CE

FIBROQUANT®

REAGENT FOR QUANTITATIVE ESTIMATION OF FIBRINOGEN

SUMMARY

At present there are known to be at least eleven factors in circulating blood, which are required for normal haemostasis. 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 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.

FIBROQUANT® kit contains lyophilized thrombin reagent and lyophilized fibrinogen calibrator to determine the quantitative reactivity of fibrinogen. **Since the reagent system contains heparin neutralizing substances, heparin levels upto 0.4 IU/ml does not interfere with test results.**

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

PRESENTATION

REF [REF]	10641020	10641040
Thrombin [IVD]	20 Tests	40 Tests
Owren's buffer	3 x 10 ml	6 x 10 ml
CAL	1 ml	1 ml
Pack insert	1	1
Graph paper	1	1

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/dl 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^{0}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 30 days when stored at 2-8^{0}C and for 4 hours at room temperature (20-25^{0}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^{0}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^{0}C and for 2 hours at room temperature (20-25^{0}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 0.01% thimerosal as preservative.
3. **FIBROQUANT®** thrombin reagent is not from a human source hence contamination due to HBsAg, HCV and HIV is practically excluded.
4. As the bovine source is from non-BSE countries, the bovine source material included in this kit is considered to be free from risk for BSE/CJD and other zoonoses. However treat the material as if infectious.
5. 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 blood products as potentially infectious.
6. It is very important that absolutely clean and dry micropipettes 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 use damaged or leaking reagents.

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 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 tri-sodium citrate (0.11 mol/l, 3.2%). Centrifuge immediately for fifteen minutes at 1500 g and transfer the plasma into a clean test tube. **Plasma must be tested within 3 hours of collection.**

ADDITIONAL MATERIAL REQUIRED

10 x 75 mm glass test tubes, pipettes, stop watch, water bath at 37^{0}C, distilled water, automated / semiautomated / mechanical / optical instrument if applicable.

PROCEDURE

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

A) Procedure for fibrinogen Calibration Curve Preparation

- The **FIBROQUANT®** thrombin reagent vial must be **reconstituted exactly with 1.0 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.
- The **FIBROQUANT®** fibrinogen calibrator vial must be **reconstituted with exactly 1.0 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 Owren's buffer as follows**:**

Test tube no.	I	II	III
Owren's buffer	NIL	800µl	900µl
Fibrinogen calibrator	200µl	200µl	100µl
Dilution (calibrator)	NIL	1:5	1:10

1. Pipette 200µl of each fibrinogen calibrator dilution into clean test tubes and prewarm for 3 minutes at 37^{0}C.
2. Add 100µl of reconstituted thrombin reagent (prewarmed at 37^{0}C for one minute) and simultaneously start stopwatch.
3. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.
4. Repeat steps 1- 3 for a duplicate test on each calibrator dilution.
5. Plot the average of the duplicate test values on **FIBROQUANT®** graph paper*.
6. Connect the points, which should produce a straight line.
7. 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.**

B) Test Procedure for sample

1. Prepare a 1:10 dilution of plasma specimen with Owren's buffer solution.
2. To a 10 x 75 mm test tube at 37^{0}C add 200µl of 1:10 dilution of plasma sample to be tested.
3. Incubate at 37^{0}C for three minutes.
4. To the test tube add 100µl of **FIBROQUANT®** thrombin reagent (prewarmed at 37^{0}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. Calculate the mean clotting time for the plasma specimen.

INTERPRETATION OF RESULTS

The fibrinogen concentration can be read off directly by interpolating the mean clotting time obtained at 1:10 dilution of the sample, from the calibration curve plotted on the graph paper provided with the FIBROQUANT® kit for fibrinogen concentration.

1. If the obtained fibrinogen concentration is > 600 mg/dl, repeat the test at 1:20 dilution of the sample. The results read of the graph will be multiplied by a factor 2 for deriving the fibrinogen concentration in the sample.

2. If the obtained fibrinogen concentration is < 80 mg/dl, repeat the test at 1:5 dilution of the sample. The results read of the graph will be divided by a factor 2 for deriving the fibrinogen concentration in the sample.

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

REFERENCE VALUES

150 - 400 mg/dl.

Each laboratory should however determine the normal reference range of a representative sample population since normal values vary from laboratory to laboratory.

PERFORMANCE CHARACTERISTICS

- Precision studies were performed on Hemostar-XF coagulometer by assaying normal and abnormal control plasmas with **FIBROQUANT®**. 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).

	Inter-assay precision			Intra-assay precision		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Normal control plasma	221	16.63	7.5	196	14.8	7.6
Abnormal control plasma	106	8.43	7.9	105	8.06	7.7

- **FIBROQUANT®** 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.

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.

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

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2. Quick A.J Hemorrhagic diseases and thrombosis, 2nd Edition, Philadelphia, Lea and Febiger, 1966.
3. CRC handbook Series in Clinical Laboratory Science, Section 1: Haematology, Volume III, 1980.
4. Dacie J.V. and Lewis S.M.: Practical Haematology, 8th Edition, Churchill Livingstone Publications, 1995.
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7. Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature limitation		Manufacturer		Contains sufficient for <n> tests
	Use by		Consult Instructions for use	BUF	Owrens Buffer / CAL Fibrinogen Calibrator
	Date of Manufacture	REF	Catalogue Number	EC REP	Authorised Representative in the European Community
LOT	Batch Number/ Lot Number	IVD	*In vitro* Diagnostic Medical Device	REAGENT	Description of reagent
	This side up	REC 1ml	Reconstitute with 1 ml distilled water	FIBRINOGEN	Test for estimation of Fibrinogen

1017/VER-01

FIBROQUANT®
Lot No.:
Exp:
Fibrinogen calibrator concentration for the lot: mg/dl

TULIP DIAGNOSTICS (P) LTD.

REGD. OFFICE: GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

MANUFACTURING UNIT: PLOT NOS. 92/96, PHASE II C, VERNA IND. EST., VERNA, GOA-403 722, INDIA.

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain