For example:

Reagent ISI=2.5	*Test Day 1*	*Test Day 2*	*Test Day 3*
Patient PT (sec)	16.0	16.0	16.0
Normal Control (10.4-12.3 sec)	11.5	10.4	12.3
INR Formula	$16.0^{2.5}$	$16.0^{2.5}$	$16.0^{2.5}$
$[R]^{*SI}$	11.5	10.4	12.3
Resulting INR	2.27	2.89	1.92

If the control time is greater than the mean normal range (MNPT), the PT ratio for any patient, PT will be smaller, potentially leading to over coagulation. If the control time is lesser than MNPT the ratio for any patient PT will be greater, leading to under coagulation.

On the other hand, MNPT for a particular laboratory using the same combination of methodology, reagent and instrument would remain constant.

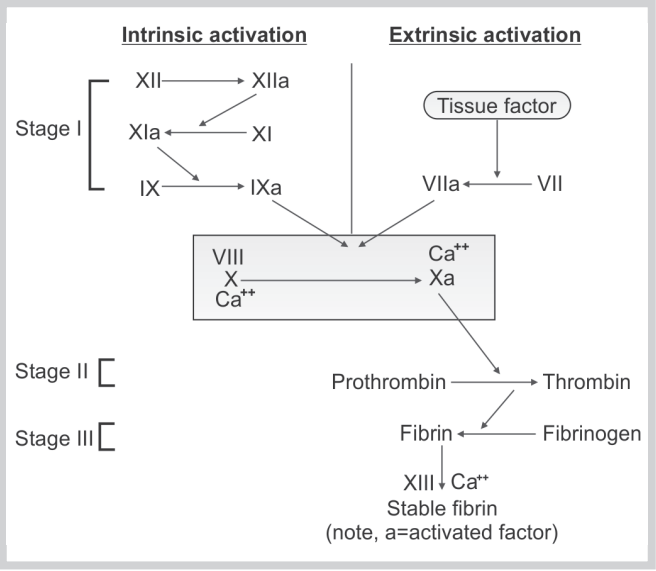
Quality Control Aspects

- Quality of water used for reconstituting lyophilized coagulation reagents must be good.
  - The water used for reconstitution of lyophilized coagulation reagents should be at least distilled twice and kept separately labeled for "coagulation studies". The reagents employed for coagulation studies are extremely delicate and inability to use good quality distilled water could lead to incorporation of metallic impurities in the reagent formulation as well as change in pH. Such changes can alter reaction kinetics and overall stability and performance of reagents.
- Quality assurance for coagulation-based reagents must be performed preferably on a daily basis.
  - Each laboratory should test coagulation reagents with normal and abnormal control plasma specimens at the beginning of each day's work to verify instruments, temperature calibration and also reagent performance.

If the control results fall within the stated limits, the test results are considered valid.

But if the results fall outside the stated control limits then the reagents, control and equipments are checked and the problem should be corrected.

Control results should be recorded and analyzed after regular intervals to ascertain the long-term validity of results.



Clotting mechanism—cascade system

PROTHROMBIN TIME (QUICK ONE-STAGE METHOD) LIQUIPLASTIN®

(*Courtesy:* Tulip Group of Companies)

Thromboplastin Reagent for Prothrombin Time (PT) Determination

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 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 patients on oral anticoagulant therapy and as a liver function test.

Reagent

Liquiplastin® 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 to 8°C. Do not freeze.
(b) The shelf-life of reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable for: 1 year at 2 to 8°C, 1 week at 18 to 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 Liquiplastin® reagent is added to normal anticoagulated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period. 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. Liquiplastin® reagent is not from human source hence, contamination due to HBsAg and HIV is practically excluded.
3. Liquiplastin® reagent contains 0.01% Thimerosal as preservative.
4. It is very important that clean and dry micropipette tips be used to dispense the reagent.
5. Avoid exposure of the reagent to elevated temperatures and contamination. Immediately replace cap after use and store at recommended temperatures only.

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 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 trisodium citrate (0.11 mol/L, 3.2%) or Profact available from Tulip; For occasional patients with hematocrit less than 20% or greater than 50%, 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 dean test tube. It should be ensured that the plasma is free from platelets (PPP). Cap the test tubes to prevent deterioration of samples. Plasma must be tested preferably immediately. However if the specimen are held at 22 to 24°C then they may be tested within 2 hours and if the specimen is held at 2 to 4°C then they may be tested within 3 hours.

Additional Material Required for Manual and Calibration Curve Methods

12 × 75 mm test tubes (plastic tubes are preferred), 0.1 mL and 0.2 mL precision pipettes, Stop watch, Water bath or heating block at 37°C, fresh normal plasmas for establishing MNPT.

Test Procedure

Manual Method

1. Aspirate from the reagent vial enough reagent for immediate testing requirements in a thoroughly clean and dry test tube (plastic test tubes are preferred).
2. Bring this reagent to room temperature before prewarming at 37°C for testing purposes.
3. Recap the reagent vial and replace immediately to 2–8°C.
4. To a 12 × 75 mm tube add 0.1 mL of plasma and place the tube in a water bath for 3 to 5 minutes at 37°C.
5. To the tube forcibly add 0.2 mL of Liquiplastin® reagent (prewarmed at 37°C for at least 3 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
6. Gently tilt 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 samples.
8. Find the average of the duplicate test values. This is the prothrombin time (PT). If a coagulation instrument is being used to perform the tests, the instrument manufacturer's instructions must be strictly adhered to.

Calculation of Results

Manual Method

The result 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':

$$R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent}}$$

Or as international normalized ratio (INR), INR = $(R)^{ISI}$ 'where ISI = International sensitivity index of the reagent (Refer reagent vial label).'

It is recommended by the 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.

Expected Values

Normal values using Liquiplastin® are between 10 and 14 seconds. Between manual and Turbodensitometric instrument results a variation of 1 to 2 seconds may be expected. For photo-optical instruments, it is recommended that each laboratory must establish their own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of Liquiplastin®.

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) 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) Glasswares and cuvettes 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, oral contraceptives, 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. (12) Homogenization of Liquiplastin® reagent suspension before use is important to achieve accurate and consistent results.

SENSITIVE THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT) DETERMINATION (ISI=1.0) UNIPLASTIN®

(Courtesy: Tulip Group of Companies)

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 VII, X, V, prothrombin and fibrinogen. During oral anticoagulant therapy most of the vitamin K-dependent factors, such as II, VII, IX, X, protein C and protein S 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, as a liver function test, determination of congenital deficiency of factors II, V, VII and X and for monitoring of patients on oral anticoagulant therapy.

Reagent

Uniplastin is a novel, highly-sensitive, low opacity, ready to use liquid calcified thromboplastin reagent, which is derived from rabbit brain. Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.