2. The expected values are obtained using replicate assay of each manufactured lot of Plasmatrol, manually and using mechanical coagulometers such as Hemostar, Hemostar XF.
3. The individual laboratory values should fall within the expected values.
4. It must however be noted that each laboratory should establish its own normal values and reference range according to GLP.

Remarks

1. When used appropriately, Plasmatrol controls are subjected to the limitations of the assay system deployed.
2. If proper values are not obtained it may indicate problems with one or more variables of the assay system.
3. Stability of the reagent is dependent on storage and handling conditions. Since these can vary between laboratories, each laboratory should determine the stability of the reagent under usual operating conditions.
4. Incorrect mixing of control plasma and reagent, insufficient preparation of plasma/reagent, contaminated reagents and glassware, etc. are a potential source of error.
5. Due to interlaboratory variations in techniques, standardization of test procedures and calibration of equipments, some variation from assigned mean values may be expected.

FIBROSCREEN THROMBIN TIME TEST FOR QUALITATIVE ESTIMATION OF FIBRINOGEN FIBROSCREEN®

(*Courtesy:* Tulip Group of Companies)

Summary

At present there are known to be at least 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.

Fibroscreen reagent is one such test reagent, which can identify the deficiency of factor I (fibrinogen). The reagent is used as a source of thrombin to determine the qualitative reactivity of fibrinogen.

Reagent

Fibroscreen reagent is a lyophilized preparation of bovine thrombin of 50 NIH/mL. Reconstitute with 1 mL of distilled water; wait for 5 minutes, do not shake and mix gently by swirling till the solution attains homogeneity. Further keep aside for 10 minutes to attain equilibrium. Gently swirl the vial while drawing the reagent for use. Once reconstituted it is ready to use reagent for the thrombin time test.

Storage and Stability

1. Store the unopened reagent vials at 2–8°C. Do not freeze.
2. The shelf-life of the reagent is as per the expiry date mentioned on the reagent vial label and carton label.
3. Once reconstituted the Fibroscreen 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 Fibroscreen reagent vial must be replaced at 2–8°C immediately upon retrieving the reagent for the day's work.

Principle

When a known quality and concentration of Fibroscreen reagent is added to citrated plasma, by observing the time required for clot formation and the quality of clot formed, a qualitative estimation of fibrinogen in the sample can be obtained.

Note

1. In vitro diagnostic reagent for laboratory and professional use. Not for medicinal use.
2. The reagent contains 0.1% sodium azide as preservative.
3. Fibroscreen thrombin reagent is not from a human source, hence contamination due to HBsAg, HIV and HCV is practically excluded.
4. It is very important that absolutely clean and dry micropipettes be used to aspirate and dispense the reagent.
5. Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace 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-M mol/L, 3.2%). Centrifuge immediately for fifteen 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 precision pipettes, stop-watch, distilled water, fresh plasma.

Procedure

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

Manual Method

Testing should be done in duplicate at room temperature (20-25°C):

1. To a clean and dry 10 × 75 mm test tube add 0.2 mL of plasma to be tested and 0.2 mL of the reconstituted Fibroscreen reagent.
2. Start a stopwatch simultaneously with the addition of the Fibroscreen reagent.
3. Shake the tube gently to mix the contents and then tilt the tube back and forth.
4. Note the time at the first appearance of the clot and for the remaining portion of 60 seconds for consistency and character of the clot formed.

Interpretation of Results

Normal plasma begins to show clot formation within 15 seconds after Fibroscreen reagent has been added. Because time of clot formation may be influenced by additional factors in the test system, estimation of approximate concentration of fibrinogen cannot be made from the initial clotting time alone but must be also made from observations of the consistency and character of the clot at 60 seconds. At 60 seconds, samples with normal fibrinogen levels will form a firm clot that adheres to the walls of the test tube when the tube is inverted. If either of these parameters are not met, (i.e. clotting time below 15 seconds or formation of a firm adhering clot after inversion of the test tube) abnormality (less than 100 mg%) of the fibrinogen reactivity should be suspected. In such cases quantitative estimation of fibrinogen using Fibroquant is strongly recommended.

Expected Values

A normal value using Fibroscreen reagent is the formation of a solid gel clot in 5-15 seconds, which adheres to the test tube wall on inversion at 60 seconds.

Remarks

1. Fibroscreen thrombin time remains normal in deficiencies of factor XIII (fibrin stabilizing factor).

INTERPRETATION OF FIRST LINE TESTS:

Test				*Condition*
PT	*APTT*	*TT*	*Platelet count*	
N	N	N	N	Disorder of platelet function, factor XIII deficiency, disorder of vascular hemostasis, normal hemostasis
Long	N	N	N	Factor VII deficiency, early oral anticoagulation
N	Long	N	N	Factor VIII: C, IX, XI, XII, prekallikrein, HMWK deficiency,von Willebrand's disease, circulating anticoagulant
Long	Long	N	N	Vitamin K deficiency, oral anticoagulants factor V, VII and II deficiency
Long	Long	N	N	Heparin, liver disease, fibrinogen deficiency, hyperfibrinolysis
Long	Long	N	N	Thrombocytopenia
Long	Long	N	Low	Massive transfusion, liver disease
Long	Long	Long	Low	DIC, acute liver disease

N-Normal

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.