Fibrinogen

INTENDED USE

Quantitative determination of fibrinogen in human plasma.

METHOD

Coagulometry – Thrombin time

CLINICAL SIGNIFICANCE

Fibrinogen is the principal plasma protein affecting the sedimentation rate. Fibrinogen concentration raises several folds during inflammation or tissue necrosis. Oestrogen ingestion, diabetus, obesity or pregnancy may also induce increased levels. Evidence as shown that plasma levels above the reference range constitute a significant independent risk factor for both coronary artery and cerebrovascular diseases. A decreased fibrinogen level in plasma is generally associated with a disturbance of liver metabolism (cirrhosis, icterus...) or with fibrinolysis and DIC (disseminated intravascular coagulation).

ASSAY PRINCIPLE

A standard amount of thrombin was added to the diluted citrate plasma sample, and the clotting time was measured. In the case of elevated thrombin concentration, the observed clotting time is inversely proportional to the fibrinogen concentration. To obtain the fibrinogen concentration, the clotting time of the sample is compared with the clotting time of a plasma dilution series with a known fibrinogen concentration. To obtain the fibrinogen concentration, the clotting time of the sample is compared with the clotting time of a plasma dilution series with a known fibrinogen concentration. Generalize. Fibrinogen is composed of buffer, bovine thrombin and reference plasma, using the method described by Claus to determine the concentration of fibrinogen in citric acid plasma. Fibrinogen can measure the concentration of clottable fibrinogen through the functional properties of the analyte, thereby providing clinically useful information. Reagent 1 is a suspension of buffered colloidal polymer. Even in a low concentration of fibrinogen, the formation of coagulum can be detected by automated and semi-automated equipment. Fibrinogen can be used in manual procedures, as well as in coagulometers that detect end-point coagulation by light-light or mechanical Spouaa methods.

REAGENTS

Thrombin reagent	Lyophilized preparation from bovine source ~ 50 NIH units per vial.
Fibrinogen calibrator	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).
Owrens buffer	Ready to use HEPES buffer pH 7.35

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 reagent 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 labels.

SPECIMEN

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 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.**

PROCEDURE

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

A) Procedure for fibrinogen Calibration Curve Preparation

1. The fibrinogen 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 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 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 FIBROGEN graph paper.
6. Connect the points, which should produce a straight line.

The calibration curve may be extended beyond the lowest and highest point.

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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 FIBROGEN 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 the.

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.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	20	20	20	20
Mean (mg/dl)	133	292	148	323
SD (mg/dl)	4.4	6.3	4.1	16.5
CV (%)	3.3	2.1	2.7	5.1

Linearity Range: between 99.5 and 871 mg/dL

Comparison with commercially available reagent:
171 plasmas located between 69 mg/dL and 910 mg/dL were tested
y = 0.9729 x - 13,847 r= 0.9900

INTERFERING SUBSTANCES

Turbidity No interference up to 0,543 abs
Low Molecular weight heparin Positive interference from 2 IU anti Xa
Non-fractionated Heparin Negative interference from 1.14 IU anti-Xa
Bilirubin Positive interference from 496 µmol/L
Hemoglobin No interference up to 261 µmol/L

BIBLIOGRAPHY

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3. CRC Handbook Series in Clinical Laboratory Science, Section 1: Haematology, Volume III, 1980.
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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
30401	20 Tests

Article # : 304 -EN
Date of Revision : 03/2021

ISO 13485:2016 CE

PERFORMANCE

Precision:

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