2. Fibrin gels may form in plasma with a fibrinogen concentration below normal. However, these gels are not firm, extrude considerable serum, and tend to slide on the side walls of the tilted test tube. Careful comparison of such gels with the firm clot with normal plasma used as a control will eliminate the possibility of confusion.
3. Fibroscreen thrombin time test is usually performed first before any specific assays are attempted, when a prolongation of (PT and APTT) cannot be explained.

FIBRINOGEN ESTIMATION-QUANTITATIVE FIBROQUANT, REAGENT FOR QUANTITATIVE ESTIMATION OF FIBRINOGEN

(*Courtesy:* Tulip Group of Companies)

Summary

At present there are known to be atleast eleven factors in circulating blood, which are required for normal hemostasis. Deficiency in any of these factors viz factors I, II, V, VII, VIII, IX, X, XI and XIII, results in a notable hemorrhagic condition, and the severity of the bleeding is proportional to the degree of deficiency. In order to treat the hemorrhagic condition, it is important to identify and quantify the deficient factor.

Fibrinogen (Factor I) is a high molecular weight glycoprotein synthesized in the liver, which plays an important role in hemostasis. For normal hemostasis to occur in response to injury or tissue damage, a sufficient concentration of fibrinogen must be present in plasma. Fibrinogen is converted into fibrin by the action of thrombin and is a key component of clot formation.

Fibroquant kit contains lyophilized thrombin and fibrinogen calibrator to determine the quantitative reactivity of fibrinogen. Since the reagent system contains heparin neutralizing substances, heparin levels up to 0.4 IU/mL does not interfere with test results.

When used as a front line test with PT, APTT, platelet count and thrombin time, fibrinogen assay helps in investigating acute hemostatic failure.

Reagent

Fibroquant kit contains:

1. Thrombin reagent, which is a lyophilized preparation from bovine source ~50 NIH units per vial.
2. Fibrinogen calibrator, which is a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on a mg basis (refer Fibroquant graph paper supplied with each kit for the value of each lot).
3. Owren's buffer, ready to use (pH 7.35).

Storage and Stability

1. Store the unopened reagent vials at 2–8°C. Do not freeze.
2. The shelf-life of the reagents is as per the expiry date mentioned on the reagent vial labels.
3. Once reconstituted the Fibroquant thrombin reagent is stable for 6 days when stored at 2-8°C and for 4 hours at room temperature (20–25°C), provided it is not contaminated. Extreme care has to be taken to maintain aseptic precautions while reconstituting, retrieving and handling reagents to prevent contamination. The reagent vial must be replaced to 2–8°C immediately upon retrieving the reagent for the day's work.
4. The reconstituted Fibroquant fibrinogen calibrator is stable for 6 hours at 2–8°C and for 2 hours at room temperature (20–25°C).

Principle

The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

Note

1. In vitro diagnostic reagent for laboratory and professional use. Not for medicinal use.
2. The individual reagents contain 0.1% sodium azide as preservative.
3. Fibroquant thrombin reagent is not from a human source hence, contamination due to HBsAg, HIV and HCV is practically excluded.
4. Fibrinogen calibrator provided in the Fibroquant kit is from a human source, which was tested and found to be non-reactive for HBsAg, HCV and HIV. However, no known test methods can assure that infectious agents are absent. Handle all human products as potentially infectious.
5. It is very important that absolutely clean and dry micropipettes be used to aspirate and dispense the reagent.
6. Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace the cap after use and store at recommended temperature.

Quality Control

A known normal control should be run in parallel with each batch of tests. This control may be Tulip plasma coagulation control Plasmatrol-I or freshly drawn normal plasma.

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 nine parts of freshly collected blood with one part of sodium citrate (0.109 mol/L, 3.2%). 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 3 hours of collection.

Additional Material Required

10 × 75 mm glass test tubes, 0.2 mL and 0.1 mL precision pipettes, stopwatch, water bath at 37°C, distilled water, automated, semiautomated/mechanical/optical instrument if applicable.

Procedure

Bring all the reagents and samples to room temperature before testing.

Procedure for Fibrinogen Calibration Curve Preparation

1. The Fibroquant thrombin reagent vial must be reconstituted exactly with one mL of distilled water; wait for 5 minutes, do not shake but gently swirl the vial till the solution attains homogeneity. Further keep the vial aside for 10 minutes to attain equilibrium. Once reconstituted it is ready to use for the fibrinogen test.
2. The Fibroquant fibrinogen calibrator vial must be reconstituted with exactly one mL of distilled water; wait for 5 minutes, do not shake, gently swirl the vial till the solution attains homogeneity. Further keep the vial aside for 10 minutes to attain equilibrium. This is the fibrinogen calibrator stock solution.
3. Dilute fibrinogen calibrator stock solution with Owren's buffer as follows:

Test tube no.	*I*	*II*	*III*
Owren's buffer	NIL	0.8 mL	0.9 mL
Fibrinogen calibrator	0.2 mL	0.2 mL	0.1 mL
Dilution (calibrator)	NIL	1:5	1:10

- Pipette 0.2 mL of each fibrinogen calibrator dilution into clean test tubes and prewarm for 3 minutes at 37°C.
- Add 0.1 mL of reconstituted thrombin reagent (prewarmed at 37°C for one minute) and simultaneously start stopwatch.
- Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.
- Repeat steps 1 to 3 for a duplicate test one each calibrator dilution.
- Plot the average of the duplicate test values on Tulip firbrinogen graph paper*.
- Connect the points, which should produce a straight line.
- The calibration curve may be extended beyond the lowest and highest point.

*The calibration curve is valid only for the same lot of Fibroquant thrombin reagent.

Test Procedure for Sample

1. Prepare a 1:10 dilution of plasma specimen with Owrens buffer solution.
2. To a 10 × 75 mm test tube at 37°C add 0.2 mL of 1:10 dilution of plasma sample to be tested.
3. Incubate at 37°C for one minute.
4. To the test tube add 0.1 mL of Fibroquant thrombin reagent (prewarmed at 37°C for one minute) and start the stopwatch simultaneously.
5. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.
6. Repeat steps 1-5 for a duplicate test.
7. If at the sample dilution of 1:10 the observed clotting time is usually between 8 and 25 seconds, the fibrinogen content is normal (Fibrinogen content between 150 and 400 mg/dL). Assay results can be read off directly from the graph paper provided with the Fibroquant kit for the fibrinogen concentration.
8. If the fibrinogen content is high the clotting time will be less than 8 seconds. In such cases repeat the test at 1:20 dilution of the sample or 1:30 dilution of the sample. The results read off the graph will be multiplied by a factor 2 or 3 for the respective dilution.

9. Conversely, if fibrinogen content is low, the clotting time will be over 25 seconds. Repeat the assay at 1:5 dilution, or if necessary at 1:2 dilution. In this case the results read off the graph will be divided by a factor of 2 or 5 for the respective dilutions.

This procedure can also be performed on an automated/semiautomated mechanical/optical instrument but the equipment manufacturer's methodology should be strictly adhered to.

Remarks

1. Significant levels of heparin and elevated levels of fibrinogen degradation products (FDP) in the patient plasma can cause falsely low fibrinogen results.
2. Insufficient prewarming of plasma and reagent or contaminated glassware may cause erroneous results.
3. EDTA should not be used as an anticoagulant.
4. Use reagents of the same lot for performing the test.
5. Do not interchange reagents from different lots.

FIBRINOLYTIC ACTIVITY

The three methods presented below are measures of fibrinolytic activity in general and are influenced by many factors. These serve as screening procedures, but the specific contribution of the various factors must be determined by other means. The lack of suitable standards makes quantitative measurements unavailable for most diagnostic laboratories.

Euglobulin Lysis Time

Principle

Euglobulin fraction of plasma contains fibrinogen and all the plasminogen activator and plasminogen of plasma but only traces of the anti-plasmins. The lysis of a fibrin clot formed by the addition of thrombin is a measure of the fibrinolytic activity.

Requirements

1. Equipment for collection of blood
2. Centrifuge
3. Topical thrombin
4. Serological pipettes
5. Carbon dioxide. A tank of CO_2 fitted with a valve to allow control of the rate of flow.

Method

1. Blood is collected in the usual manner and mixed immediately with 0.11 M sodium citrate in a ratio of 1 part citrate solution to 9 parts blood.
2. Plasma is obtained by centrifugation.
3. 0.4 mL plasma is placed in a test tube and 7.6 mL distilled water is added.
4. CO_2 is bubbled into the solution through a capillary tube for 30 seconds.
5. The precipitate which forms is collected by centrifugation at about 3,000 rpm for 15 minutes.
6. The precipitate is dissolved in 1 mL M/15 phosphate buffer, pH 7.2.
7. To the euglobin in phosphate buffer, 0.1 mL thrombin (topical thrombin diluted to 100 units per mL with saline) is added. The solution is mixed.
8. Clotting should be rapid. After clotting has occurred, the tube is placed in water bath (37°C) and observed for lysis of clot, which is the end-point.

Result

In normal plasma, a period of 2 to 4 hours is required for euglobin clot lysis to occur (the technique should be standardized in each laboratory).

Dilute Blood Clot Lysis Time

Principle

Plasmin inhibitors lose activity on dilution to a greater extent than fibrinolytic activity. Whole blood is diluted with a buffer solution and clotted by the addition of thrombin. The clot is observed for lysis of the clot.

Requirements

1. Equipment for collection of blood sample
2. Test tube
3. Timer
4. Phosphate buffer, pH 7.4. To 1000 mL distilled water, 9.47 g Na_2HPO_4 is added and dissolved. This is mixed with 250 mL distilled water containing 3.02 g KH_2PO_4
5. Topical thrombin (or any other make) diluted to 100 units per mL with normal saline.

Method

1. Tubes containing 1.70 mL buffer and 0.1 mL thrombin solution are placed in an ice bath.
2. Collect blood sample in standard manner using a syringe that can deliver accurately 0.2 mL aliquots of blood.
3. Add 0.2 mL blood to each of two tubes containing buffer and thrombin and mix.
4. Clotting should occur promptly.
5. Tubes are placed in refrigerator (4°C) for 30 minutes and then transferred to a water bath at 37°C.