SENSITIVE THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT)
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. 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.

REAGENT
UNIPLASTIN® 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.

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

PRINCIPLE
Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When UNIPLASTIN 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.

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 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 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 tubes 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 tested within 3 hours.

ADDITIONAL MATERIAL REQUIRED
Micropipettes for transferring reagents into the reagent cups. Fresh normal plasmas for establishing MNPT / calibrating % activity, isotonic saline.

TEST PROCEDURE
The test procedure for UNIPLASTIN® system pack has been preprogrammed on CoaLAB 6000. MNPT for every lot of UNIPLASTIN® system pack must be determined and edited in the CoaLAB 6000 PT programme. The ISI value for every lot of UNIPLASTIN® system pack has to be edited in the CoaLAB 6000 PT programme.

Determination of MNPT
- Bring all the reagents to room temperature.
- Enter the Routine Menu.
- Load the required numbers of normal plasmas into separate sample cups and assign them appropriate identity nos.
- Create Job list of PT test for the loaded samples.
- Load the required amount of UNIPLASTIN® system pack reagent in the reagent cup.
- Measure the prothrombin time for all the loaded samples using the existing PT programme.
- Calculate the mean PT value of the total number of samples. This is the MNPT value.
- Edit the test parameters of the existing PT test programme with the obtained MNPT value as mentioned below:
  1. Enter the Test menu in CoaLAB6000:
  2. Modify test: Enter
  3. Select test: PT: Enter
  4. PT parameter: 2° conversion: Enter
  5. INR: Enter the MNPT value in seconds for 100%

* It is recommended by WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT results are dependent on the combination of reagent lot, instrument and technique followed at each laboratory. Usually plasma from at least 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.

The MNPT is applicable only for the same Lot of UNIPLASTIN® system pack reagent.

Programming of ISI value
Follow steps 1-5 as mentioned in procedure for determination of MNPT;
- Enter
- ISI: Enter the ISI value mentioned at the end of the UNIPLASTIN® system pack package insert

The ISI is applicable only for the same Lot of UNIPLASTIN® system pack reagent.

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 µl</td>
<td>150 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>FNP</td>
<td>600 µl</td>
<td>300 µl</td>
<td>450 µl</td>
<td>700 µl</td>
</tr>
</tbody>
</table>

- Enter the Routine Menu.
- Load each of the neat and diluted FNP in separate sample cups and assign them appropriate identity nos.
- Load the required amount of UNIPLASTIN® system pack reagent in the reagent cup as per the PT programme
- Measure the prothrombin time for each of the neat & diluted FNP using the existing PT programme.
- Edit the test parameters of percentage activity with the obtained PT time for the respective concentration of FNP as mentioned:
1. Enter the **Test menu** and edit data as mentioned below:
2. Modify test: Enter
3. Select test: PT: Enter
4. PT parameter: 1st conversion: Enter
5. Curve interp: Enter
6. Enter the obtained PT time in seconds for the respective % activity

These test parameters are applicable only for the same lot of UNIPLASTIN® system pack reagent.

**Test procedure for the samples**
1. Enter the **Routine Menu**
2. Load the required number of sample plasmas in separate sample cups giving appropriate identity nos. and create joblist of PT test for sample plasmas.
3. Load the required amount of UNIPLASTIN® system pack reagent in the reagent cup.
4. Measure the thromboplastin time for the samples.

**EXPECTED VALUES**

Normal values using UNIPLASTIN® system pack reagents are between 10-15 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 its own normal range based on procedure and instrument in use. It is mandatory that each laboratory must establish its own MNPT for each lot of UNIPLASTIN® system pack.

**Oral Anticoagulant Therapeutic Range:** INR = 2.0 - 3.5.

**REMARKS**

1. It is recommended that controls (PLASMATROL H-I/II 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 hemolysed 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, butabarbital, 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.

**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**