STA® - LIQUID FIB

Quantitative determination of fibrinogen according to Clauss

- Kit Containing 12 x 4-ml Vials

(REF 00673) IVD CE

March 2014 *English 2*

1/ INTENDED USE

The **STA® - Liquid Fib** kit is intended for use with STA-R®, STA Compact® and STA Satellite® for the quantitative determination of fibrinogen levels in plasma by the clotting method of Clauss (1).

2/ SUMMARY AND EXPLANATION

Fibrinogen is a glycoprotein of a molecular weight of approximately 340000 daltons (5), present in plasma at a concentration in the range of 2 to 4 g/l (200-400 mg/dl) (2). It is synthesized in the liver (1.7 to 5 g/day) (5). The synthesis of fibrinogen is controlled by the gene which codes for the β chain synthesis (3). Due to the existence of a genetic polymorphism for this gene, the plasma level of fibrinogen varies among individuals (3). The half-life of fibrinogen is about 3-5 days (5).
Fibrinogen is composed of six chains: 2 Aα, 2 Bβ and 2γ (4). Thrombin (factor IIa) breaks up the fibrinogen molecule to split out 2 fibrinopeptide A (FPA) fragments from the Aα chains and 2 fibrinopeptide B (FPB) fragments from the Bβ chains (4). The fibrin monomers that are produced from these reactions then aggregate to form fibrin, which is subsequently stabilized by factor XIIIa (3, 4). The first step of this stabilization consists of the binding of two γ chains of two fibrin monomers (4). This binding is the origin of D-dimer, the degradation product that is specific of fibrin (4).
An increase of fibrinogen level is found in cases of diabetes, inflammatory syndromes, obesity (3, 5); a decrease of the fibrinogen level is observed in DIC, fibrinogenolysis (3).
Furthermore, fibrinogen seems to be involved in the pathogenicity of thrombotic cardiovascular events (3, 5).

3/ TEST PRINCIPLE

In the presence of an excess of thrombin, the clotting time of a diluted plasma sample has a direct bearing on the level of plasma fibrinogen (3).

4/ KIT REAGENT

An Assay Value insert with a barcode is provided in the box. This barcode contains the following information: lot number, kit code number, reagent code number, expiration date and calibration values.

STA® - Liquid Fib: titrated human calcium thrombin (approx.100 NIH units/ml) containing a specific heparin inhibitor to allow the assay of fibrinogen in heparinized plasma samples.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
The reagent provided in this kit contains materials of human and/or animal origin. Whenever human plasma is required for the preparation of this reagent, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, 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.

5/ CAUTION

Store at 2-8 °C. For *in vitro* diagnostic use only. This reagent is to be used only by certified medical laboratory personnel authorized by the laboratory.
The STA® - Liquid Fib kit is designed for use with analyzers of the STA® line suitable with this reagent. Read the Reference Manual of the analyzer model carefully before starting.
Exercise great care in the handling of this reagent and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

6/ SPECIMEN COLLECTION AND TREATMENT

Sample collection must be in conformity with the recommendations for haemostasis tests.

- Blood (9 vol.) is collected in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.).
- Centrifugation: 15 minutes at 2000-2500 g.
- Plasma storage: 8 hours at 20 ± 5 °C.

7/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use. Swirl the vial gently; then place a new STA® - mini Reducer (REF 00797) in the vial and the perforated cap on top.

  This reagent contains a 3:1 mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. At the concentration provided (< 0.06 %), this mixture is classified as sensitising.
  Warning
  May cause an allergic skin reaction.
  Wear protective gloves/protective clothing/eye protection/face protection.
  IF ON SKIN: Wash with plenty of soap and water.

- **Storage**
  The reagent in intact vials is stable until the expiration date indicated on the box label when stored at 2-8 °C.
  Once opened, the reagent, with a new STA® - mini Reducer and the perforated cap in place, remains stable for 10 days on STA-R®, STA Compact® and STA Satellite®.
  After first opening, the remaining solution stored at 2-8 °C in its original vial with the cap on top is stable for 2 months when free of any contamination.
  Do not freeze.

  NB: Considering the numerous combinations of storage conditions (partly on board, partly at 2-8 °C), each laboratory should establish its own stability durations according to its practices. These durations should not exceed the above mentioned figures which have been determined under controlled conditions.
  In case of storage at 2-8 °C, allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use.

8/ REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - Owren-Koller** (REF 00360).
- For calibration: **STA® - Unicalibrator** (REF 00675), in case the pre-calibration is not selected.
- For quality control: **STA® - Coag Control** N + P (REF 00679), **STA® - Coag Control** N + ABN* (REF 00676), **STA® - Coag Control** (N + ABN) **PLUS** (REF 00677), **STA® - System Control** N + P (REF 00678) or **STA® - Routine QC 2 ml** (REF 00554).
- STA-R®, STA Compact® or STA Satellite®.
- STA® - mini Reducer (REF 00797).
- Common clinical laboratory equipment and materials.

* Available in the United States only.

9/ PROCEDURE

9.1. Calibration

- **Pre-calibration Protocol**
  The pre-calibrated fibrinogen values are identical for all the reagents of the same lot. The pre-calibration has been determined with a secondary standard of the 09/264 International Standard established in 2011.
  To enter the calibration data on the analyzer, scan the barcode printed on the Assay Value insert across the instrument barcode reader. The calibration data will be validated for the lot being used once the two fibrinogen control levels have been determined.
  The calibration curve can be examined on the screen of the analyzer in the "Calibration" menu (see the Reference Manual).

- **Calibration Protocol with STA® - Unicalibrator**
  As an alternative to the pre-calibration protocol, the calibration may also be performed with STA® - Unicalibrator, provided that the correct test setup has been chosen (see the Reference Manual). Prepare the STA® - Unicalibrator and scan the information contained in the barcode of the Assay Value insert into the instrument.
  The standards are automatically prepared by the analyzer by dilution with STA® - Owren-Koller according to the parameters entered in the instrument for the assay.
  The calibration curve can be examined on the screen of the analyzer in the "Calibration" menu (see the Reference Manual).

9.2. Patients' Plasmas

Patients' plasmas are tested undiluted. They are loaded in the instrument (see the Reference Manual of the analyzer model). The analyzer automatically prepares the dilutions with STA® - Owren-Koller. Then select the test(s) to be performed.

9.3. Quality Control

It is necessary to run controls in order to ensure accuracy and reproducibility of the results. Two different levels of control should be used. Prepare the control reagents and scan into the instrument the information contained in the barcode printed in their respective Assay Value inserts. These controls are used undiluted.

9.4. Assay

Refer to the "Standardized Operating Procedures" of the instrument for full details on how to proceed from this point.
The fibrinogen assay of the plasmas to be tested is automatically carried out by the analyzer as soon as the samples have been loaded. If any of the patient results falls outside the linearity range of the assay, the instrument automatically retests the sample in question at an appropriate dilution, provided that this option has been entered in memory in the test setup (see the Reference Manual).

10/ RESULTS

The fibrinogen level of the plasmas being tested is displayed, in the unit selected by the operator, in the "Test Panel/Test Status" screen of the analyzer (see the Reference Manual). The result is to be interpreted according to the patient's clinical and biological states.

Ensure that the values obtained for the controls are within the ranges stated in the Assay Value inserts provided in the control box. If the control values are outside the stated ranges, check all components of the test system to ensure that all are functioning correctly, i.e., assay conditions, reagents, integrity of the plasmas being tested, etc. If necessary, repeat the assays.

11/ LIMITATIONS

- When the fibrinogen assay is to be performed on samples collected from patients receiving thrombolytic therapy and without addition of an anticoagulant mixture containing a plasmin inhibitor in the collection tube, fibrinogen results may be underestimated.
- The STA® - Liquid Fib procedure is insensitive to the following substances: fibrin degradation products (up to 120 µg/ml), hirudin (up to 3 µg/ml), heparins (UFH and LMWH) (up to 2 IU/ml), dabigatran (up to 500 ng/ml) and rivaroxaban (up to 1.2 µg/ml).

12/ REFERENCE INTERVAL

The normal plasma fibrinogen level in the adult population is usually in the range of 2-4 g/l (200-400 mg/dl) (2). However, each laboratory should determine its own normal range.
During pregnancy there is an increase in fibrinogen level (3, 5).

13/ PERFORMANCE CHARACTERISTICS

- **Linearity Range - Measuring Range**
  When the plasma to be tested is diluted 1:20, the STA® - Liquid Fib assay has a linear range of 1.0-8.0 g/l (100-800 mg/dl) on STA-R®, STA Compact® and STA Satellite®. The measuring range can be extended down to 0.4 g/l (40 mg/dl) and up to 12.0 g/l (1200 mg/dl) when the plasma is automatically retested at an appropriate dilution (see section 9.4).

- **Reproducibility**
  Different samples were used for the intra-assay and inter-assay reproducibility studies. Results obtained by the STA-R® are shown below:

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
n	21	21	10	10
$\bar{X}$ (mg/dl)	283	103	284	103
SD (mg/dl)	6	5	6	3
CV (%)	2.1	4.9	2.1	3.2

REFERENCES

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3. MACKIE I.J., KITCHEN S., MACHIN S.J., LOWE G.D.O.:
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4. MOSESSON M.W.:
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Significant changes are indicated by dotted lines in the margin.

DIAGNOSTICA STAGO S.A.S.
9 rue des Frères Chausson
92600 Asnières sur Seine (France)
+33 (0)1 46 88 20 20
webmaster@stago.com

English