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: 10/10/2018

PRINCIPLE OF THE METHOD [5] [6]

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: Fibrinogen REAG
Lyophilized reagent
Thrombin of animal origin.

Once reconstituted: Thrombin reagent is not classified as dangerous

R2: Fibrinogen DIL SPE
Hepes Buffer, pH 7.35 (for dilution of plasma), stabilizer
According to 1272/2008 regulation, this reagent is not classified as dangerous

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

PREPARATION OF REAGENTS

R1: Freeze dried reagent.
Working reagent: Open a vial of R1 and add promptly the amount of distilled water indicated on the label of R1. Cap the vial and mix gently until complete dissolution.
R2: Plasma dilution buffer, ready for use.

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.

LIMITS [2] [3] [8]

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

ADDITIONAL EQUIPEMENT

General laboratory equipment
Automatic Coagulation analyzer or semi-automated analyser
Distilled or demineralized water for reagent reconstitution

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.

QUALITY CONTROL

REF NP01: CONTROL PLASMA Level I and Level II
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:
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.

PROCEDURE

Let stand the working reagent reach room temperature (18-25 °C).

Manual method on semi-automated analyzer
Dilute samples and controls: 1/10 in DIL SPE Buffer
Calibration: prepare dilutions as indicated in § Calibration.

- Diluted Plasma (calibrators, controls, plasmas): 200 µL

Incubate for 2 minutes at 37 °C

- Working Reagent (mix before use): 200 µL

The automatic countdown timer will start immediately after working reagent addition and stop when the clot is formed.

Automated method on Thrombolyzer series
Refer to the full detailed application specific to the automated system.
Note:

- Performances and stability data have been validated on Thrombolyzer Compact X (available on request).
- With manual procedure and on other automated coagulation analyzer, performances and stability data must be validated by user.
- Other validated applications or proposal applications are available on request

CALIBRATION

Use REF CA01: Cal plasma traceable to WHO International Standard NIBSC Code: 98/612
Manual method on semi-automated analyzer: Prepare a calibration curve with dilution 1/5, 1/10, 1/15 and 1/20 in DIL SPE Buffer. Measure in triplicate the clotting time of each level.
Automated method on Thrombolyzer series: Perform a calibration with REF CA01 using automatic dilutions indicated in the specific application.

CALCULATION

Manual method on semi-automated analyzer
Enter the mean of the clotting time found for each dilution of REF CA01, and the corresponding Fibrinogen concentration (mg/dL) in the system. Fibrinogen concentration will be calculated automatically according to calibration curve.
Automated method on Thrombolyzer series: Fibrinogen concentration (mg/dL) will be calculated automatically according to calibration curve.

PERFORMANCES

The **within run and between run studies** were performed with normal and abnormal plasma on Thrombolyzer Compact X:

Within run ***N = 20***	Normal Plasma	High Plasma
Mean (mg/dL)	145	278
S.D. (mg/dL)	4.2	3.6
C.V. %	**2.9**	**1.3**

Between run ***N = 20***	Normal Plasma	High Plasma
Mean (mg/dL)	152	307
S.D. (mg/dL)	3.4	10.4
C.V. %	**2.3**	**3.4**

Linearity Range: between 99.5 and 871 mg/dL
Comparison with commercially available reagent: 173 plasmas between 80 mg/dL and 1109 mg/dL were tested:
y = 1.0065 x – 25.597 r= 0.9875
Interferences:

Turbidity	No interference up to 731 mg/dL triglycerides
Low Molecular weight heparin	No interference up to 2 IU anti Xa
Unfractionated heparin	Negative interference from 1.66 UI anti Xa
Bilirubin	No interference up to 496 µmo/L
Hemoglobin	No interference up to 261 µmol/L

Other substances may interfere with the results (see § Limits)
Calibration Stability: 6 days; **On board stability**: 8 days (8 h/days on board)
Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

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
REAG Reagent	DIL SPE Diluent specimen	H_2O Distilled water	

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