FIBRIPHEN 2

REF CK572K

R1 6 x 2 mL

IVD

Clotting method for quantitative determination of Fibrinogen.

HYPHEN BioMed

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INTENDED USE:

The FIBRIPHEN 2 kit is a clotting method for *in vitro* quantitative determination of Fibrinogen in human citrated plasma (Clauss method).

SUMMARY AND EXPLANATION:

Fibrinogen is a 340 Kd soluble plasma glycoprotein, synthesized in the liver, containing 6 peptidic chains, with a 2 to 2 symmetry, and linked by disulfide bridges (2 Aα, 2 Bβ and 2 γ chains). Thrombin clots fibrinogen and forms fibrin, which is stabilised by activated factor XIII in presence of calcium. Fibrinogen is lysed by plasmin to fragments X and Y, first, then D and E.[2,4,5]
Fibrinogen concentration in normal human plasma is usually in the range 2 to 4 g/L[5]. Elevated fibrinogen concentrations (> 4g/L) are observed in clinical situations associated with inflammation[4] and have also been studied as a risk factor for cardiovascular disease and thrombosis[3,4].
Hypofibrinogenemia is mainly associated with severe liver disease, and excessive consumption of fibrinogen (DIC, hyperfibrinolysis)[4]. Numerous variants of fibrinogen have been described, associated to asymptomatic cases, or to cases with bleeding and/or thrombosis[4].

PRINCIPLE:

In the presence of a constant and in excess amount of thrombin, the clotting time obtained for diluted citrated plasma depends on the plasma fibrinogen concentration.

REAGENTS:

R1 **Calcium Thrombin**, from bovin origin (about 100 NIH/mL), lyophilized in presence of an heparin neutralizing substance, preservatives and stabilizers. Contains BSA.
6 vials of 2 mL.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- The bovine plasma used to prepare the reagent and the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- Waste should be disposed of in accordance with applicable local regulations.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- For *in vitro* diagnostic use.

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

R1 **Reagent 1**: **Calcium Thrombin**
Reconstitute the contents of each vial with exactly **2 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **14 days** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **1 month** frozen at -20°C or less*

*Thaw only once, as rapidly as possible at 37°C, adapting the incubation period to the volume of reagent**.** The stability of the thawed reagent should be checked under laboratory work conditions.

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021A/AR021K/AR021L).
- Specific calibrators and controls with known titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

Materials:

- Electromagnetic water-bath, semi-automatic or automatic instrument for clotting assays.
- Stopwatch; Calibrated pipettes; test tubes.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[6] guidelines for further information concerning specimen collection, handling and storage).

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage[7]:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

The kit is a clotting method, automated or manual methods. Perform the test at **37°C** and the clotting time, triggered by addition of the FIBRIPHEN reagent, is measured.

Automated methods:
Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method:
1. Reconstitute the calibrators and controls as indicated in the specific instructions. Prepare 2 mL of calibrator **diluted 1:5** in Imidazole buffer (note: by definition, the 1:20 dilution of the calibrator corresponds to a concentration of "C" g/L of Fibrinogen). For the calibration curve, dilute the calibrator in Imidazole buffer as described below ("C" defines the concentration of Fibrinogen):

Fibrinogen (g/L)	C:2	C	2C	4C
Dilution	1:40	1:20	1:10	1:5
Volume of calibrator at 1:5	0.125mL	0.250mL	0.500mL	1mL
Volume of Imidazole Buffer	0.875mL	0.750mL	0.500mL	0mL

2. Tested controls and plasma must be **diluted 1:20** in Imidazole buffer.

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens within 2 hours. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

To ensure optimal performances of the assay, perform all assays (calibration, samples, controls) extemporaneously and successively without interruption.

3. Principle: Method (manual) using a mechanical coagulation indicator, such as a metal ball or index, or balancing, is used for detecting clotting.
Dispense the following to a small test tube, or in the reaction cuvette of the coagulation instrument incubated at **37°C**:

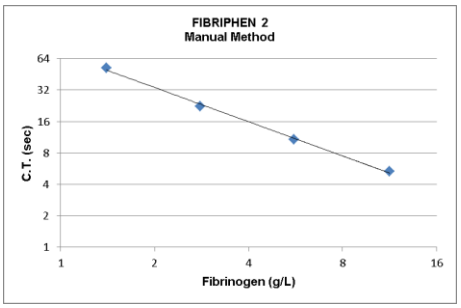
	Volume
Calibrator, tested plasma or controls diluted at **1:20**	200 µL
Incubate **at 37°C** for **2 minutes**, then add the following (starting the stop-watch):	
R1 **FIBRIPHEN.** Pre-incubated at **37°C**	100 µL
Record the clotting time CT (in seconds).	

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The plasma calibrator covering the dynamic test range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

The calibration curve shown below, obtained on manual method is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be defined, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual method, plot the calibration curve log-log, with the clotting time (sec) along the Y-axis and the Fibrinogen concentration, expressed as g/L, along the X-axis.
- The concentration of Fibrinogen in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in g/L.
- The results should be interpreted according to the patient's clinical and biological condition

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Various drugs or therapies can affect the results (eg: anti-thrombin substances may interfere in the assay and prolong the obtained clotting time). An additional investigation should be conducted to determine the origin of each unexpected or abnormal result.
- A “repeat” clotting time for a sample even with the same reagent lot can vary slightly according to the instrument used, and the clot detection mode and instrument setting (clot detection sensitivity). Each laboratory should check and validate its own usual range, as well as target values and acceptance ranges for each new lot of controls, in its specific test conditions.
- The FIBRIPHEN reagent contains a heparin neutralizing substance. For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed for Heparin concentration up to 2 IU/mL, Arixtra concentration up to 2 µg/mL, hirudin concentration up to 5 µg/mL, Fibrin degradation products (FDP) up to 130 µg/mL and Argatroban® concentration up to 2 µg/mL, by plasma overload tests).

EXPECTED VALUES:

The normal plasma Fibrinogen level in the adult population is usually in the range of 2 to 4 g/L[5]. However, each laboratory has to determine its own normal range.

PERFORMANCE:

- The measuring range depends on the analytical system used (about 1 to 12 g/L of Fibrinogen on STA-R®-series, for sample assayed at the 1:20 dilution).
- For a better accuracy, samples measured ≤ 1 g/L can be tested at the twice-concentrated dilution (1:10), and obtained results divided by 2; for samples measured >12g/L, an additional two-fold dilution (1:40) can be used and obtained results multiplied by 2.
- The indicative clotting times observed for this assay are in the range 4-7 seconds, and about 22±5 seconds, respectively for the 12 g/L or 3 g/L Fibrinogen concentrations, using the water bath or STA-R® method.
- Performance studies were conducted internally using a STA-R®. Performance was assessed using laboratory controls. The following results were obtained:

	Fng (g/L)	N	Intra assay CV (%)	Inter assay CV (%)
Sample 1	2.67	10	2.3%	2.3%
Sample 2	1.47	10	2.6%	3.7%

REFERENCES:

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3. Henschen-Edman AH. On the identification of beneficial and detrimental molecular forms of fibrinogen; 29: 179. Haemostasis 1999-186.
4. Lord ST. Fibrinogen. In: Molecular basis of thrombosis and haemostasis. High KA and Roberts HR. ed. Marcel Dekker Inc, 1995: 51-74.
5. Marguerie G. Le fibrinogène, facteur multifonctionnel de l’hémostase. Medecine/Sciences. 1986.
6. CLSI Document H21-A5: “Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline”. 2008
7. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.