9. 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 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':

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

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 atleast 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 Uniplastin are between 11–15 seconds. Between manual and turbodensitometric instrument results a variation of 1–2 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 Uniplastin.

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 hemolysed 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 to 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, 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 UNIPLASTIN reagent suspension before use is important to achieve accurate and consistent results.

THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT) DETERMINATION, LYOPLASTIN® (LYOPHILIZED REAGENT, ISI=1.0)

(*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

Lyoplastin is a sensitive, lyophilized 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.

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 reconstituted Lyoplastin reagent can be used for 10 days when stored at 2–8°C provided it is not contaminated.
d. It is strongly recommended that enough reconstituted reagent should be retrieved for the days use and the unused reagent should be immediately replaced to 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 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 reagent for laboratory and professional use only. Not for medicinal use.
2. Lyoplastin reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.
3. It is very important that scrupulously clean and dry micropipette tips be used to aspirate/dispense the reagent.
4. Avoid exposure of the 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.

Additional Material Required

12 × 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 (25–30°C) prior to reconstitution. Lyoplastin® reagent is reconstituted with 3 mL de-ionized, distilled water as follows: (a) Add accurately 3 mL of distilled water to the lyophilized Lyoplastin® reagent, (b) Gently mix to dissolve, (c) Keep for 10 minutes and mix again gently ensuring complete resuspension of the lyophilized reagent. Avoid froth formation, (d) Thorough mixing should be ensured before withdrawing material every time for test purposes.

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 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. 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.

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 the reagent to room temperature before prewarming at 37°C for testing purpose.
3. Recap the reagent vial and replace immediately 2–8°C.
4. To a 12 × 75 mm tube add 0.1 mL of plasma (PPP) 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 Lyoplastin 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 sample.
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 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':

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

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 Lyoplastin® are between 11-15 seconds. Between manual and turbo densitometltric instrument results a variation of 1-2 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 Lyoplastin®.

Oral anticoagulant therapeutic range: INR = 2.0-3.5.

Remarks

1. lt 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 $\pm$ 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, 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.

Clinical Implications

Conditions accompanied by an increased prothrombin time (PT) include:

- Prothrombin deficiency
- Vitamin K deficiency
- Hemorrhagic disease of the newborn
- Liver disease (e.g. alcoholic hepatitis)
- Anticoagulant therapy
- Biliary obstruction
- Salicylate intoxication
- Hypervitaminosis A
- DIC disease.

Interfering Factors

1. *Diet*: Excessive amounts of green, leafy vegetables will increase body's absorption of vitamin K.
2. *Alcohol*: PT is increased due to liver disease.
3. *Diarrhea and vomiting*: These increase PT.
4. *Quality of venipuncture*: It is important that a clean and careful venipuncture is done, otherwise the PT can be shortened.
5. Many drugs can alter PT.

Clinical Alert

1. If PT is excessively prolonged, vitamin K is given intramuscularly. Ordinarily, intramuscular injections are contraindicated during anticoagulant therapy because large painful hematomas may form at the injection site. As values get into danger zones, assess carefully for bleeding, including: (i) craniotomy checks, (ii) lung auscultation (especially of upper lobes), and (iii) occult blood in the urine.
2. Patients who are being monitored by PT for long-term anticoagulant therapy should not take any drugs unless absolutely necessary.
3. When unexpected changes in anticoagulant doses are needed to maintain a stable PT, or when there is a consistent change in PT, a drug interaction should be suspected.
4. Blood for PT should be drawn for a base line and prior to administration of anticoagulants.
5. Protamine sulphate is the antidote for heparin.

The INR Method of Reporting Results

By definition INR represents the PT ratio which would have been obtained for a particular patient sample as if the WHO reference thromboplastin itself (ISI=1.0) had been used in the PT determination.

$$INR = [R]^{ISI}$$

$$INR = \left[\frac{\text{Patient PT in seconds}}{\text{Mean of the normal range}}\right]^{ISI}$$

A PT ratio is obtained by dividing the patient PT in seconds by the "mean of the normal range" (MNPT). This ratio is then "normalized" by raising the results to the power of the ISI of the PT reagent used. Lower the ISI of the reagent used, closer will be the INR to the observed PT ratio. Ideally, when the ISI of the reagent is 1.0 then the INR is a simple PT ratio since $(R)^{1.0} = R$.

Currently many coagulation instruments are available that can perform this exponential calculation by entering the ISI of the reagent in use. Alternatively a table is provided by reagent manufacturers for reading off "INR" directly for the given patient PT ratio, corresponding to the ISI value of the reagent used.

Recommended Therapeutic Ranges for Oral Anticoagulant Therapy

Indications	INR	Intensity
• Prophylaxis of venous thrombosis (high risk surgery) • Treatment of venous thrombosis • Treatment of pulmonary embolism • Prevention of systemic embolism – Tissue heart values – Acute myocardial infarction (to prevent systemic embolism) – Valvular heart disease	2.0–3.0	
• Atrial fibrillation • Mechanical prosthetic values (high risk) • Prevention of myocardial infarction recurrent	2.5-3.5	High

INR CONVERSION TABLE

ISI							
R	1.00	1.05	1.10	1.15	1.20	1.25	1.29
1.0	1.00	1.00	1.10	1.00	1.00	1.00	1.00
1.1	1.10	1.11	1.11	1.12	1.12	1.13	1.13
1.2	1.20	1.21	1.22	1.23	1.24	1.26	1.27
1.3	1.30	1.32	1.33	1.35	1.37	1.39	1.40
1.4	1.40	1.42	1.45	1.47	1.50	1.52	1.54
1.5	1.50	1.53	1.56	1.59	1.63	1.66	1.69
1.6	1.60	1.64	1.68	1.72	1.76	1.80	1.83
1.7	1.70	1.75	1.79	1.84	1.89	1.94	1.98
1.8	1.80	1.85	1.91	1.97	2.02	2.08	2.13
1.9	1.90	1.96	2.03	2.09	2.16	2.23	2.29
2.0	2.00	2.07	2.14	2.22	2.30	2.38	2.45
2.1	2.10	2.18	2.26	2.35	2.44	2.53	2.60
2.2	2.20	2.29	2.38	2.48	2.58	2.68	2.77
2.3	2.30	2.40	2.50	2.61	2.72	2.83	2.93
2.4	2.40	2.51	2.62	2.74	2.86	2.99	3.09
2.5	2.50	2.62	2.74	2.87	3.00	3.14	3.26
2.6	2.60	2.73	2.86	3.00	3.15	3.30	3.43
2.7	2.70	2.84	2.98	3.13	3.29	3.46	3.60
2.8	2.80	2.95	3.10	3.27	3.44	3.62	3.77
2.9	2.90	3.06	3.23	3.40	3.59	3.78	3.95
3.0	3.00	3.17	3.35	3.54	3.74	3.95	4.13
3.1	3.10	3.28	3.47	3.67	3.89	4.11	4.30
3.2	3.20	3.39	3.59	3.81	4.04	4.28	4.48
3.3	3.30	3.50	3.72	3.95	4.19	4.45	4.67
3.4	3.40	3.61	3.84	4.09	4.34	4.62	4.85
3.5	3.50	3.73	3.97	4.22	4.50	4.79	5.03
3.6	3.60	3.84	4.09	4.36	4.65	4.96	5.22
3.7	3.70	3.95	4.22	4.50	4.81	5.13	5.41
3.8	3.80	4.06	4.34	4.64	4.96	5.31	5.60
3.9	3.90	4.17	4.47	4.78	5.12	5.48	5.79
4.0	4.00	4.29	4.59	4.92	5.28	5.66	5.98
4.1	4.10	4.40	4.72	5.07	5.44	5.83	6.17
4.2	4.20	4.51	4.85	5.21	5.60	6.01	6.37
4.3	4.30	4.63	4.98	5.35	5.76	6.19	6.58
4.4	4.40	4.74	5.10	5.50	5.92	6.37	6.76
4.5	4.50	4.85	5.23	5.64	6.08	6.65	6.96
4.6	4.60	4.96	5.36	5.78	6.24	6.74	7.16
4.7	4.70	5.03	5.49	5.93	6.40	6.92	7.36
4.8	4.80	5.19	5.62	6.07	6.57	7.10	7.56
4.9	4.90	5.31	5.74	6.22	6.73	7.29	7.77
5.0	5.00	5.42	5.87	6.37	6.90	7.48	7.97
5.1	5.10	5.53	6.00	6.51	7.06	7.66	8.18
5.2	5.20	5.65	6.13	6.66	7.23	7.85	8.39
5.3	5.30	5.76	6.26	6.81	7.40	8.04	8.60
5.4	5.40	5.88	6.39	6.95	7.57	8.23	8.81
5.5	5.50	5.99	6.52	7.10	7.73	8.42	9.02
5.6	5.60	6.10	6.65	7.25	7.90	8.61	9.23
5.7	5.70	6.22	6.78	7.40	8.07	8.81	9.44
5.8	5.80	6.33	6.91	7.55	8.24	9.00	9.66
5.9	5.90	6.45	7.05	7.70	8.41	9.20	9.87
6.0	6.00	6.56	7.18	7.85	8.59	9.39	10.09

Other Factors Influencing the INR

The variability in the responsiveness of the PT reagents, is corrected through the "ISI" calibration, however, three additional technical factors influence the INR:

- Derivation of MNPT
- Magnitude of difference in the ISI value of test thromboplastin and IRP (ISI=1.0)
- Method of clot detection employed during PT test.

MNPT

MNPT is a critical requirement in the derivation of INR. Ideally each laboratory must derive its own MNPT from 20 or more normal patients for a given PT reagent and lot under use. This corrects within laboratory test variables that influence PT results. If "normal control plasmas" are used in place of patient plasma for arriving at the MNPT it can effect the evaluation of the patients level of anticoagulation. 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 $[R]^{ISI}$	$\frac{16.0^{2.5}}{\quad}$	$\frac{16.0^{2.5}}{\quad}$	$\frac{16.0^{2.5}}{\quad}$
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.

ISI Value of PT Used and Method of Clot Detection

INR loses some precision when comparisons are made with thromboplastins with markedly different ISI values as against the IRP (ISI=1.0) and different methods of clot detection, e.g. manual, mechanical, optical, etc.

Therefore, manufacturers must provide ISI values adapted to the method used for clot detection. Also the reagent used for reporting results should be ideally as close to 1.0 as possible.

Advantages of the INR system

- Major advantage of the INR system is that it helps alleviate confusion in the interpretation of PT results. Usually laboratory changes like change in thromboplastin and/or equipments could go unnoticed

by the attending physicians. the INR remains constant even with such changes.
- INR system affords comparison of PT results between laboratories.
- INR system provides a more accurate and convenient mean of monitoring patients who travel extensively.
- INR therapeutic ranges for different clinical conditions are based on international collaborative studies. Usage of standardized dosage reduces the risk of thrombotic episodes or secondary bleeding.

Disadvantages of the INR System

- The prothrombin time test is always a part of the preoperative screening panels. It is also frequently used to evaluate other hemostatic disorders, such as liver disease, DIC, LA, hereditary factor deficiencies and acquired vitamin K deficiency. Since these disorders have been excluded from the derivation of the ISI, INR has a diagnostic and therapeutic value mainly applicable for patients stabilized on oral anticoagulants. Therefore, laboratories may prefer to report both the INR and patients time in seconds depending on clinical application.
- The INR systems effectiveness would still depend on the calibration of the coagulation instruments as well as thromboplastin reagents used.
- Derivation of the correct MNPT and use of the mean normal range in each laboratory.
- Usage of thromboplastin reagents with ISI of preferably 1.0 or as close to 1.0 as possible.
- The correct use of the formula to compute the INR.
- Uniform understanding of the INR system by clinicians as well as laboratorians.

Patient Variables in PT/INR Testing

There are many factors that can influence the results of the PT/INR tests so that they do not reflect the patient's usual coagulation state. Coagulation tests are susceptible to errors introduced by suboptimal specimen quality because of a number of factors such as blood collection technique, labile state of several coagulation proteins, and laboratory transportation factors. In order to get acceptable accuracy it is important to understand and control these factors as much as possible.

Factors that Influence Coagulation Test Results

Age and Gender

Age specific reference ranges are critical for correct interpretation of coagulation data. Bleeding time declines with age and many coagulation factors increase with age as do markers of coagulation activation. Age and gender can also influence platelet function. Females tend to have longer bleeding times than males.

Blood Type

Type O individuals have significantly lower von Willebrand factor and factor VIII activity than subjects with type A, B, or AB. This causes increased bleeding and dotting times.

Within Day Variation

Incidences of platelet activation are highest in the mornings, resulting in increased coagulation activation.

Seasonal Variation

Increased coagulation activity has been described in cold weather.

Intraindividual Variability

Many coagulation analytes are less precise than other analytes and thus can give variable results within the same individual.

Diet, Alcohol and Smoking

Cardiac risk factors can increase coagulation factor level/activation. Smoking elevates plasma fibrinogen. Von Willebrand factor, thrombin generation and platelet activation may all have an effect causing variability. Moderate ethanol intake inhibits platelet reactivity and increases fibrinolysis and INR.

Medications

A number of other medications, including hormone replacement therapy, selective estrogen receptor, modifiers and oral contraceptives can alter coagulation and raise the INR. In addition, non-steroidal anti-inflammatory drugs, antibiotics and fluoroquinolones can also alter the INR.

Menstrual Cycle, Pregnancy

Significant hormonally determined changes in coagulation factors, inhibitors, fibrinolysis and activation markers must be considered as interpretation of the results.

Diseases

States, which lead to anemia, polycythemia or hemolysis or uremia, can also interfere with coagulation tests.

Physical and Emotional Stress

These are commonly associated with increased coagulation and platelet activation.

Posture

Values can change from supine to upright positions due to the shift of water and subsequent reduction in plasma volume. Hence, standardization of posture is recommended.

Venous Occlusion

Traumatic or prolonged phlebotomy accentuates the hemostatic activation, producing artificially altered coagulation times.

Vitamin K

Certain fat substitutes in some snackitems contain unspecified amount of vitamin K. Green, leafy vegetables and green tea also contain high levels of vitamin K. This can have an impact on serum vitamin K levels and the INR can drop as a result. Alternative medicines: According to the AANA (American Association of Nurse Anesthetists) some sources, certain herbal drugs can cause interference in coagulation cycles, falsely elevating the INR.

Anticoagulant Therapy

It is of utmost importance to bear in mind that patients on heparin will show inaccurate INR results.

While certain pre-analytical factors are not entirely controllable, every effort must be made to ensure that most conditions have been stable for a period of time. Patient preparation and blood collection should be standardized according to the guidelines.

Prothrombin Determination (Two-stage Method)

Principle

Prothrombin in the presence of optimal procoagulants and calcium will form thrombin. The amount of thrombin formed can be calculated by determining the dilution of plasma that will clot a standard fibrinogen reagent in a specific period of time. The amount of thrombin formed is a measure of the amount of prothrombin present in the starting sample.

The test consists of two stages. In the first stage, prothrombin is incubated with a standard mixture containing thromboplastin, calcium, a buffer and a source of procoagulants. In the second stage, samples of the incubating mixture are added to a standard fibrinogen solution and the clotting time is determined.

Results

1. The object of the procedure is to determine the dilution of plasma from which will evolve one unit of thrombin under optimal conditions. A unit of thrombin is defined as that amount which will form a clot of 1 mL of fibrinogen in 15 seconds under standard conditions.
2. If varying amounts of thrombin are added to standard amounts of fibrinogen the clotting time of the mixture is an index of the thrombin concentration within a specific range. When thrombin concentrations are plotted against clotting times, the results describe a hyperbolic curve. With thrombin concentrations between 0.80 and 1.34 units, there is good correlation between thrombin concentration and clotting time. With greater amounts of thrombin, there is little change in the speed of clotting, with relatively large changes in thrombin concentration. With lesser amounts of thrombin, small changes in thrombin concentration result in large changes in the speed of clotting.

APTT/PTTK CEPHALOPLASTIN REAGENT FOR PARTIAL THROMBOPLASTIN TIME (APTT) DETERMINATION USING ELLAGIC ACID AS ACTIVATOR LIQUICELIN-E®

(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 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. Activated partial thromboplastin time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, IX, VIII, X, V, II and fibrinogen. Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy.

Reagent

Liquicelin-E® is a liquid ready to use activated cephaloplastin reagent for the determination of activated partial thromboplastin time. It is a phospholipid preparation derived from rabbit brain with ellagic acid as an activator.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.