REAGENT FOR QUANTITATIVE ESTIMATION OF FIBRINOGEN ON AUTOMATED COAGULOMETERS

SUMMARY
Fibrinogen (Factor I) is a high molecular weight glycoprotein synthesized in the liver, which plays an important role in haemostasis. For normal haemostasis 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.

Low levels of fibrinogen are found in:
- Liver disease.
- Increased fibrinogen consumption due to the prolonged presence of disseminated intravascular coagulation.
- Hypermucoidosis in patients with neoplasia, acute promyelocytic leukemias & Obstetric complications such as premature detachment of placenta or abruptio placentae, amniotic fluid embolism, retention of dead fetus.
- Dysfibrinogenemia (Functionally defective fibrinogen due to an abnormal molecular form, but the levels remain normal) found either congenitally or acquired in liver disease.

An increase in synthesis of fibrinogen along with hyperfibrinogenemia occurs during an acute phase response in tumors, trauma or burns. Chronic active inflammatory processes such as rheumatic diseases and collagen vascular diseases are associated with prolonged hyperfibrinogenemia.

Studies such as the Framingham study, the Northwick Park Heart Study have demonstrated that an increased fibrinogen concentration is an independent risk factor for atherosclerotic diseases such as myocardial infarction or stroke.

Fibrinogen estimation is also useful in monitoring of thrombolytic therapy. A pronounced decrease in fibrinogen (< 0.1 g/L) is observed in the case of Streptokinase and Urokinase whereas a moderate decrease in fibrinogen is observed in the case of t-PA and Prourokinase.

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

FIBROQUANT® System pack 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.

PRESENTATION

<table>
<thead>
<tr>
<th>REF</th>
<th>10642120</th>
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</thead>
<tbody>
<tr>
<td>Assays</td>
<td>120</td>
</tr>
<tr>
<td>Bovine Thrombin</td>
<td>6 x 1 ml</td>
</tr>
<tr>
<td>Owrens buffer (BUF)</td>
<td>6 x 10 ml</td>
</tr>
<tr>
<td>CAL</td>
<td>1 ml</td>
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<tr>
<td>Pack insert</td>
<td>1</td>
</tr>
</tbody>
</table>

REAGENT

FIBROQUANT® System pack kit contains:
1. Thrombin reagent (Bovine Thrombin), which is a lyophilized preparation from bovine source ~ 50 NIH units per vial.
2. Fibrinogen calibrator (CAL), which is a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen (C) on a mg/dl basis (refer end of package insert for the value of each lot).
3. Owrens buffer (BUF), ready to use (pH 7.35).

STORAGE AND STABILITY

1. Store the reagent 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 thrombin reagent is stable for 30 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® System pack 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. The thrombin reagent provided in the FIBROQUANT® System pack is not from a human source hence contamination due to HBsAg, HCV and HIV is practically excluded.
4. Fibrinogen calibrator provided in the FIBROQUANT® System pack 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 blood products as potentially infectious.
5. It is important to ensure that the reagent cup is clean and dry before dispensing the reagent into the reagent cup.
6. It is very important that absolutely clean and dry micropipettes / disposable tips be used to aspirate and dispense the reagent.
7. Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace cap after use and store at recommended temperature.
8. Do not transfer the reagent from the reagent cup into the reagent vial at the end of day's work. Discard the left over reagent.
9. Do not mix reagents of different lots in the reagent cups.
10. It is necessary to use Teflon stirrers in reagent cups for homogenizing the reagent to obtain accurate and consistent results.
11. The test procedure in this package insert has been designed for application on CoaLAB 6000 only. However these reagents can be programmed on other automated coagulometers, also provided the reagents have been standardised on them.

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 H-I, available from Tulip (Cat. No. 11040061) 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 venepuncture 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 (0.11 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
Test tubes, appropriate micropipettes and disposable tips, distilled water, Kaolin suspension, available from Tulip Cat. No. 20401010.

TEST PROCEDURE
A defined application for the FIBROQUANT® System pack must be installed in accordance with the general instrument settings given below. For instructions refer the respective instrument manual.

B) Procedure for Preparation of Fibrinogen Calibration Curve

For use on CoaLAB 6000: The thrombin reagent vial must be reconstituted exactly with 500 µl of kaolin suspension and 500 µl 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.

For use on other instruments: The thrombin reagent vial must be reconstituted exactly with 1000 µl 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.

The 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.
Dilute fibrinogen calibrator stock solution with Owrens buffer as follows:

<table>
<thead>
<tr>
<th>Test tube no.</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owren's buffer</td>
<td>NIL</td>
<td>450µl</td>
<td>700µl</td>
</tr>
<tr>
<td>Fibrinogen calibrator</td>
<td>600µl</td>
<td>150µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Dilution (calibrator)</td>
<td>NIL</td>
<td>1:4</td>
<td>1:8</td>
</tr>
<tr>
<td>Fibrinogen concentration (C)</td>
<td>4 X C</td>
<td>2 X C</td>
<td>C</td>
</tr>
</tbody>
</table>

Enter Routine Menu.
- Load each of the prepared calibrator dilutions into separate sample cups and assign proper identity nos.
- Create Job list of FBG test for the calibrator dilutions.
- Load the required amount of FIBROQUANT® thrombin reagent and Washing solution (marketed by Tulip) in the respective reagent positions.
- Measure the clotting time for the calibrator dilutions using the existing FBG programme.
- Edit the test parameters of the existing FBG test programme with the obtained clotting time values as mentioned below:
  1. Enter the Test menu in CoaLAB 6000
  2. Modify test: Enter
  3. Select test: FBG: Enter
  4. 1st conversion: Enter
  5. Curve interp: Enter
  6. Edit the clotting time in seconds corresponding to the fibrinogen concentration with the obtained clotting time in seconds for the respective fibrinogen concentration.

*The calibration curve is valid only for the same lot of FIBROQUANT® System pack reagent.

B) Test Procedure for samples
1. Dilute the plasma samples 1:8 with Owrens buffer.
2. Load each of the diluted sample plasmas into separate sample cups with appropriate identity nos. and create the job list of FBG test for the sample plasmas.
3. Load the required amount of FIBROQUANT® thrombin reagent and Washing solution in the respective reagent positions.
4. Determine the fibrinogen concentration for the sample plasmas using the FBG programme.

INTERPRETATION OF RESULTS
1. The fibrinogen concentration in 1:8 diluted plasmas represents the 100% fibrinogen concentration of the sample.
2. Samples with Fibrinogen mean error (FME) results must be retested.
3. Samples with NCF (no clotting found) must be retested with 1:4 dilution of the plasma.
4. Samples with Fibrinogen concentration < 80 mg/dl must be retested with 1:4 dilution of the plasma. The fibrinogen concentration reported by the instrument divided by 2 represents the fibrinogen concentration of the sample.
5. Samples with Fibrinogen concentration > 600 mg/dl must be retested with further dilution such as 1:12. The fibrinogen concentration reported by the instrument multiplied by the appropriate dilution factor represents the fibrinogen concentration of the sample.

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. EDTA should not be used as an anticoagulant.

PERFORMANCE CHARACTERISTICS
- Precision studies were performed on CoaLAB 6000 coagulometer by assaying normal and abnormal control plasmas with FIBROQUANT® System pack. One normal control plasma and one abnormal control plasma in replicates of 10 were used to determine inter assay and intra-assay precision of the clotting times (seconds).

<table>
<thead>
<tr>
<th></th>
<th>Inter-assay precision</th>
<th>Intra-assay precision</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Normal control plasma</td>
<td>239</td>
<td>21.3</td>
</tr>
<tr>
<td>Abnormal control plasma</td>
<td>123</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* FIBROQUANT® System pack was evaluated by estimating the fibrinogen concentration of control plasmas of two different manufacturers with known concentration of fibrinogen. The values obtained were within the expected range described in the respective control plasma assay value sheets.
WARRANTY
This product is designed to perform as described on the pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

SYMBOL KEYS

**TULIP DIAGNOSTICS (P) LTD.**

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