CE

Quantia® FIBRINOGEN

QUANTITATIVE TURBIDIMETRIC IMMUNOASSAY FOR ESTIMATION OF FIBRINOGEN

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.

Low levels of fibrinogen are found in;

- Liver disease,
- Increased fibrinogen consumption due to the prolonged presence of disseminated intravascular coagulation,
- Hyperfibrinolysis in patients with neoplasia, acute promyelocytic leukemia's & 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.

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

Quantia®-FIBRINOGEN is an antigen immunoassay for the quantitative determination of fibrinogen in human plasma.

REAGENT

1. **Quantia®-FIBRINOGEN** Activation buffer (R1): ready to use buffer.
2. **Quantia®-FIBRINOGEN** reagent (R2): ready to use solution of anti-fibrinogen antibody.
3. **Quantia®-FIBRINOGEN** calibrator: lyophilized preparation of plasma equivalent to the stated amount of Fibrinogen on a mg/dl basis, when hydrated appropriately.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2 – 8 °C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial labels.
3. The reconstituted **Quantia®-FIBRINOGEN** calibrator is stable for 7 days at 2 – 8 °C and 48 hours at 25 – 30°C (RT).

PRINCIPLE

Quantia®-FIBRINOGEN is a turbidimetric immunoassay for the determination of fibrinogen and is based on the principle of agglutination reaction. The test specimen is mixed with **Quantia®-FIBRINOGEN** antibody reagent (R2) and activation buffer (R1) and allowed to react. Presence of fibrinogen in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at wavelength 340 nm. The increase in turbidity corresponds to the concentration of fibrinogen in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. All the reagents derived from human source have been tested for HBsAg and HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Do not use vortex mixers for mixing reagents. Gently mix the reagents and samples during test procedures.
6. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
7. Calibrators of different manufacturers must not be used with **Quantia®-FIBRINOGEN** reagents.

8. The **Quantia®-FIBRINOGEN** assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
9. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.

SPECIMEN COLLECTION AND PREPARATION

Though no special preparation of the patient is required prior to sample collection by approved techniques, it is preferable that patients are not heavily exercised before blood collection. Fasting or only light non-fatty meals prior to blood collection provide samples with a desirable opacity.
Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11mol/l, 3.2%) PROFACT available from TULIP; Cat No. 10660020. Centrifuge immediately for 15 minutes at 1500-3000 rpm (approximately 1500 g) on a laboratory centrifuge and transfer the plasma into a clean test tube. Cap the test tubes to prevent deterioration of the samples. **Plasma must be tested preferably immediately.** However if the specimen is held at 22-24^0 C then they may be tested within 8 hours and if the specimen are held at 4-8^0 then they may be stored upto one week, provided they are not contaminated.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 340 nm wavelength filter and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable cuvettes such as **Quantiamate semi micro cuvettes**/ glass cuvettes.

TEST PROCEDURE

Bring reagents and samples to room temperature before use.
Assay conditions;

Wavelength	340 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of Fibrinogen Curve

The **Quantia®-FIBRINOGEN** calibrator must be reconstituted exactly with 1.0 ml of distilled water, wait for 10 minutes, gently swirl the vial till the solution attains homogeneity and is at RT (25°C – 30°C). Once reconstituted it is ready to use for Fibrinogen test. The Concentration (S) of Fibrinogen in the reconstituted calibrator is as mentioned at the end of this package insert.
Prepare 1.0 ml of 50 mg/dl Fibrinogen **working standard (SW)** from the reconstituted calibrator as mentioned at the end of this package insert. The working standard (Sw) is stable for eight hours at 2-30°C.
Dilute the calibrator as mentioned below for preparation of calibration curve.

Test Tube No.	1	2	3	4	5
Calibrator dilution No.	D1	D2	D3	D4	D5
Working standard (Sw)	100 µl	100 µl D1	100 µl D2	100 µl D3	15 µl D1
Isotonic Saline	-	100 µl	100 µl	100 µl	485 µl
Conc. of Fibrinogen in mg/dl	S	S/2	S/4	S/8	S/32

The above five dilutions of the calibrator including the highest 50 mg/dl (D1) and lowest 1.5mg/dl concentrations of measuring range **must** be used for the preparation of the calibration curve.

Test procedure for preparation of calibration curve

(Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.)

1. Zero the instrument with distilled water
2. Pipette 500 µl of **Quantia®-FIBRINOGEN** activation buffer (R1) and 20µl working standard (D1) in the measuring cuvette. Mix well and incubate for 5 minutes at 37°C.
3. Read absorbance (A1).
4. Add 100 µl of **Quantia®-FIBRINOGEN** reagent (R2), mix gently (do not vortex) and start the stopwatch simultaneously.
5. Read absorbance (A2) at the end of **exactly** five minutes.
6. Repeat steps No. 2– 5 for each diluted working standard (D2 to D5) for preparing calibration curve.
7. Calculate ΔA (A2 – A1) for each dilution of the working standard (D1 to D5). Plot a graph ΔA versus concentration of fibrinogen on the graph paper provided with the kit.

"The calibration curve" so obtained is valid only for the same lot of Quantia®-FIBRINOGEN reagent.

Test procedure for specimen

For determination of fibrinogen concentration in the test specimen, dilute the test specimen 1:10 with normal saline.

1. Follow steps 2– 5 as mentioned in above procedure for calibration curve using the diluted test specimen in place of the working standard.
2. Calculate ΔA (A2 – A1) for the diluted test specimen.

VALIDATION CRITERIA

If the ΔA of the diluted test specimen is less than ΔA obtained for the standard of highest concentration (D1) then proceed directly for calculations of results.

If the ΔA of the diluted test specimen is higher than ΔA of standard with highest concentration (D1) then the test has to be rerun by carrying out further dilution of test specimen such as 1:20, 1:40, 1:60 etc., till the ΔA of the diluted specimen is less than ΔA of D1.

Calculations

1. Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the Fibrinogen concentration 'C' of the diluted test specimen.
2. Multiply the Fibrinogen concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of Fibrinogen in the neat test specimen.

Concentration of Fibrinogen in the neat test specimen in mg/dl = C x F

(Where 'F' is the dilution factor of the test specimen, for e.g. 10 for 1:10 dilution of test specimen and so on)

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The **Quantia®-FIBRINOGEN** reagent has been designed to measure Fibrinogen concentrations in the range is about 15-500 mg/dl and is linear between the measuring range. The exact range is dependent on the Calibrator value, which is lot specific.

Detection limit / Analytical Sensitivity

Detection limit: 1.5 mg/dl

The detection limit represents the lowest measurable Fibrinogen concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed upto a concentration of 750 mg/dl of Fibrinogen.

Precision

Inter-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	10	480	2.2	4.5%
Sample 2	10	125	0.3	2.6 %
Sample 3	10	15.2	0.12	8%

Intra-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	10	480	1.0	2.2
Sample 2	10	160	0.8	5.0
Sample 3	10	31	0.16	5.1

Interference

No interference was observed with:

Interference factor	No interference upto
Glucose	500 mg/dl
Albumin	10 g/dl
Bilirubin	50 mg/dl
Haemoglobulin	500 mg/dl
Triglycerides	1000 mg/dl
Heparin	0.4 IU/ml

REFERENCE VALUES

The reference values of Fibrinogen in normal population are 150-400 mg/dl
Each laboratory should define its own assay specific reference range for relevant population.

REMARKS

1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When ΔA obtained for the test specimen is greater than the ΔA of the standard with highest concentration then, it indicates that the concentration of Fibrinogen concentration in the test specimen is beyond the measuring range of the **Quantia®-FIBRINOGEN** assay. Such specimens should be rerun with further dilutions.
3. Do not read results beyond five minutes.
4. An increase in synthesis of fibrinogen 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.
5. It is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis.
6. In patient suspected with dysfibrinogenemia, it is recommended that the sample be tested with a functional assay (clot based assay) for fibrinogen.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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3. Haemostasis and thrombosis: Basic principles and clinical practice, 3rd Ed., J B Lippincott company, Philadelphia, 1994, Pg 1262.
4. Laboratory issues in diagnosing abnormalities of Protein C, Thrombomodulin, and Endothelial cell protein C receptor, CAP consensus conference XXXVI.
5. Data on file: Tulip Diagnostics (P) Ltd.

Quantia®-FIBRINOGEN Calibrator

Lot No.:		
Conc. (S) on reconstitution with 1.0 ml distilled water		**mg/dl**
To prepare 1.0 ml working standard (Sw)	**Vol. of normal saline**	**µl**
	+	
	Vol. of reconstituted calibrator	**µl**