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LIAPHEN™ Fibrinogen

REF 120102

R1 4 x 5 mL

IVD

Immuno-turbidimetric method for fibrinogen assay,
With ready to use liquid reagent.

HYPHEN BioMed

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INTENDED USE:

LIAPHEN™ Fibrinogen kit is an immuno-turbidimetric assay for *in vitro* quantitative determination of Fibrinogen antigen (Fib:Ag) on human citrated plasma or in purified medium using a manual or automated method. Reagents are in the liquid presentation, ready to use.

SUMMARY AND EXPLANATION:

Technical:[1-3]

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 then stabilized by activated Factor XIII in presence of calcium. Fibrinogen is lysed by plasmin to fragments X and Y, first, then D and E.

Clinical:[3-7]

Fibrinogen concentration in normal human plasma is usually in the range 2 to 4 g/L. Elevated fibrinogen concentrations (> 4g/L) are observed in clinical situations associated with inflammation and have also been considered as a risk factor for cardiovascular disease and thrombosis.

Hypofibrinogenemia is mainly associated with severe liver disease, or excessive consumption of fibrinogen (DIC, hyperfibrinolysis).

Numerous variants of fibrinogen have been described, associated to asymptomatic cases, or to cases with bleeding and/or thrombosis.

PRINCIPLE:

LIAPHEN™ Fibrinogen is an immunoturbidimetric method, based on antigen-antibody reaction: fibrinogen antigen of the sample reacts with polyclonal rabbit anti-human fibrinogen antibodies, leading to latex particles agglutination. This agglutination can be directly detected by a change of absorbance. The absorbance change is directly proportional to the amount of fibrinogen in the sample.

REAGENTS:

R **Latex**, liquid form. Contains BSA and small amounts of sodium azide (0.9 g/L)

4 vials of 5 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R Reagent is ready to use, homogenize the reagent prior to use, by gentle inversion while avoiding formation of foam and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

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.

R Reagent stability after opening, excluding any contamination or evaporation, and stored in the original vial, is of:

- **6 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Dilution buffer: Imidazole buffer (AR021B/AR021K/AR021L/AR021M/ AR021N) or Tris NaCl BSA (0.05M; 0.15M; 1%) buffer, pH 7.40 (TBSA). The same buffer must be used for all the tests performed.
- Specific calibrators and controls with known Fib:Ag titration, such as:

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

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or automatic instrument for immuno-turbidimetric assays.
- Stopwatch; Calibrated pipettes; plastic test cuvettes for spectrophotometer.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[8] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references[8,9].

PROCEDURE:

The kit can be used for kinetics methods, automated or manual methods. Perform the test at **37°C** and the turbidimetry is measured **at 620nm** (other wavelengths can be used, preferentially between 405 and 700nm).

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. Reconstitute the reference preparation (assay in purified milieu) or plasma calibrator, and plasma controls, as indicated in the specific instructions or according to internal practice.

For a calibrator or a reference preparation with a known Fib:Ag concentration (C) in µg/mL, the **20 µg/mL** concentration is obtained using the following dilution factor : **D = C:20 with C in µg/mL**. (For example, for a standard at 3.000 µg/mL, the dilution factor **D** will be **D=** 3.000:20 = 150.)

Prepare **3 mL** of the **20 µg/mL Fib:Ag** dilution (**C1**) in the dilution buffer.

Prepare the calibration curve by preparing serial dilutions as follows:

Standard	C1	C2	C3	C4	C5	C6
Fib:Ag (µg/mL)	**20**	**15**	**10**	**5**	**2.5**	**0**
Volume of standard	1000µL of **C1**	750µL of **C1**	500µL of **C1**	250µL of **C1**	125µL of **C1**	0µL
Volume of Buffer	0 µL	250µL	500µL	750µL	875µL	1000µL

For the manual method, a calibration curve must be performed for each test series.

2. Dilute the specimens and controls in the dilution buffer, as described in the table below (manual method):

Specimens	Reference	Dilution
BIOPHEN™ Normal Control Plasma	223201	**1:300**
BIOPHEN™ Abnormal Control Plasma	223301	**1:300**
Specimen (≈ 1 to 6 g/L)	n.a	**1:300**

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

3. Dispense the following to the plastic test cuvette incubated at **37°C**:

	Volume
Specimen, calibrator or control diluted	100 µL
R **Latex reagent,** Pre-incubated at **37°C and homogenized before use**	400 µL
Mix and incubate **at 37°C** for **exactly 15 minutes**, **and immediately after:**	
Mix and read the absorbance at **620nm** against the dilution buffer. **Respect the same overall reaction time for each sample.**	

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.

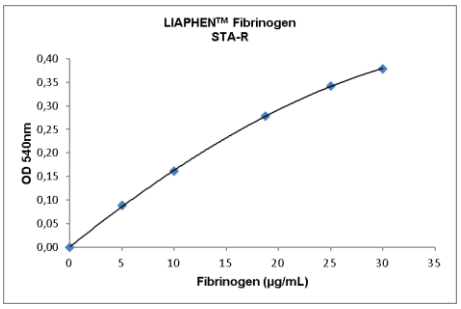
For elevated concentrations (> 6 g/L), it is recommended to use a specimen dilution of 1:1000 and for low concentrations (< 1 g/L), use a specimen dilution of 1:100.

CALIBRATION:

LIAPHEN™ Fibrinogen assay can be calibrated for the assay of Fibrinogen (antigen). The plasma calibrator covering the calibration 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 range is about 0 to 30 µg/mL (on STA-R®).

The calibration curve shown below 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 acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve, with the OD 620 nm along the Y-axis and the Fib:Ag concentration, expressed as µg/mL, along the X-axis, by choosing the most suitable interpolation mode. The Fib:Ag concentration (µg/mL) in the test specimen is inferred from the calibration curve, and multiplied by the dilution factor used (300 at the standard dilution).
- If other dilutions are used, the level obtained is the measured level, multiplied by the dilution factor used.
- 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.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- The presence of rheumatoid factor may result in an overestimation of the Fibrinogen concentration.[10]
- Various drugs or treatments can affect the results. An additional investigation should be realized to determine the origin of each unexpected or abnormal result.
- For the possible influence of Hook effect, refer to the specific application guide for the analyzer used (no significant effect is observed for fibrinogen concentrations up to 90 µg/mL in the test dilution).

EXPECTED VALUES:

The reference range was measured on healthy adult subjects (n=56) on STA-R® (Central 90%, 95th percentile) between 1.94 g/L and 4.17 g/L of Fib:Ag. Each laboratory has to determine its own normal range.

PERFORMANCES:

- The measuring range depends on the analytical system used (about 1 to 30 µg/mL of Fib:Ag in the test dilution on STA-R®-series, ie 0.2 to 6 g/L).
- Specificity: serums are assayed below 0.2 g/L (mean).
- Performance studies were conducted internally on STA-R®-series. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 2 repetitions within each series for a control level. The following results were obtained:

Control	**Intra assay**				**Inter assays**			
	n	**Mean**	**CV%**	**SD**	**n**	**Mean**	**CV%**	**SD**
Level 3	20	5.04	3.8	0.19	20	5.04	7.7	0.39
Level 2	20	2.58	3.1	0.08	20	2.58	9.8	0.25
Level 1	20	1.31	3.4	0.04	20	1.31	9.8	0.13

- Correlation with reference method (LIAPHEN™ Fibrinogen vs FIBRIPHEN™ on STA-R®) :
  n = 70 y = 0.97x - 0.06 r = 0.977
- Interferences: No interference, on the STA-R® analyzer was observed with the molecules and up to following concentrations :

Hemoglobin	200 mg/dL	**Heparin (UFH/LMWH)**	2/2 IU/mL
Bilirubin	20 mg/dL	**Intralipids (Triglycerides equivalent)**	2000 mg/dL

  Also refer to the specific application guide of the analyzer used.
- Cross-reactivity: The LIAPHEN™ Fibrinogen reacts also with Fragment DD (DDimer), Fibrinogen fragment D and Fibrin Degradation Products (FDPs); it does not react with Fibrin Fragment E.

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SYMBOLS:

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

Changes compared to the previous version.