>12 x 3 ml</td>
<td>12 x 4 ml</td>
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<td>INR conversion table</td>
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<td>1</td>
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<td>Peak insert</td>
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</table>

REAGENT

LYOPLASTIN® is a sensitive, Lyophilized Calcified Thromboplastin Reagent, which is derived from rabbit brain.

REAGENT VIAL LABEL
- (a) Add accurately stated amount of distilled water to the lyophilized LYOPLASTIN® reagent. (b) Gently mix to dissolve. (c) Keep for 10 minutes and mix again gently ensuring complete reconstitution of the lyophilized reagent. Avoid foam formation. (d) Thorough mixing should be ensured before withdrawing a sample at any time for test purposes.

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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, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, oral contraceptives, asparagine, doxifluridine, erythromycin, 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.

12. Homogenization of LYOPLASTIN® reagent suspension before use is important to achieve accurate and consistent results.

**PERFORMANCE CHARACTERISTICS**

**Precision**

The Precision of Prothrombin time determination is highly dependent on the method used. Precision studies were performed on Hemostar-XF coagulometer by assaying normal and abnormal control plasmas with LYOPLASTIN®. One normal control plasma and one abnormal control plasma in replicates of 10 were used to determine inter assay and intra-assay precision of the clotting times (seconds).

<table>
<thead>
<tr>
<th></th>
<th>Inter-assay precision</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Normal control plasma</td>
<td>14.6</td>
<td>0.45</td>
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<tr>
<td>Abnormal control plasma</td>
<td>33.8</td>
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</table>

**Factor Sensitivity**

LYOPLASTIN® is useful for measuring the deficiencies of factors of the extrinsic pathway. The factor sensitivity of LYOPLASTIN® 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 LYOPLASTIN® with factor deficient plasmas (seconds)</th>
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<tbody>
<tr>
<td></td>
<td>Factor VII</td>
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<tr>
<td>100</td>
<td>23.6</td>
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<td>50</td>
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<tr>
<td>25</td>
<td>35.2</td>
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<td>12.5</td>
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<td>6.25</td>
<td>56.1</td>
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<td>3.12</td>
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</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.

**WARRANTY**

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

**BIBLIOGRAPHY**

5. WHO Expert Committee on Biological Standardization, No. 687, 1983.
7. Data on file: Tulip Diagnostics (P) Ltd.