



LIAPHEN Fibrinogen # 120102

Turbidimetric latex immunoassay for the quantitative determination of Fibrinogen

For *in vitro* diagnostic use only (IVD)

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INTENDED USE:

LIAPHEN Fibrinogen kit is a latex immunoassay for measuring Fibrinogen in human citrated plasma or in purified milieu, using a manual or automated method, *in vitro* exclusively.

CLINICAL APPLICATIONS:

Diagnosis of quantitative deficiencies of fibrinogen. Measurement of fibrinogen on plasma, (elevated concentrations) with an immunoassay.

SPECIMEN:

Human citrated plasma.
Cell culture supernatants.
Any biological fluid where fibrinogen must be present.

ASSAY PRINCIPLE:

LIAPHEN Fibrinogen is an immuno-turbidimetric assay using latex particles coated with polyclonal rabbit anti-human fibrinogen antibodies, for the *in vitro* determination of Fibrinogen. When the tested sample is mixed with the latex reagent (R1), the anti-Fibrinogen antibodies coupled onto latex particles react with Fibrinogen present in the sample and agglutination occurs. The amount of agglutination is directly proportional to the amount of fibrinogen in the sample and is measured by light absorption (A620nm or specific automate wavelength).

Latex + Fibrinogen → Aggregates (A620nm,...)

REAGENTS:

R1: Reagent 1: Latex reagent

Latex microparticles coated with polyclonal rabbit anti-(h)-fibrinogen antibodies. Ready to use liquid reagent, containing BSA and sodium azide (0.9 g/L).
4 vials of 5 ml

Warning:

- Bovine Serum Albumin (BSA) was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.
- Sodium azide (0.9 g/l) may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Tris NaCl BSA 1% pH 7.50 buffer (TBSA). (ex: TBSA #AR005A)
- Plasma Calibrator titrated for Fibrinogen (ex: BIOPHEN Plasma Calibrator #222101).
- Or Reference material for Fibrinogen (international or internal).
- Normal and Abnormal Quality Control Plasmas (ex: BIOPHEN Normal Control Plasma #223201, and BIOPHEN Abnormal Control Plasma (#223101) or BIOPHEN Plasma Fibrinogen Control Low #SC070K) titrated for Fibrinogen.

Material:

- Spectrophotometer, with a wave-length set up at 620 nm, photometer or automates for chromogenic assays.
- Stop watch.
- Calibrated pipettes.
- Cuvette or microplate

TRACEABILITY TO THE REFERENCE MATERIAL:

The concentration of the Internal reference standard for Fibrinogen determined with a clotting method (Fibrigen) has been verified against the SSC/ISTH secondary coagulation standard lot #3 from NIBSC. No differences have been noticed between the concentrations measured with the clotting method and those measured with the Liaphen Fibrinogen (immunologic determination).

STORAGE CONDITIONS:

Reagents must be stored at 2-8°C, in their original packaging box. They are then stable until the expiration date printed on the box.

Note: Stability studies for 3 weeks at 30°C show that the reagent can be shipped at room temperature for a short period without damage.

PREPARATION AND STABILITY OF REAGENTS:

R1: Reagent 1: Latex reagent

- Let to homogenize for 30 minutes at room temperature (18-25°C).
- Mix gently before each use.

Stability of opened reagent R1, kept in its original vial, hermetically closed, provided any evaporation or contamination is avoided:

- 7 days at room temperature (18-25°C)
- 6 months at 2-8°C

Cautions:

- Reagents must be handled with care, in order to avoid any contamination during use.
- After use, vials should be closed with their specific caps.

TESTED SPECIMEN:

Human citrated plasma or any biological fluid where Fibrinogen must be assayed.
Collection and preparation: Blood (9 vol.) must be collected on 0.109M (or 0.129M) citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g.
Storage: citrated plasma should be tested within 8 hours or stored frozen at -20°C or colder for up to 6 months, and thawed for 15 min. at 37°C just before use. Do not use plasmas that have already been frozen and thawed once.

Note: Refer to GEHT or CLSI recommendations for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect (haemolysed, lipaemic aspect...).

TEST PROCEDURE:

LIAPHEN Fibrinogen kit is designed for being used with automated kinetic methods but it can also be used with the manual method. Adaptations to the various automates are available upon request.

Using the manual method, the assay is performed at the controlled temperature of 37°C and the agglutination development is measured at 620 nm.

CALIBRATION:

The kit can be calibrated with a commercially available plasma calibrator, titrated for Fibrinogen concentration (e.g. Biophen Plasma Calibrator ref 222101), or with internal or international reference material for Fibrinogen, or with purified fibrinogen titrated for Fibrinogen.

If calibration is performed with a commercially available plasma calibrator or with purified fibrinogen, with a known Fibrinogen concentration (C) in µg/ml, the point 20 µg/ml, is obtained by diluting the calibrator using the following dilution factor $D = C:20$, if C is expressed in µg/ml. (For example, for a plasma calibrator with a fibrinogen concentration C of 3.000 µg/ml, the dilution factor D to be applied is $D = 3.000:20 = 150$.)

Prepare 3 ml of the 20µg/ml dilution (C1) in Tris-NaCl-BSA pH 7.50 (TBSA), the calibration curve can then be obtained by preparing serial dilutions as follows:

Standard	C1	C2	C3	C4	C5	C6
Fibrinogen (µg/ml)	20	15	10	5	2.5	0
Vol of Fbg standard	1000µL of C1	750µL of C1	500µL of C1	250µL of C1	125µL of C1	0µL
Vol of TBSA	0 µL	250µL	500µL	750µL	875µL	1000µL

In order to get the full assay performances, the calibration curve must be prepared just before running the assay in order to avoid any Fibrinogen degradation which could lead to erroneous results.

TESTED SAMPLES AND CONTROLS:

Normal Plasma samples and controls (up to 6 g/L) are assayed at the 1:300 dilution in TBSA. Pathological plasmas that contain elevated or low fibrinogen level must be diluted in order to obtain a concentration ranging from 5 to 20 µg/ml of fibrinogen, after dilution. As an example, use a 1:1000 dilution in case of hyperfibrinogenemia (6 to 20 g/L), or a 1:100 dilution in case of hypofibrinogenemia (<1 g/L). For assaying cell culture supernatants, the appropriate dilution must be determined by testing various dilutions. The diluted samples must be tested within 2 hours.

ASSAY PROTOCOL:

• Manual Method:

Reagents	Cuvette method	Microplate method
Calibrators, or diluted tested plasmas, or Controls	100 µl	50µl
R1 Latex preincubated at 37°C and homogenized before use	400 µl	200µl
Mix and incubate for 15 min at 37°C exactly and, immediately after:		
Mix and read the absorbance at 620nm against TBSA. Respect the same overall reaction time for each sample.		

Cautions:

- Using manual method, a calibration curve must be performed for each test series.

Note:

- Other wavelengths than 620nm can be used (from 405 to >700nm). Higher is the wavelength and lower is the blank value and the absorbance of plasma lipids, but the assay response is decreased.

• Automated methods:

Adaptations to the various analysers are available upon request. The assay is then performed kinetically. Sample blanks are automatically subtracted. **Reagent volumes and working dilution can vary according to the automate used. Refer to the specific adaptation and specific cautions for each instrument.**

QUALITY CONTROL:

Using commercially available quality control plasmas titrated for fibrinogen allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. The calibration curve is acceptable when the concentrations measured for controls are within the acceptance range.

Various control plasmas are available: **BIOPHEN Normal Control Plasma (#223201)** and **BIOPHEN Plasma Fibrinogen Control Low (#SC070K)**. Each laboratory should verify its own target value and acceptance range, in the exact working conditions, for each new lot of controls.

Note :

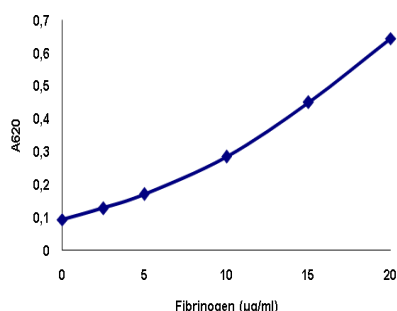
- A new calibration curve must be carried out better with each series, and for each new lot of reagents, after each important maintenance of the analyzer, or when measured values for the quality controls are out of the acceptance range determined for the method.
- Each laboratory can establish its own acceptance ranges, according to the instruments and protocols used.

RESULTS:

- For the manual method, plot on abscissae the fibrinogen concentration (in µg/ml) and on ordinates the corresponding absorbance (A620nm) and draw the calibration curve. Using an adapted software draw the calibration curve that best fits your values (3rd order polynomial, best fit, Akima...). Calibration is validated when controls are measured in compliance and $r^2 \geq 0.98$.
The Fibrinogen concentration in the tested sample is obtained by multiplying the the concentration obtained by the dilution factor used using the calibration curve (e.g. for a 1:300 dilution factor, multiply the result by 300).

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is an example only, obtained with the manual method. Only the calibration curve generated for the series of assays performed must be used for calculating the concentrations.



PERFORMANCE CHARACTERISTICS:

- Dynamic range:** using manual method, from 0 to 20 µg/ml of human fibrinogen in the test dilution (i.e. 0 to 6 g/L in the test sample).
- Limit of Quantification:** < 1 µg/ml (i.e. 0.2 g/L in the test sample), defined as the lowest concentration giving a CV < 20% for repeatability on N=15 replicates.
- Specificity:** serums are assayed below 0.2 g/L.
- Precision:** 3 plasmas with high, medium and low fibrinogen concentrations have been assayed twice a day during 6 days. Results are presented in the table below:

	Fibrinogen concentration (g/L)	Within-run repeatability	Total precision
Level 3	5.04	3.8%	7.7%
Level 2	2.58	3.1%	9.8%
Level 1	1.31	3.4%	9.8%

• Method comparison:

The comparison study with Fibriphen (Fibrinogen Clotting Assay, #CK575K, Hyphen BioMed) performed on STA-R instrument (Stago) gave the following results:

$$N = 69 \quad Y = 0.97X - 0.06 \quad R = 0.977$$

The comparison study with Multifibren U® (Fibrinogen Clotting Assay, Siemens) performed on BCS instrument (Siemens), gave the following results:

$$N = 96 \quad Y = 1.02X + 0.19 \quad R = 0.958$$

- Cross-reactivity:** The LIAPHEN Fibrinogen reacts also with Fibrinogen fragment D, Fragment DD (DDimer), and Fibrin Degradation Products (FDPs) prepared from plasma or from purified fibrinogen; it does not react with Fibrin Fragment E.
- High-dose hook effect:** No hook effect is observed for Fibrinogen concentrations below 90µg/ml (corresponding to 18 g/L in the test sample on STA-R instrument and 27 g/L using manual method), when the standard plasma dilution are used.
- Limitations of the procedure:**
 - No interference is observed for: Unfractionated Heparin and Low molecular weight Heparin ≤ 2 IU/ml, Bilirubin ≤ 0.2 g/L, Haemoglobin ≤ 2 g/L, Intralipid® ≤ 0.75% (corresponding to 20 g/L of triglycerides).
 - The presence of rheumatoid factor may result in an overestimation of the Fibrinogen concentration.
 - For patients receiving thrombolytic therapy, blood samples must be collected with an anticoagulant mixture containing a plasmin inhibitor (eg: aprotinin).

USUAL VALUES AND PATHOLOGICAL VARIATIONS:

The reference range in normal plasma, obtained on N= 56 healthy individuals on STA-R instrument is from 1.9 to 4.3 g/L of fibrinogen. Systematic deviations from this range may be determined by the particular device. It may be necessary to calculate a reference range specific to the laboratory.

Low fibrinogen levels can be observed in the following clinical situations: acute liver failure, congenital afibrinogenemia or hypofibrinogenemia, disseminated intravascular coagulation, primary fibrinolysis, secondary fibrinolysis, treatment with thrombolytic drugs (streptokinase, urokinase, tissue plasminogen activator, L-Asparaginase)

Elevated fibrinogen concentrations (> 5mg/ml) are observed in clinical situations associated with inflammation.

GENERAL INFORMATION AND BIOCHEMISTRY:

Fibrinogen is a 340 Kd glycoprotein, 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.

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