FIBRINOGEN (CLAUSS method)

Determination of Fibrinogen in plasma using manual and automated procedure

TEST PRINCIPLE

The determination of fibrinogen with thrombin clotting time is based on the method originally described by Clauss; in the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma.

SAMPLES

Collected, into the plastic or siliconized glass tube, 9 parts of freshly draw venous blood and 1 part of trisodium citrate 3.8%.
The plasma was separated after centrifugation of the mixture for 10 'at 1500 x g.
Stabilità: 4 ore a 15-25°C oppure 24 ore a -20°C.
Do not use EDTA or heparin.

REAGENTS

Bovine thrombin:
Buffered lyophilized bovine thrombin, preservatives.

Plasma Calibration "Cal-Fib":
Lyophilized human plasma, stabilizers and preservatives.

Control Plasma "Pat-Fib":
Lyophilized human plasma, stabilizers and preservatives.

Calibration and Control Plasma concentrations are shown on the attached certificate.

REAGENTS PREPARATION AND STORAGE

Reconstitute one vial of bovine thrombin with 2 ml of distilled water. Stopper the bottle and mix gently by inversion to avoid foaming.
Stability: 8 hours at 15-25°C, 7 days at -20°C

Reconstitute one vial of Calibration Plasma with 1 ml of distilled water. Stopper the bottle and mix gently by inversion to avoid foaming.
Stability: 8 hours at 15-25°C, 2 days at -20°C

Reconstitute one vial of Control Plasma with 1 ml of distilled water. Stopper the bottle and mix gently by inversion to avoid foaming.
Stability: 8 hours at 15-25°C, 2 days at -20°C

MATERIAL REQUIRED BUT NOT SUPPLIED

Test tubes for analysis, chronometer, thermostat at 37°C, pipettes 0.1, 0.2 and 1 ml, serological pipettes, Diluent.

PRECAUTION

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
The blood of donors of materials used for the preparation of the reagents has been tested and found negative for HBsAg and anti-HIV and HCV.
However no known test can offer complete assurance that products derived from human blood can not transmit hepatitis, HIV or other infectious agents, therefore has to take all necessary precautions for handling potentially infectious.

PROCEDURE (Manual Application)

Plasma Dilution
Dilute the samples and control plasma "Pat-Fib, 1:10 with diluent or distillate water (1 plasma + 9 diluent)

Calibration curve

a) Reconstitute 1 vial of Plasma Calibration "Cal-fib" as indicated above.
b) Prepare a dilution series as shown in the diagram below:

	150%	100%	50%	25%
Diluent (ml)	0,85	0,90	1,90	3,90
Cal-Fib (ml)	0,15	0,10	0,10	0,10

c) For each dilution to determine at least in duplicate, the clotting time.
d) On bilogarithmic graph paper back in the abscissa the concentrations expressed in mg/dl or g/l corresponding to the 4-point calibration curve and the ordinate times achieved in seconds. Combining the points you must obtain a straight line.
e) Use the graph to calculate the concentrations in mg/dl or g/l of the samples.
f) The calibration curve must be repeated at every change kit lot Fibrinogen.

Place the bovine thrombin and the sample incubated at 37 ° C.
In a test tube for analysis, transfer 200 µl of sample.
Add 100 µl of bovine thrombin and measure the time to clot formation.

INTERPRETATION OF RESULTS

Each laboratory should provide for determining the calibration curve as described above, and then by interpolation to determine the concentration of the sample analysis.

To improve the detectability of a clot on optical instruments, reconstitute the reagent thrombin as described and then add 0.1 ml of suspension of Kaolin.
For concentrations above 600 mg/dl is recommended to dilute the sample 1:20 (0.05 ml + 1.95 ml diluent), multiplied by 2 the value at mg/dl obtained.
For concentrations below 100 mg/dl is recommended to dilute the sample 1:5 (0.2 ml + 0.8 ml diluent), dividing by 2 the value in mg/dl obtained.

EXPECTED VALUES

Each laboratory must provide the definition of reference values for the population under investigation. Bibliographical lists which reference interval:

Fibrinogen 200 - 400 mg/dl (2 - 4 g/L)

CLINICAL SIGNIFICANCE

Fibrinogen concentration values outside the reference were observed in acute inflammation and during pregnancy (elevated), in the thrombolytic therapy in liver disease, in congenital disfibrinogemia in DIC and pancreatitis (lower values).

NOTE

- As with any diagnostic procedure, if the results are inconsistent with the clinical presentation, the physician should evaluate data obtained using this test in light of other clinical information.
- Only for in vitro diagnostic use.

CALIBRATION / QUALITY CONTROL

The kit contains the control plasma "Pat-fib" to be used to verify the correct functionality of the reagents.

TEST PERFORMANCE

Interferences
Heparin concentrations less than 1 U/ml did not interfere. Interferences were detected in samples with degradation products of fibrinogen and total bilirubin concentrations higher than 15 mg/dl.
High levels of paraprotein, antibodies to thrombin and drugs that activate the fibrinolytic system may interfere with the analysis of fibrinogeno.
Severe inflammatory reactions may increase the circulating factor I (fibrinogen).

Precision

INTRASERIE Repeat Values in mg/dl			
n = 10	Mean	SD	CV%
FIB. Low	118.2	2.573	2.18
FIB. Normal	243.2	3.852	1.58
FIB. High	639.0	4.242	0.66

INTERSERIE Repeat Values in mg/dl			
n = 10	Mean	SD	CV%
FIB. Normal	309.4	7.167	2.32
FIB. Pathological	119.1	1.728	1.45

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 100 plasma samples in 3 sessions:
Fibrinogen Clauss Intermedical = y
Fibrinogen Clauss competition = x
n = 100
y = 12,04235 + 0,92254x r = 0,98894

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CG-02

Bovine Thrombin	4 x 2 ml (liophile)
Calibration Plasma	1 x 1 ml (liophile)
Control Plasma	1 x 1 ml (liophile)
Kaolin	1 x 1 ml (liquide)

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