FIBRINOGEN

VITRO SCIENT.

INTENDED USE

THROMBIN TIME TEST FOR QUANTITATIVE ESTIMATION OF FIBRINOGEN

BACKGROUND

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 hemostasis. For normal hemostasis to occur in response to injury or tissue damage, 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.

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

ASSAY 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.

REAGENTS

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 FIBROGEN graph paper supplied with each kit for the value of each lot).
3. **Owrens buffer,** ready to use (pH 7.35).

REAGENT STORAGE AND STABILITY

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 (0.109 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.**

SPECIMEN

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PROCEDURE

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

A) Procedure for fibrinogen Calibration Curve Preparation

1. The FIBROGEN 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.
2. The FIBROGEN 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.
3. Dilute fibrinogen calibrator stock solution with Owrens buffer as follows:

Test tubes No.	I	II	III
Owrens buffer	NIL	800 µl	900 µl
Fibrinogen calibrator	200 µl	200 µl	100 µl
Dilution (calibrator)	NIL	1:5	1:10

1. Pipette 200 µl of each fibrinogen calibrator dilution into clean test tubes and prewarm for 3 minutes at 37°C.
2. Add 100 µl of reconstituted thrombin reagent (prewarmed at 37°C for one minute) and simultaneously start stopwatch.
3. Stop the stop watch 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 FIBROGEN 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.

Medical Device Safety Services
MDSS GmbH
Burckhardtstr. 1
30163 Hannover, Germany
Tel.: +49 511 6262 8630
Fax: +49 511 6262 8633

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B) Test Procedure for sample

1. Prepare a 1:10 dilution of plasma sample with Owrens buffer solution.
2. To a 10 x 75 mm test tube at 37°C add 200 µl of 1:10 dilution of plasma sample to be tested.
3. Incubate at 37°C for one minute.
4. To the test tube add 100 µl 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. Calculate the mean clotting time for the plasma specimen.

Interpretation of first line tests:

The fibrinogen concentration can be read off directly by interpolating the mean clotting time at 1:10 dilution of the sample, from the calibration curve plotted on the graph paper provided with FIBROGEN 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 if < 80 mg/dl, repeat the test at 1:5 dilution of the sample. The results read off the graph will be divided by a factor 2 deriving the fibrinogen concentration in the sample.

EXPECTED 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.

WARNING & PRECAUTION

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. FIBROGEN thrombin reagent is not from a human source hence contamination due to HBsAg, HCV and HIV is practically excluded.
4. Fibrinogen calibrator provided in the FIBROGEN 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 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 cap after use and store at recommended temperature.
7. Significant levels of heparin and elevated levels of fibrinogen degradation products (FDP) in the patient plasma can cause falsely low fibrinogen results.
8. Insufficient prewarming of plasma and reagent or contaminated glassware may cause erroneous results.
9. EDTA should not be used as an anticoagulant.
10. Use reagents of the same lot for performing the test.
11. Do not interchange reagents from different lots.

BIBLIOGRAPHY

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
3031	20 Test

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
info@vitroscient.com

orders:
+202 26439698
order@vitroscient.com

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