FIBRI-PREST® AUTOMATE
Quantitative Determination of Fibrinogen

FIBRI-PREST® AUTOMATE ②

- Kit Containing 12 x 2-ml Vials for Approx. 240 Tests

(REF 00613)

FIBRI-PREST® AUTOMATE ⑤

- Kit Containing 12 x 5-ml Vials for Approx. 600 Tests

(REF 00854) CE

English 3 - Revised July 2003

1/ INTENDED USE

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 340 000 daltons, present in plasma at a concentration in the range of 2 to 4 g/l (200-400 mg/dl) (5). It is synthesized in the liver (1.7 to 5 g/day) (4) and by megakaryocytes (5). The synthesis of fibrinogen is controlled by the gene which codes for the β chain synthesis (5). Due to the existence of a genetic polymorphism for this gene, the plasma level of fibrinogen varies according to the individuals (5). The half-life of fibrinogen is about 3-5 days (4).
Fibrinogen is composed of six chains: 2 Aα, 2 Bβ and 2 γ (5). 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 (5). The fibrin monomers that are produced from these reactions then aggregate to form fibrin (6), which is subsequently stabilized by factor XIIIa. The first step of this stabilization consists of the binding of two γ chains of two fibrin monomers (5). This binding is the origin of D-dimer, the degradation product that is specific of fibrin (5).
Fibrinogen can be degraded by plasmin (5).

An increase of fibrinogen level is found in cases of diabetes, inflammatory syndromes, obesity (8); a decrease of the fibrinogen level is observed in DIC, fibrinogenolysis (5).
Furthermore, fibrinogen seems to be involved in the pathogenicity of thrombotic cardiovascular events (7, 8).

3/ TEST PRINCIPLE

In the presence of an excess of thrombin, the clotting time of a diluted plasma has a direct bearing on the level of plasma fibrinogen (2, 3).

4/ KIT REAGENT

Fibri-Prest® Automate: freeze-dried titrated human calcium thrombin (approx. 80 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 regulatory safety precautions in the manipulation of these biological materials as if they were infectious.

5/ CAUTION

For *in vitro* diagnostic use only. Store at 2-8 °C. This reagent is to be used by certified medical laboratory personnel only. The disposal of waste materials must be carried out according to current local regulations.

6/ SPECIMEN COLLECTION AND TREATMENT

- Blood (9 vol.) is collected in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.) (in the USA follow NCCLS guidelines H3-A3 and H21-A3).
- Centrifugation: 10 minutes at 2,500 g.
- Plasma storage: 8 hours at 20 ± 5 °C.

7/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Reconstitute the Fibri-Prest® Automate reagent as follows:
  – each vial from REF 00613 with 2 ml distilled water
  – each vial from REF 00854 with 5 ml distilled water.
  Then, allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Swirl vial gently before use.

- **Storage**
  The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.

  Once reconstituted, they remain stable for:
  7 days at 20 ± 5 °C } in their original vial
  14 days at 2-8 °C } or in a plastic tube.

8/ REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - Owren-Koller** (REF 00360).
- **Unicalibrator** (REF 00625).
- **Coag Control N + P** (REF 00621) or **System Control N + P** (REF 00617): control plasmas, normal and abnormal levels.
- Common clinical laboratory equipment and materials (centrifuge, distilled water...).

9/ PROCEDURE

9.1. Calibration

Use STA® - Owren-Koller to prepare at least 3 dilutions of the Unicalibrator (1:5, 1:10 and 1:20 dilutions or 1:10, 1:20 and 1:40 dilutions). At the dilution that is recommended by the instrument manufacturer for patients' samples, the Unicalibrator has the fibrinogen level indicated in the Assay Value insert.

9.2. Patients' Samples

Again, use STA® - Owren-Koller buffer to prepare 1:10 dilution (1 vol. plasma + 9 vol. buffer) or 1:20 dilution (1 vol. plasma + 19 vol. buffer) of patients' plasmas exactly as recommended by the manufacturer of the instrument being used.

9.3. Quality Control

Similarly, use STA® - Owren-Koller buffer to prepare the same dilution of the control as described above for the patients' plasmas, i.e., 1:10 or 1:20 dilution, depending on the instrument manufacturer's instructions.

9.4. Assay

Keep the Fibri-Prest® Automate reagent on the bench at room temperature (18-25 °C). Follow the instrument manufacturer's instructions for fibrinogen assay. For instance:

• Test sample (standard, patient's or control)	0.2 ml
• Incubate at 37 °C for	2 min.
• Fibri-Prest® Automate	0.1 ml
Read the clotting time (seconds).	

10/ RESULTS

Use log-log graph paper. Plot the fibrinogen level for each calibration point on the x-axis and the corresponding clotting times (seconds) on the y-axis. Draw the best-fit calibration line.
Deduce the fibrinogen level of the plasma being tested from the calibration line. The calibration table found in the kit is provided only as a guide. It is recommended that each laboratory perform assay calibration with the instrument being used.

If the clotting time for the 1:10 (or 1:20) dilution of the plasma being assayed is:
– less than 5 s, retest the plasma at 1:20 (or 1:40) dilution; in this case the test result read from the calibration line will be multiplied by 2
– longer than 30 s, retest the plasma at 1:5 (or 1:10) dilution; in this instance the test result read from the calibration line will be divided by 2.

Ensure that the values obtained for the controls are within the ranges stated in the insert provided in the Coag Control N + P or System Control N + P 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, calibration, integrity of the plasmas being tested, etc. If necessary, repeat the test-run.

11/ LIMITATIONS

- When the fibrinogen assay is to be performed on samples collected from patients receiving thrombolytic therapy, the blood samples must be collected with an anticoagulant mixture containing a plasmin inhibitor (such as aprotinin, REF 00820).
- The Fibri-Prest® Automate procedure (1:20 dilution of the tested plasma) is insensitive to the following substances: fibrin degradation products (up to 130 µg/ml), hirudin (up to 3 µg/ml) and heparin (up to 2 IU/ml).

12/ EXPECTED VALUES

The normal plasma range of fibrinogen in the adult population is usually between 2 and 4 g/l (200-400 mg/dl) (5). However, each laboratory should determine its own normal range.

13/ PERFORMANCE CHARACTERISTICS

- **Working Range**
  With the calibration table provided in the kit, the procedure has a working range of 90-1000 mg/dl.
- **Reproducibility**
  Different samples were used for the intra-assay and inter-assay reproducibility studies. Results obtained by ST art® are shown below:

	Intra-assay reproducibility		Inter-assay reproducibility	
	Sample 1	Sample 2	Sample 3	Sample 4
n	24	24	10	10
$\bar{X}$ (mg/dl)	313	112	292	100
SD (mg/dl)	5	4	7	3
CV (%)	1.7	3.7	2.3	3.3

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