Fibrinógeno

Reagent for the determination of fibrinogen levels in plasma

SUMMARY

Fibrinogen is a glycoprotein present in plasma and α platelet granules. It is the coagulation factor found in highest concentration of plasma (200-500 mg/dl).
In the presence of a trauma or vascular injury, the thrombin cleaves fibrinogen to form fibrin monomers. These monomers spontaneously form polymers and are stabilized producing the insoluble fibrin. A decrease in fibrinogen level is found in cases of hereditary disorders such as hypofibrinogenemia, afibrinogenemia, dysfibrinogenemia, and also in other circumstances such as hepatic disease, extended intravascular clotting, fibrinolytic syndromes, etc.
An increase in fibrinogen level is found in cases of diabetes, inflammatory syndromes, etc.
In addition, high fibrinogen levels are now recognized as a risk factor for the development of cardiovascular disease.

PRINCIPLE

This assay is based on the Clauss method, designed as a reference procedure by the NCCLS (National Committee for Clinical Laboratory Standards).
In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the plasma fibrinogen concentration. The resultant clotting time is compared to a standard fibrinogen preparation.

PROVIDED REAGENTS

A. Reagent A: vials containing lyophilized thrombin. Once reconstituted the concentration is approximately 100 NIH units of thrombin/ml.
B. Reagent B: 0.05 M, pH 7.3 imidazole solution
Calibrator: vial containing lyophilized plasma. See assigned Fibrinogen value in label.

NON-PROVIDED REAGENTS

Bidistilled or deionized water.

INSTRUCTIONS FOR USE

Reagent A and Calibrator: remove the aluminum seal and open the vial withdrawing the rubber stopper to avoid loss of material. Add the bidistilled or deionized water indicated in the label. Cap, allow the material to stand for 30 minutes and then swirl gently (without agitating) until dilution is complete before use. Date.
It is recommended to maintain the Reagent A in its original vial after reconstitution and during use.
Reagent B: ready to use. Avoid contamination. Keep in its original vial properly capped.

WARNINGS

The provided reagents are for “in vitro” diagnostic use.
The Calibrator has been prepared from human plasma, which has been tested by an FDA approved method and found non-reactive for HBsAg, antibodies to HIV and HCV. However, no known test method can offer complete assurance of the absence of infectious agents. The Calibrator, Controls and patient samples should be handled as potentially infectious biological material.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10ºC) until expiration date indicated on the box.
Reconstituted Reagent A: stable 5 days refrigerated (2-10ºC) or 30 days frozen (-20ºC). Thaw rapidly at 37ºC. Do not refreeze. Allow the reagent to stand at room temperature before using again. Avoid extended warming.
Reconstituted Calibrator: stable for 8 hours refrigerated (2-10ºC).

SAMPLE

Plasma
a) Collection: obtain blood samples carefully avoiding stasis or foaming, mix gently in a tube with anticoagulant in 9 + 1 proportion (example: 4.5 ml blood + 0.5 ml anticoagulant). Centrifuge and remove plasma before 30 minutes. Perform the extraction with plastic syringes.
b) Additives: Anticoagulante TP from Wiener lab. or sodium citrate 3.8% or 3.2% could be used to obtain plasma. Do not use EDTA or heparin.
c) Known interference substances:
- Icteric, lipemic or hemolyzed samples may generate erroneous results.
- High levels of fibrinogen or fibrin degradation products may extend the coagulation period, especially when the fibrinogen levels are lower than 150 mg/dl.
- Therapeutic heparin levels do not interfere with the assay, however high levels may cause falsely low fibrinogen results.

See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be stored in plastic tubes until testing to reduce the contact activation effect that can occur with glass tubes. Plasma should be stored in refrigerator (2-10ºC) until testing. This

period should not be extended more than 4 hours. In case this process could not be performed, plasma should be frozen at -20°C. This process should be performed rapidly, alike the thawing (immersing in a 37°C bath) prior to testing.

REQUIRED MATERIAL

1- Provided

- Double logarithm paper sheet

2- Non-provided

- Hemolysis tubes
- Plastic tubes for preparing solutions
- Pipettes and micropipettes to measure indicated volumes
- Water bath at 37°C
- Stopwatch
- Light source for clot observation

PROCEDURE

I- CALIBRATION CURVE

1- Prepare dilutions of Calibrator 1:5, 1:10, 1:15, 1:20, 1:30, using 0.1 ml of reconstituted Calibrator and 0.4, 0.9, 1.4, 1.9, and 2.9 ml Reagent B respectively. Plasma diluted 1:10 represent 100% of the assigned value.
2- Prewarm 0.2 ml of each dilution to 37°C for 2 minutes.
3- Set stopwatch with the addition of 0.1 ml reconstituted Reagent A (do not warm thrombin reagent) to the pre-warmed dilutions and time clot formation.
4- Calculate the average clotting time for each dilution, in duplicate.
5- Use all of the points to construct a log-log curve that plots fibrinogen concentration vs. clotting time. Draw the straight line of best fit. The final curve must consist of at least 3 consecutive points.

Dilution	Reagent B	Calibrator	Fibrinogen concentration(*)	Dilution factor
1:5	0.8	0.2	---- mg/dl	x 2 =
1:10	0.9	0.1	---- mg/dl	x 1 =
1:15	1.4	0.1	---- mg/dl	x 0.67 =
1:20	1.9	0.1	---- mg/dl	x 0.5 =
1:30	2.9	0.1	---- mg/dl	x 0.33 =

(*) fibrinogen concentration indicated on the label of the Calibrator

The fibrinogen value of every curve dilution is determined multiplying the fibrinogen concentration in the Calibrator by the dilution factor. For example, if there is a fibrinogen level of 260 mg/dl, indicated in the Calibrator, then the dilutions 1:5, 1:10, 1:15, 1:20 and 1:30 have 520, 260, 173, 130 and 87 mg/dl respectively.

II- PATIENT SAMPLES AND CONTROLS

1- Prepare dilutions 1:10 of the patients' plasmas or control plasmas in Reagent B.
2- Prewarm 0.2 ml of each dilution to 37°C for 2 minutes.
3- Add 0.1 ml Reagent A rapidly and register clotting time.
4- Repeat testing and calculate the mean result for each sample.

CALCULATIONS

Given the coagulation time of the patient or control, enter this value to the standard curve and interpolate the fibrinogen value for every case.

INTERPRETATION OF RESULTS

If the sample's coagulation time is too short (for example, less than 7 seconds) dilute plasma 1:20 with Reagent B and assay again. Multiply the result by 2.
If the sample's coagulation time is too much (for example, more than 35 seconds) dilute plasma 1:5 with Reagent B and assay again. Multiply the result by 0.5.

QUALITY CONTROL METHOD

Control Plasma normal/pathologic

REFERENCE VALUES

The observed range of values for normal patients oscillates between 200-400 mg/dl.

Each laboratory should establish its own Normal Reference Range from individuals representing the population being tested.

SI SYSTEM UNITS CONVERSION

Fibrinogen (mg/dl) x 0.01 = Fibrinogen (g/l)

PROCEDURE LIMITATIONS

- A new calibration curve should be performed with every change of reagent's lot or any instrument change.
- Failures in the reconstitution of reagents may generate erroneous results.
- Sample collection: samples and their dilutions should be placed in plastic tubes or siliconized borosilicate glass. It is important to maintain the blood-anticoagulant relation as well as the citrate concentration used.
- It must be checked that the assay be performed at 37°C and that the test tubes be absolutely clean.

PERFORMANCE

a) Reproducibility: processing replicates from the same samples during the day, the following results were obtained:

Level	S.D.	C.V.
12.1 sec	± 0.3 sec	2.7 %
20.4 sec	± 0.6 sec	3.2 %

b) Linearity: the reaction is linear between 100 and 600 mg/dl.

PARAMETERS FOR AUTOANALYZERS

Fibrinogen reagent is suitable for use with manual, mechanical, and photo-optical methods. For semi-automatic and automatic instrumentation follow the instrument manufacturer's instructions.

WIENER LAB. PROVIDES

Kit for 100 tests (Cat. No. 1705006) containing:

- Reagent A: 10 x → 1 ml
- Reagent B: 2 x 60 ml
- Calibrator: 1 x → 1 ml

REFERENCES

- Clauss, A. - Acta Haematol. 17:237, 1957.
- Koepke, J.A. - Am. J. Clin. Pathol. 63:984, 1975.
- Collet, J.P. - Blood 82/8:2462, 1993.
- Ernest, E. - Ann. Intern. Med. 118/12:956, 1993.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
Σ	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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