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 antibiotics, barbiturates, 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 can 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
6. WHO Expert Committee on Biological Standardization, No.687, 1983.
7. Data on file: Tulip Diagnostics (P) Ltd.
withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20SWG.
The venipuncture 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 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**

Applications suitable for Hemostar Auto and Coalab are available on request.

**General Application parameter set up**

A defined application for the UNIPLASTIN® system pack must be installed in accordance with the general instrument settings given below. For instructions refer to the respective instrument manual.

**General instrument settings**

**Parameters/Suggested Applications**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Start</th>
<th>Incubation</th>
<th>Read clotting time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chat</td>
<td>60-90 secs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Determination of MNPT**

Bring all the reagents to room temperature. 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 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. 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 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>
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</table>

Determine the clotting time of each of the above dilutions using the instrument protocol.

**Test procedure for the samples**

- Load the required number of sample plasmas giving appropriate identity nos. and create joblist of PT test for sample plasmas.
- Load the required amount of reagent in the reagent cup.
- Measure the thromboplastin time for the samples.

**Quality control**

- The performance of Uniplastin 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. Ensure that sufficient amount of sample and reagents are present as per the requirement of the instrument protocol.

**CALCULATIONS**

The results are automatically calculated by the analyzer and may be reported in Seconds, Ratio, INR and % activity as per the programmed protocol.

**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 their 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.

**PERFORMANCE CHARACTERISTICS**

The Precision of Prothrombin time determination is highly dependent on the method used. Precision studies were performed on Coalab 6000 coagulometer by assaying normal and abnormal control plasmas with UNIPLASTIN® system pack reagent. 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).

**Determination of MNPT**

Bring all the reagents to room temperature. Load the required amount 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 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. 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 reagent.

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Determine the clotting time of each of the above dilutions using the instrument protocol.

**UNIPLASTIN® system pack**

is useful for measuring the deficiencies of factors of the extrinsic pathway. The factor sensitivity of UNIPLASTIN® system pack 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.

**Activity of Factor (%)**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Clotting time with UNIPLASTIN® system pack with factor deficient plasma (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VII</td>
<td>100</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>50</td>
</tr>
<tr>
<td>Factor IX</td>
<td>25</td>
</tr>
<tr>
<td>Factor X</td>
<td>12.5</td>
</tr>
<tr>
<td>Factor V</td>
<td>5.25</td>
</tr>
<tr>
<td>Factor II</td>
<td>3.12</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.

**REMARKS**

1. It is recommended that controls (PLASMATROL H-II Available from Tulip Cat. No. - 11040261, 11041001) 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, stercor, Iepisic 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.