Fibrinogen in Plasma by Rate of Clot Formation on the STA-Compact

University of Washington Medical Center
Department of Laboratory Medicine
Immunology Division
Director: Mark Wener M.D.
Supervisor: Phyllis Daum M.T. (ASCP)
Authors: Phyllis Daum M.T. (ASCP)
Joanne Estergreen M.T. (ASCP)

0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
l11_b	LBXFB	Fibrinogen (mg/dL)	Fibrinogen
l11_b	LBDFBSI	Fibrinogen (g/L)	

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

On the STA-Compact, the Fibrinogen concentration in plasma is determined quantitatively by the Clauss clotting method. This test method involves measuring the rate of fibrinogen to fibrin conversion in diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content is rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact the clotting time is inversely proportional to the level of fibrinogen in the plasma.

Clot detection by the STA-Compact involves an electromagnetic- mechanical system. The oscillation of a steel ball within the cuvette with the thrombin and diluted plasma is monitored by the STA-Compact. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time in seconds. The time is translated into Fibrinogen concentration from a Fibrinogen Standard curve, stored on the STA Compact.

Increased fibrinogen levels are observed in cases of diabetes, inflammatory syndromes and obesity. A decrease of the fibrinogen level is observed in DIC, fibrinolysis and hereditary diseases.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with Staphene or bleach solution. Dispose of all biological samples and diluted specimens in a biohazard bag at the end of the analysis.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal of personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for reagents used in this procedure are kept in the Coagulation Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Each shipment of specimens received from the NHANES IV mobile unit arrives with a corresponding transmittal sheet and a Send File (a comma delineated text file) transmitted electronically (labeled *boxnum.*shp). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX

b. The information from the shipping file is imported into a result file with the following format:

Results File: Fibrinogen -Vessel ID 10

Field	Format	Type	Item ID
Sample ID	XXXXXXXXX	Int	
Slot Number	XXX	smallint	
Sample Collection Date	mm/dd/yy hh:mm:ss	Smalldatetime	
MEC Comment Code	XX	Smallint	
Date of Receipt	mmddyyyy	Smalldatetime	LBXFBDR

Field	Format	Type	Item ID
Fibrinogen Run num	{test code}mmddyy.x(letter)	Char(10)	LBXFBBT
Fibrinogen Run Date	mmddyyyy	Smalldatetime	LBXFBDA
Fibrinogen Result	XXXX	Char(5)	LBXFB
Fibrinogen Comment Code	XX	Smallint	LBXFBLC
Fibrinogen Tech id	XXX	Char(3)	LBXFBTK
Fibrinogen 2.5% repeat	XXXX	Char(5)	LBCFB

c. After the testing is completed, the run number, date of analysis, Fibrinogen result, Fibrinogen comment, Fibrinogen analyst, and the Fibrinogen 2.5% repeat results are entered into the result file.

d. Data entry is checked for errors.

e. The result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. Blood is collected in 3.2% trisodium citrate in the proportion of nine volumes of whole blood to one volume of anticoagulant.

b. Centrifuge the blood at 3500 rpm for seven minutes.

c. If testing is not done within twelve hours of collection the plasma may be stored at -70 ^{o}C for nine months.

d. Unacceptable specimens include samples that are short draws, clotted samples or hemolyzed samples as these may yield incorrect results.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Reagents and standard materials

1. STA-Fibrinogen: Freeze-dried titrated human calcium thrombin (approx. 100 NIH unit/ml) containing a specific heparin inhibitor. Reconstituted Stability on the STA-Compact is 4 days. Stable until manufacturer's expiration date when stored at 2 - 8 ^{o}C. Cat #00674, (Diagnostica Stago, Manufactured in Paris, France Distributed from Parsippany , NJ, USA)

2. Owren-Koller buffer is ready to use buffer. It is used by the STA Compact to perform dilutions of controls and patients' plasmas. Stability is 120 hours on the STA Compact and until the expiration date when stored at 2-8 ^{o}C. Cat #00360, (Diagnostica Stago, Parsippany , NJ)

3. Desorb U: 15 mL vials of this reagent requiring no reconstitution. The Desorb U is a decontamination reagent for the STA-Compact. Store at 2-8 ^{o}C until use. Stable until manufacturer's expiration date. Cat # 00975 (Diagnostica Stago, Parsippany , NJ)

4. Cleaner solution comes in large plastic bottles and is loaded into the side of the instrument. Store at room temperature prior to use. Cat #00973 (Diagnostica Stago, Parsippany , NJ)

5. STA-Coag control N+P control plasmas, normal and abnormal levels Cat #00679 (Diagnostica Stago, Parsippany , NJ)

6. Distilled water (University of Washington, Seattle, WA).

b. Reagent preparation

1. STA-Fibrinogen: Reconstitute each vial with 5.0 mL of reagent grade water. Let sit 30 minutes at room temperature. Swirl gently. Reconstituted Stability on the STA-Compact is 4 days. Stable until manufacturer's expiration date when stored at 2 - 8 ^{o}C.

c. Instrumentation

1. STA Compact Analyzer

   The STA Compact is an automated laboratory instrument designed to perform in vitro tests which aid in the diagnosis of coagulation abnormalities as well as to assist in monitoring anticoagulant therapy. The instrument is capable of performing clotting assays as well as chromogenic and immunological assays on plasma samples. (Diagnostica Stago, Parsippany , NJ)

2. STA Compact Cuvette roll Cat # 6453 (Diagnostica Stago, Parsippany , NJ)

3. Centrifuge (Jouan, Winchester, VA)

4. Computers (Dell Computer Corporation, Round Rock, Texas).

5. Pipettors and disposable tips 1000-uL, Pipetteman (Gilson, France).

6. Latex gloves, disposable (Safeskin, San Diego, CA).

d. Standards/Calibrator Preparation

A manufacturer supplied bar-code is provided in each box of fibrinogen reagent. This bar-code contains the following information: lot number, kit code number, reagent code number, expiration date, calibration values. With this bar-code it is not necessary to use a fibrinogen calibrator in the fibrinogen assay performed on the STA analyzers using the STA Fibrinogen reagent.

The pre-calibrated fibrinogen values are identical for all vials of each lot. When using the pre-calibration parameters provided by the insert bar-code for the first time, verify that local conditions (e.g. samples collection) are such the results obtained with this pre-calibrated reagent system are identical with those obtained with the laboratory's own calibration procedure.

e. Preparation of Quality Control materials

Coag Control N + P: Control N is citrated normal human plasma and Control P is citrated abnormal human plasma, both freeze-dried. Reconstitute each vial with 1.0 mL reagent grade water. Let sit 30 minutes at room temperature. Swirl gently. Reconstituted stability on the STA Compact is 8 hours. Store at 2 - 8 ^{o}C prior to reconstitution, good until manufacturer's expiration date. Cat #00679 (Diagnostica Stago, Paris, France)

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. The pre-calibrated fibrinogen values are identical for all the vials of each lot and are provided by the manufacturer.

b. Entering the data for the calibration curve: The database of the STA Compact monitors all reagent lot numbers. When the operator scans a new lot of fibrinogen reagent, the STA Compact will request the operator to scan the bar code printed on the bar code insert across the STA Compact bar code reader. By entering the bar coded information the calibration curve (stored in the bar code) is entered into the memory of the STA- Compact

c. The calibration curve will be validated for the lot being used when the two fibrinogen control levels have been run. If the validation controls are outside the assayed range, the STA-Compact will not run patient samples.

d. To examine the calibration curve on the STA Compact screen go through the MAIN MENU under CAL./CONTROL select CALIBRATION. Move the cursor to FIB and press “Enter”. Curve will appear on the STA-Compact screen.

e. To print the calibration curve: While examining the curve on the STA-Compact screen, press ESC for options. Select PRINT. Press “Enter” to execute the print command. The STA-Compact cannot print a calibration curve while the STA-Compact is running.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

1. Quick thaw frozen specimens, QC plasma samples, and calibration plasma samples in a 37 ºC water bath and mix by inversion for 10 sec.

2. Prepare a sufficient reagent and cuvettes for the samples being tested.

b. Instrument Set-up

1. The parameters for the STA Compact plus are as follows:

STA Compact Parameters

Parameter	Setting
Sample volume	0.10 ml (1/20 dilution in Owen Koller Diluent)
STA Fibrinogen	0.050 ml
Min time	4 sec
Maximum time	66 sec
Mean Time	30 sec
Primary units	mg/dL
Redil condition	1/4 <100.00 mg/dL 1/40 > 900.00 mg/dL

2. Refer to the START-UP procedure for the STA-Compact before running patient specimens on the STA-Compact at the start of each shift.

c. Instrument Operation (see operator's manual for STA Compact for more details).

1. Request quality control: Through the MAIN MENU under CAL./CONTROL, select QUALITY CONTROL and press “Enter”. Cursor to the FIB test. Select FIB by pressing **F1** and then **F10** to run QC. Enter the posted access code and press “Enter” when prompted. After moving out of the QUALITY CONTROL menu the controls will begin running.

2. Load patients’ samples: Access the sample drawer through the MAIN MENU under LOADING. After the drawer opens, identify the type of specimen, such as microsample, with **F8**, or stat, with **F12**. Identify the sample by bar coding in or typing in the patient account number and then placing the specimen into the drawer. Samples may also be loaded when in the Test Panel screen by pressing **F1** to access the sample drawer and then follow the previous instructions.

3. In MANUAL MODE, the operator must order the test(s) from the test or test profile menu. Cursor to the desired test or test profile and press “Enter” to select. When all desired tests are selected, press **F10** to save.

4. In AUTO MODE, the STA Compact will automatically order the test(s) selected in the AUTO MODE profile.

5. If TELELOADING is selected as the AUTO MODE profile, the STA Compact will query the host computer and download the test(s) as well as assign the status (i.e. stat).

6. As soon as the sample drawer closes, the TEST STATUS screen will appear. If there is not enough reagent(s) to run the test(s), the suspect reagent(s) will appear in red with the amount of deficiency. This deficiency will BLOCK the SAMPLE PIPETTING. When this occurs, add the necessary reagent(s) to run the samples by responding N (NO) to the warning message ‘NEW TESTS ARE DELAYED - REACTIVATE?' Reagents can then be loaded in the drawer. By responding Y (YES) to the warning message ‘NEW TESTS ARE DELAYED - REACTIVATE?' the instrument will continue to perform all tests for which there is sufficient reagent (i.e. while waiting for reagents to stabilize after reconstitution).

7. All dilutions of controls and patients’ samples are automatically prepared by the STA-Compact according to the parameters entered in TEST SET UP. If the patients’ results fall outside the assay range, the STA Compact automatically retests the sample in question at an appropriate dilution provided that the option has been entered in TEST SET UP under the redilute conditions. (See test system parameters)

8. All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected in SYSTEM STATUS. Results are reported to the nearest whole number.

9. For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and press “Enter”. This will display the FILE PROCESSING screen. Follow the options on the left hand side of the screen(i.e. **F3** - rerun test, **F5** - insert or add-on test).

d. Calculations

The STA-Compact automatically converts the clotting time in seconds to the Fibrinogen

concentration in mg/dL using a [log-log] Fibrinogen standard curve. The assay uses a dilution of 1:20 sample plasma in Owren Koller buffer. The STA Compact automatically redilutes the sample 1:4 if a sample has a concentration <100 mg/dL and 1:40 if the value is >900 mg/dL

e. Recording of Data

1. Specimen results are entered into the Assay Specific Results table using the Excel program. Each box has a corresponding Results Table (labeled *boxnum.testname.*xls) that can be located in the Pending Results folder. A copy of this table is printed out and checked for accuracy of data entry.

2. Control results are entered to the Assay Specific QC/Levy-Jennings Table using the Excel program. A copy of this table is printed out and checked for accuracy of data entry.

f. Replacement and Periodic Maintenance of Key Components

1. Weekly Maintenance:
   - Clean air filters
   - Clean needles with mandrels
   - Clean washing wells with 10% bleach
   - Check Peltier reservoirs liquid level
   - Clean drawers and measurement plate with warm water
   - Clean measurement and incubation wells with cotton swab soaked in ethanol, remove any debris
   - Clean suction tip with warm water

2. Monthly Maintenance:
   - Replace syringe tip and check o-ring if necessary

3. Periodic Maintenance to be performed by the manufacturer's service engineer on a regular periodic schedule.

9. Reportable Range of Test Results

a. The lowest reportable fibrinogen result is 20 mg/dl.

b. If the Fibrinogen result is >MAX the clotting time used to ascertain the Fibrinogen concentration is > 66 sec. This translates into a Fibrinogen of <25 mg/dL (once the test has been repeated diluted 1:4). Report the Fibrinogen as <25 mg/dL" (>Max) or numerical result if one is given.

c. Specimens with results > 900 mg/dl are automatically repeated by the instrument using a 1:40 dilution.

10. QUALITY CONTROL (QC) PROCEDURES

a. Bench quality controls are used in this analytical method. Bench quality control specimens are tested with each analytical run (a set of consecutive assays performed without interruption) so that judgements may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

b. Two levels of control material are used. The acceptable QC ranges are set by running 20 parallel runs with previously established controls.

c. QC can be run automatically at pre-set intervals (in TEST SET UP) or by ordering manually from the Quality Control Menu at the start of each shift.

d. All control ranges are monitored automatically by the STA- Compact. If any controls are outside the 2 SD range, the STA Compact will audibly and visually alarm the operator. Otherwise, the results can be found in the individual QC files. Control results are automatically filed in the STA Compact QC file. All results for a 24 hour period will be reduced to a "mean" value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean.

e. The system is out of control if any of the following events occur for any one of the quality control materials

- The mean from a single pool falls outside the 99% confidence limits.
- The means from two pools fall either both above or both below the 95% confidence limits.
- The means from eight successive runs for one pool fall either all above or all below the center line.

11 REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. In patients receiving drugs that affect the fibrinolytic system, the plasma levels of fibrinogen degradation products (FDP) may be extremely high. FDPs may inhibit both thrombin action on fibrinogen and fibrin polymerization. At normal fibrinogen concentrations, FDPs have a minimal effect on the fibrinogen assay. At fibrinogen concentrations below 150 mg/dl, FDPs greater than 100 g/mL increasingly inhibit the thrombin clotting rate assay. High levels of paraproteins may interfere with the polymerization of fibrin monomers too.

b. The clinical use of topical bovine thrombin has led to the generation of antibodies in some patients. These antibodies may lead to artifactual prolongation of the thrombin clotting rate assay of fibrinogen.

c. Heparin may interfere with this assay. However, the STA-Fibrinogen reagent contains a specific inhibitor of heparin. Any prolongation of the assay is, therefore, related to a real coagulation factor deficiency of Fibrinogen.

d. 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 at a final concentration of 0.2 TIU (trypsin inhibitor unit) per mL of blood.

13. REFERENCE RANGES (NORMAL VALUES)

150-400 mg/dL The range is from published data and was confirmed by the coagulation Division using 72 normal samples in September of 1998.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Westat will be immediately notified vie e-mail in the event of any sample observed to have a fibrinogen less than < 100 mg/dL.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are thawed and mixed prior to testing, and are kept at room temperature until analysis. The specimens are refrozen at ≤ - 70 º C. after testing.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There are no acceptable alternative methods of analysis. Specimens may be stored at 4-8 ^{o}C for no longer than 72 hours. Otherwise, specimens should be stored at ≤ -70 ^{o} C until the system is returned to functionality.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Westat will be immediately notified vie e-mail in the event of any sample observed to have a fibrinogen less than < 100 mg/dL.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record keeping should be used for tracking specimens. The primary results include daily test results as well as stored quality control results.

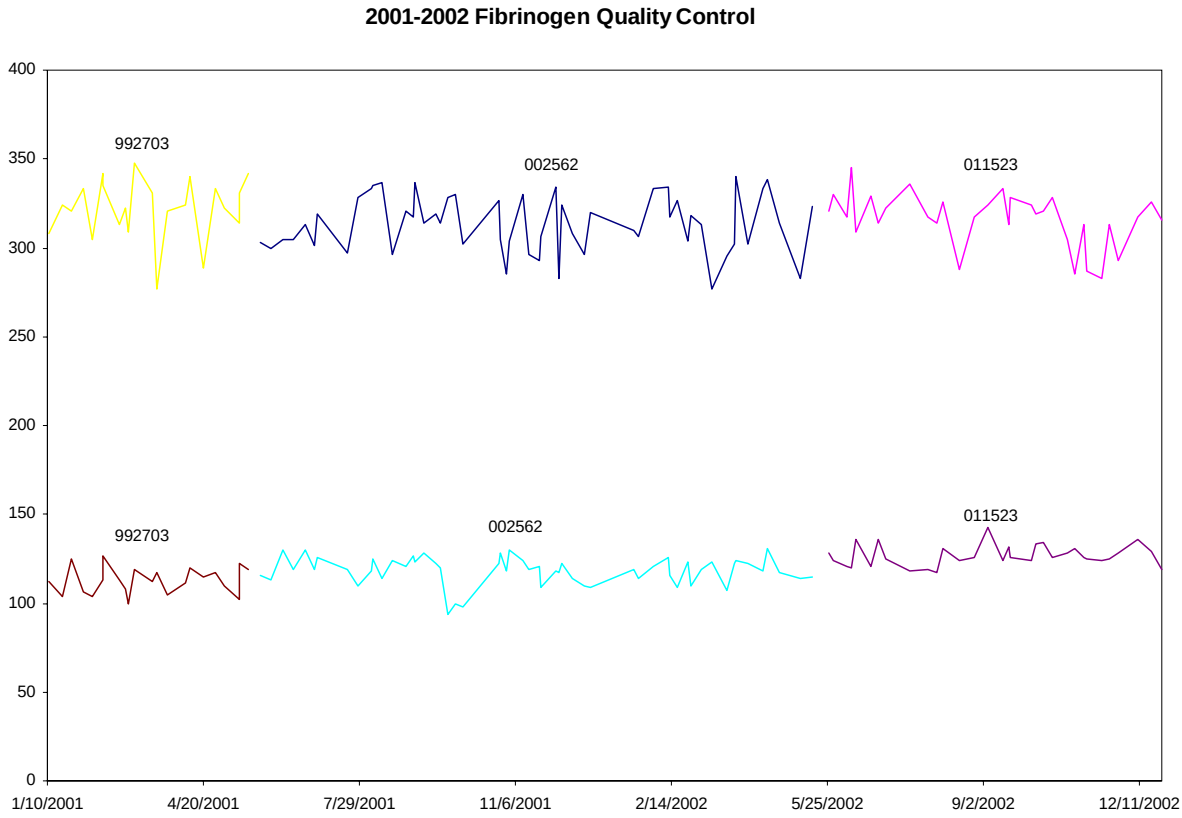
The original NHANES IV file is copied into a template Excel file and onto the hard drive of a PC computer. After the results are entered into the database and assay results transmitted electronically. Files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the result files are transferred onto a CD along copies of original ship files and QC information.

The residual plasma is stored at ≤ -70 ^{o} C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Fibrinogen by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
992703	22	1/11/2001	5/19/2001	322	17.3	5.4
992703	22	1/11/2001	5/19/2001	113	7.5	6.7
002562	55	5/26/2001	5/15/2002	313	16.1	5.1
002562	55	5/26/2001	5/15/2002	118	7.8	6.6
011523	32	5/26/2002	12/25/2002	316	15.0	4.8
011523	32	5/26/2002	12/25/2002	127	6.1	4.8



REFERENCES

1. Clauss A. "Gerrinnungsphysiologische Schnellmethods zur Bestimmung des Firinogens", ACTA Haematol,. 17,237-246, 1957.

2. Destain, F., Duzer, A., Ferrand, B., Protier, A., "Dosage du fibrinigene par la micr-methods de coagulation de von A. Clauss", Pathol. Biol., 8, 17.18, 1615-1621, 1960

3. Soria, J., "Contribution a l'etude de la physiopathologie de la fibrinoformation", These de doctorat d'etat en pharmacie, Paris 1968

4. Koepke, J.A,. Gilmer, P.R. Jr., Filip, D.J., Eckestein, J.D., Sibley, C.A., "Studies of fibrinogen measurement n the CAP survey program", Am. J. Clin. Pathol., 64, 984-989, 1975

5. Hantagen. R.R., Franci, C.W., Scheragia, H.A., Marder, V.J., "Fibrinogen structure and physiology in Hemostasis and Thrombosis Basic Principles and clinical practice", Colman, R.W., Hirsh, J., Marder, V.J., Salzman, E.W., Philadelphia: J.B. Lipincott company, 269-288, 1987

6. Samama, M., Conrad, J., Horellou, M.H., Lecompte, T., "Physiologie et exploration de l'hemastase" Paris: Doin, 1990

7. Collet, J.P., Soria, J., Mirshahi, M., Dagonnet, F.B. Caen,J., Soria,C. "Dusart syndrome a new concept of the relationship between fibrin clot architecture and fibrin clot degradability: hypofibrinolyisis related to an abnormal clot structure". Blood, 82,8,2462-2469, 1993.

8. Ernst, E., Resch, K.L. "Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature". Ann. Intern .Med., 118, 12 956-963, 1993

9. Alessi, M..C., Aillaud, M.F., Juhan-Vague, I., “Facteurs de risque thrombogenes et atherosclerose” Feuil. Bilol, XXXV, 197, 39-41, 1994

Other Sources

STA-Fibrinogen, Quantitative Determination of Fibrinogen by STA® Analyzers. Package insert 26363-Revised December 1997.

STA-Coag Control N + P or STA System Control N + P, Control Plasma for Coagulation Tests on STA® Analyzers. Package insert 26379 - Revised July 1997 and 26384 - Revised May 1997, respectively.