CE
Usage
Diagnostic
In Vitro

Abliance

FIBRINOGEN

Reagent for quantitative determination of Fibrinogen in human plasma

REF FI02 : R1 (5 x 2 mL) + R2 (1 x 60 mL)
REF FI05 : R1 (10 x 5 mL) + R2 (2 x 125 mL)
Made in France Revision: 23/12/2014

PRINCIPLE OF THE METHOD [5] [6]

The method uses Von Clauss and al. method.
In the presence of an excess of thrombin, the time of formation of the fibrin clot in the plasma (pre-diluted) is reversely proportional to the amount of fibrinogen in the sample.
The clotting time is measured at 37°C.

CLINICAL SIGNIFICANCE [1] [2]

Fibrinogen is a glycoprotein (340 KDa) synthesised by liver and present in the plasma at a level within 2 to 4 g/L.
The concentration of fibrinogen is increased in infections, estrogens ingestion, tissue necrosis, obesity, pregnancies and diabetes. An increase of fibrinogen is also involved as a risk factor for coronary artery disorders and cerebrovascular diseases.
A decrease of fibrinogen in plasma is associated with:

- liver diseases (cirrhosis, jaundice)
- Fibrinolysis or disseminated intravascular coagulation (DIC)

REAGENTS

R1: Thrombin Reagent REAG
Lyophilized Thrombin of animal origin.

R2 : Buffer for dilution of plasma DIL SPE
Hepes pH 7.35, stabilizer

PREPARATION OF REAGENTS

Working reagent:
Open a vial of R1; add promptly into the contents the amount of distilled water indicated on the label of R1.
Recap vials and mix gently the R1 reagent until complete dissolution, avoiding foam.
Bring to 37°C the necessary volume of R1 for 15 minutes before work.
Store immediately the remaining volume of R1 and R2 well recapped, at 2-8°C.

STABILITY AND STORAGE

Unopened vials stored at 2-8°C are stable until the expiry date stated on the label.
R1: after reconstitution the working reagent is stable 7 days at 2-8°C, or 24 hours at room temperature.
R2: once opened, if stored at 2-8°C and free from contamination, contents of the vial is stable until the expiry date stated on the label.
Discard any reagent:

- after expiry date
- if quality controls values are out of the range

SAMPLES COLLECTION AND HANDLING [2] [6]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (trisodium citrate solution 0.109M).
Mix immediately the blood with anticoagulant.
Avoid drawing with a syringe that could result in the formation of micro-clots.
Centrifuge 10 minutes at 2500g.
The sample is stable 4 hours after collection, at room temperature (15-25°C).
Collection on Citrate Hepes tube increases the sample stability to 8 hours.

INTERFERENCES [2] [3] [8]

This test is protected against fibrinolysis by a fibrinolysis inhibitor.
It is advised to dilute sample promptly after venipuncture
Following Interferences have been tested on Option 4 Coagulometer:

Interferent	Results
Haemoglobin	No interference up to 327 µmol/L
Turbidity	No interference up to 2%
Bilirubin	Positive interference above 450 µmol/L

For a more comprehensive review of factors affecting this assay, refer to the publication of Young D.S.

ADDITIONAL EQUIPEMENT

General laboratory equipment
Coagulation analyzer or stopwatch and bath (37°C+/- 0.5)
Reference plasma
Control plasmas

SAFETY CAUTIONS

ABLIANCE reagent kits are designated for professional in vitro diagnostic use.
Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples (to manipulate as potentially infectious)
For further information, Material Safety datasheet is available upon request.
Waste disposal: Respect legislations in place in the country

QUALITY CONTROL

At least once a run, when changing reagent vial or after maintenance of the analyzer, it is advised to use 2 levels of control plasmas:
REF NP01 includes normal and pathological plasmas
or any other assayed control plasmas referring to the same method.

If the controls results are out the defined range, perform consecutively until correction: repetition of the test with fresh control plasma, calibration with a new vial of reagent, use of a new vial of reference plasma. If no solution is found, contact your local supplier or Abliance technical support.

EXPECTED VALUES [1] [2]

The normal range for fibrinogen in adult plasma is usually 200-400 mg/dL
However, it is advised to each laboratory to establish its own reference range of expected values.

CALIBRATION

Use reference plasma REF CA01 to prepare a calibration curve as follows:
1/5, 1/10, 1/15 and 1/20 dilutions with Reagent R2.
Measure in triplicate the clotting time of each level.
Prepare the reference curve on a paper graph or enter the mean of the clotting time found for each dilution, and the corresponding Fibrinogen concentration (mg/dL) in the analyzer.

PERFORMANCES

Results of Performances Assays on Thrombolyzer Compact X:

Repeatability (within run):

Mean (mg/dL): 75	CV%: 2.3	n= 21
Mean (mg/dL): 169	CV%: 1.4	n= 21
Mean (mg/dL): 329	CV%: 2.2	n= 21

Reproducibility (run to run):

Mean (mg/dL):294	CV% :3.1	n=32
Mean (mg/dL):162	CV%: 4.5	n=32
Mean (mg/dL): 77	CV%: 5.3	n=32

PROCEDURE

Semi-automated and automated method:
Refer to the appropriate instrument Operator's manual for detailed instructions.
Manual method:
Dilute samples and control: 1/10 in R2 Reagent (Buffer for dilution)
Calibrators: prepare dilutions as indicated in § Calibration.
Pre-incubate the Thrombin Reagent to reach a temperature of 37°C.
Mix gently the reagent before pipetting.

- Diluted Plasma: 200 µL

Incubate for 2 minutes at 37°C

- Thrombin (pre-warmed at 37°C): 200 µL

Start a stopwatch immediately, and record the time required for clot formation in seconds.

CALCULATION

Plot the concentrations (mg/dL) of each dilution of reference plasma versus the clotting time (sec).
Example for calibrator at 300mg/dL:

Dilutions	1/5	1/10	1/15	1/20
Concentrations (mg/dL)	600	300	200	150
Clotting times (sec.)	To be assayed			

Calculate the concentration in fibrinogen of sample by interpolation of its clotting time in the calibration curve.

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Curtis, E.R. Ashwood, W.B. Saunders (1999) p. 1133, 1145, 1740-41, 1813, 1846.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.404-405*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-260 à 3-261*
(4) *VON CLAUSS A. ACTA HAEMATOLOGICA 1957. **17**, 237-246.*
(5) *DESTAING F-DUZER A. PATHOLOGIE ET BIOLOGIE 1960, **8**, 1615.*
(6) *HURLET A.-JOSSO F: PATHOLOGIE BIOLOGIE 1972, **20**, 3-4,165-173*
(7) *CAEN-LARRIEU-SAMAMA : L'HEMOSTASE, 1968, EXPANSION SCIENTIFIQUE.*
(8) *Technique en hématologie, Flammarion médecine-sciences, 2nd éd . 1978, p.184-186*

Manufacturer	Use by	IVD In vitro diagnostic	Temperature limitation
REF Catalogue number	See insert	LOT Batch number	→ Dilute with

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