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.

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

Principle

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions. APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation; inhibitors like heparin.

Note

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Liquicelin-E, 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 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

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without 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 tubes, after detaching the needle from the syringe.

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; Centrifuge immediately for 15 minutes at 3000 rpm (approximately 2000 g) and transfer the plasma into a clean test tube. Plasma must be tested within three hours of blood collection. For heparin determination, platelet deficient plasma should be used, hence higher centrifugation time is required.

FNP Collection

Prepare a plasma pool (FNP) of freshly collected blood from at least five normal healthy donors and process as above. Plasma must be tested within three hours of blood collection.

Additional Material Required*

12 × 75 mm test tubes; 0.1 mL, 0.2 mL and 2.0 mL precision pipettes; Stopwatch; Water bath or heating block 37°C; Fresh normal pooled plasma; $CaCl_2$ (0.02 mol/L).

*Available from Tulip Diagnostics.

Test Procedure

Manual Method

1. Before use, the reagent should be mixed well by gentle swirling. Do not shake.
2. Aspirate from the reagent vial enough reagent for the immediate testing requirement in a thoroughly clean and dry test tube. Bring this reagents to room temperature before prewarming at 37°C for testing purposes.
3. Separate test tubes containing Liquicelin-E® and Tulip's calcium chloride solution should be brought to 37°C (depending on volume, approximately 5 to 10 minutes required). Do not incubate the test plasma.
4. To a 12 × 75 mm test tube, add 0.1 mL test plasma and 0.1 mL Liquicelin-E®. Shake tube briefly to mix the reagent and plasma, place tube at 37°C for 3 to 5 minutes.
5. Following incubation period, add forcibly 0.1 mL prewarmed calcium chloride into the plasma and Liquicelin-E® mixture, simultaneously start a stopwatch. Shake tube briefly to mix contents, keep at 37°C for 20 seconds.
6. Following 20 seconds incubation, remove the tube, gently tilt back and forth until a gel clot forms, stop the watch, record time.
7. Repeat steps 2–4 for a duplicate test using the same test plasma.
8. Find the average from the duplicate test values. This is the activated partial thromboplastin time (APTT of patient plasma).
9. Similarly repeat steps 2-4 twice, and record duplicate values using FNP in place of test plasma (APTT of FNP).

If a coagulation instrument is being used to perform the tests, the instrument manufacturer's instructions must be strictly adhered to.

Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/mL.
2. Mix 0.2 mL of 10 U/mL diluted heparin with 1.8 mL of FNP to give a heparin standard of 1 U/mL concentration.
3. Dilute the heparin standard as prepared above (1 U/mL) with FNP as follows :

Test tube No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Heparin standard (1 U/mL) in mL	0.5	0.4	0.3	0.2	0.1	0.1	-
FNP in mL	-	0.1	0.2	0.3	0.4	0.9	0.5
Heparin concentration (U/mL)	1	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 mL each of the seven heparin dilutions into clean test tubes.
5. Add 0.1 mL Liquicelin-E® reagent to each test tube.
6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
7. Forcibly add 0.1 mL calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.
8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds.
9. Repeat steps 4–8 for each dilution for duplicate test, and find the average of the duplicate test values.
10. Plot the mean of the double determination in 'seconds' against each heparin concentration using Liquicelin-E® graph paper.
11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/mL.

Calculation and Reporting of Results

Manual Method

a. The result may be reported directly in terms of the mean of the double determination of the APTT of the test plasma.

OR

b. As a ratio R as follows:

$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$$

Calibration Curve Method

Heparin concentration in the test sample can be directly obtained from the Liquicelin-E® calibration curve by interpolating the test plasma clotting time against the heparin concentration in U/mL.

Expected Values

Normal values using Liquicelin-E® reagent are between 21 and 29 seconds at 3 minutes activation time. 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.

Remarks

1. Due to inter and intralaboratory variations users must establish their own normal population range as well as normal and abnormal range.
2. It is recommended that controls with known factor activity should be run simultaneously with each test series routinely.
3. Incorrect mixture of blood and trisodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware, etc. are potential source of errors.
4. Incorrect dilutions of heparin is also a potential source of error.
5. Oxalated plasma may induce prolonged clotting times.
6. 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.
7. Abnormalities of coagulation factor VII, factor XIII and platelets are not detected by this test procedure.
8. For automated equipment, it is strongly recommended that the equipments manufacturer's methodology is strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the in vivo half-life of heparin is approximately 1.5 hours. When it is administered intravenously, it has an immediate anticoagulant effect but its efficacy decreases rapidly with time.
10. Platelet factor IV, a heparin-neutralizing factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in vitro the specimen should be collected with a minimum of trauma.
11. Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

Clinical Implications of APTT

1. The APTT is prolonged in all coagulation defects of stage I (includes platelet activity and thromboplastin).
2. The APTT is usually prolonged in Willebrand's disease and is accompanied by a consistently diminished factor VIII level.
3. The APTT and PT will detect 95% of coagulation abnormalities. When APTT is performed in conjunction with a prothrombin time (PT), a further clarification of

coagulation defects is possible. For example, a normal PT and abnormal APTT means that the defect lies in the first stage of the clotting mechanism.

Causes of prolonged APTT

- Hemophilia
- Vitamin K deficiency
- Liver disease
- Presence of circulating anticoagulants
- DIC disease (chronic or acute).

Shortened APTT occurs in:

- Extensive cancer, except when liver is involved
- Immediately after acute hemorrhage
- Very early stages of DIC.

Circulating Anticoagulants

Usually occurs as an inhibitor of a specific factor (e.g. factor VIII). Most commonly seen in the development of anti-factor VIII or anti-factor IX in 5 to 10% of hemophiliacs. Anticoagulants that develop in the treated hemophiliac are detected by prolonged APTT. Circulating anticoagulants also can be detected in some cases:

- Following repeated plasma transfusions
- Drug reactions
- Tuberculosis
- Chronic glomerulonephritis
- Systemic lupus erythematosus
- Rheumatoid arthritis.

NORMAL AND ABNORMAL CONTROL PLASMAS FOR COAGULATION ASSAYS PLASMATROL H-I/II®

(*Courtesy:* Tulip Group of Companies)

Summary

Tulip Plasmatrol H-l and Plasmatrol H-ll are two level human plasma controls that are suitable for use as normal and abnormal control plasma for PT, APTT, TT and fibrinogen testing using clot based methods. Coagulation controls provide a means of day-to-day quality control in the hemostasis laboratory for control of accuracy and precision.

Reagent

Plasmatrol is a stabilized and freeze dried preparation of selected human plasma with values determined and assigned for specific clot based tests, which are lot specific. The plasma controls are assayed using Tulip coagulation reagents.

Reagent Storage and Stability

Unopened vials should be stored at 2–8°C and are stable up to the expiry date mentioned on the vial labels. After reconstitution the shelf life of the control plasma is 3 hours at 25–30°C and 8 hours when stored at 2–8°C.

Principle

The properties of the control plasma are similar to those of pooled fresh plasmas. Since, the plasma controls have assigned values, when substituted in place of a sample, in clot based coagulation assays, they can be used for laboratory quality assurance.

Note

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The source material used for preparation of the reagent is screened by third generation assays for HBsAg, HCV and HIV antibodies and are found to be non-reactive. However, handle the material as if it is infectious, as no known test method can assure that infectious agents are absent.

Preparation of the Reagent

1. Reconstitute the control plasma with exactly 1 mL of bi-distilled water. Avoid using water-containing preservatives.
2. Re-stopper the vial and allow to stand until, the hydration is complete (usually 5–7 minutes).
3. Mix by gently swirling and inversion, avoiding froth formation. Do not shake.
4. Allow to stand and equilibrate for a further 15 minutes before use.
5. Use the reconstituted plasma within 3 hours of reconstitution.

Test Procedure

1. Use the reconstituted Plasmatrol controls in the same manner as freshly prepared titrated platelet poor plasma from a patient.
2. Use the procedure as laid out in the Uniplastin, Liquiplastin®, Liquicelin-E®, Fibroscreen, Fibroquant pack inserts.

Expected Values

1. The expected value of specific assays are provided on the assay value sheet accompanying each kit, and are lot specific.