Quest Diagnostics

TRAINING UPDATE

Lab Location:	SGAH & WAH	**Date Distributed:**	11/6/2012
Department:	Core	**Due Date:**	12/1/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Fibrinogen SGAH.G05, WAH.G05 V002

Description of change(s):

Section	Reason
3.2	Delete frozen storage
4.1	Remove Millipore water

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training at all sites (version 002)

Technical SOP

Title	**Fibrinogen**
Prepared by	Ashkan Chini Date: 4/7/2011
Owner	Robert SanLuis Date: 4/7/2011

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Annual Review		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Fibrinogen, Quantitative	Clot based assay / STA® Compact	FIBR

Synonyms/Abbreviations
FIB

Department
Coagulation

2. ANALYTICAL PRINCIPLE

The STA® Fibrinogen kit is intended for the quantitative determination of fibrinogen in plasma by the clotting method of Clauss. In the presence of an excess of thrombin, the clotting time of diluted plasma is inversely proportional to the level of plasma fibrinogen. The clot is detected by the STA® Compact. The STA® Compact is a fully automated coagulation instrument that uses an electromagnetic mechanical clot detection 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 read from a stored curve on the STA® Compact. An increase of the fibrinogen level is observed in cases of diabetes, inflammatory syndromes and obesity. A decrease of the fibrinogen level is observed in DIC, fibrinolysis, thrombolytic therapy and hereditary diseases.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting plasma may be used for samples to be analyzed by this method. Vacutainer tube must be filled to the line to ensure the proper ratio of blood to anticoagulant.
Special Collection Procedures	Hematocrit of >55% or < 20%: Recollect the specimen using the correct volume of anticoagulant determined by the following formula: 100 – HCT equals the volume of anticoagulant (60 x 0.5) to mix with blood for a total of 5.0 ml
Other	When the fibrinogen assay is to be performed on samples collected from patients receiving thrombolytic therapy, the blood samples must be collected with an anti-coagulant mixture containing a plasmin inhibitor (See section 13).

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Whole Blood (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum **- Minimum**	4.5 mL (9:1 blood to anticoagulant) in 5ml tube 2.7 mL whole blood (9:1 blood to anticoagulant) in 3.0 mL tube

Criteria	
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.
Stability & Storage Requirements	Room Temperature: 8 hours (20 ± 5° C) Refrigerated: Not recommended Frozen plasma: Not recommended
Specimen preparation	Centrifuge whole blood for specified time /speed documented on each centrifuge for preparing platelet-poor plasma.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Clotted or under-filled tubes are not accepted. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message.
Compromising Physical Characteristics	Moderate to gross hemolysis. Reject sample and request a recollection. Credit the test with appropriate LIS English text code HMM (Specimen moderately hemolyzed) or HMT (Specimen markedly hemolyzed) Lipemia: Acceptable Icterus: Acceptable
Other Considerations	None

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
STA – Fibrinogen	Diagnostic Stago (REF 00674)
STA – Owren-Koller Buffer	Diagnostic Stago (REF 00360)
Pure Reagent Grade water	NERL Diagnostics (Cat. No. 0015)

4.2 Reagent Preparations and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent 1	**STA – Fibrinogen**
Container	Manufacturer supplied vial
Storage	2-8°C
Stability	Stable until expiration date indicated on the box label. Once reconstituted, the reagent is stable: • 5 days with perforated plastic cap in place. • 14 days at 2-8°C in its original capped vial.
Preparation	Reconstitute each vial with 5 mL of distilled water. Allow the reconstituted reagent to stand at room temperature (18-25°C) for 30 minutes. Swirl vial gently. Then place the perforated plastic cap on the vial.

Reagent 2	**STA – Owren-Koller Buffer**
Container	Manufacturer supplied vial
Storage	2-25°C
Stability	The buffer solution in intact bottles is stable until the expiration date indicated on the box label. After opening it remains stable for 3 days.
Preparation	If the buffer solution is refrigerated, allow it to stand at room temperature (18-25°C) for 30 minutes before use.

Reagent 3	**NERL Reagent Grade water**
Container	Manufacturer supplied vial
Storage	Room temperature.
Stability	Stable 30 days after opening.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

1. Fibrinogen does not require calibration. Reagent kits are pre-calibrated; this pre-calibration is identical for all the reagents of each lot.
2. 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 insert across the STA® Compact bar code reader.
   - 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.

3. Examine calibration curve on STA®/STA® Compact screen:
   - Through the MAIN MENU under CAL. /CONTROL select CALIBRATION.
   - Move the cursor to FIB and press **Enter** ↵. Curve will appear on STA®/STA® Compact screen.

4. Print calibration curve:
   - While examining the curve on the STA®/STA® Compact screen, press ESC key for options.
   - Select PRINT press **Enter** ↵. Select Execute.
   - Press **Enter** ↵ to execute the print command.

   Note: The STA®/STA® Compact cannot print a calibration curve while the STA®/STA® Compact is running.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
STA® Coag control N + ABN	Diagnostic Stago (REF 00676)

6.2 Control Preparations and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) time prepared, (5) expiration date and time, (6) initials of tech, and (7) any special storage instructions; check for visible signs of degradation.

Control	**Coag Control N + ABN**
Preparation	Reconstitute each vial of Reagent 1 or 2 with exactly 1 mL of Reagent Grade water. Allow the reconstituted material to stand at room temperature for 30 minutes. Then, swirl the vial gently before use.
Storage/Stability	2-8° C The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8° C. Once reconstituted, Reagents 1 and 2 remain stable for 8 hours on analyzers of the STA® line.

1. After the reconstitution period, request the product drawer to open through the MAIN MENU under LOADING and bar code the controls. Place the controls into the appropriate drawer.
2. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.

3. All control ranges are monitored automatically by the STA® Compact. If any controls are outside the ± 2 SD range, the instrument 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 are converted 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.

6.3 Frequency

Both controls are run at the beginning of each shift, every 4 hours after, and with the change of any reagent used in test performance.

6.4 Tolerance Limits

Step	Action
1	The established QC ranges are in the QC file of the STA Compact. The quality control results from the instrument are transmitted to the LIS and can be viewed in the OEM function. Any out-of-range QC results will be flagged by the LIS.
2	If all controls are within QC parameters all sample results can be reported.
3	Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed. Supervisor may override rejection of partial or complete runs only with detailed documentation that follows criteria that is approved by the Medical Director.
4	Corrective action documentation must include the following: QC rule(s) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information. See the QA SOP "QC Responsibilities and Review" for more detail.
5	If the assay is down and results will not be reported in the scheduled turnaround time, clients will be notified of the situation.

6.5 Review Patient Data

Technologist must review each result print-out for error messages. Refer to the STA® Compact system manual "Error messages" section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

STA® Compact – Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge calibrated for preparing platelet-poor plasma

7.3 Supplies

- Cuvette Roll – Diagnostic Stago
- STA – black adaptors
- STA – brass adaptors
- Plastic micro cups
- Plastic transfer pipettes
- Micro sample tube – Diagnostic Stago

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection is required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	Instrument Set-up Protocol
1	At the start of each shift, verify instrument temperatures and availability of cuvettes and cleaner solution by accessing the System Status screen from the main bar.
2	Record the temperatures on the maintenance sheet. If the reagent arm 2, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patients being run.
3	Make sure that there is an adequate supply of reagents in the analyzer, and they are in date.
4	Load cuvettes and cleaner/wash solution on the analyzer if needed.

8.2	Analytical Procedure
1	Refer to START-UP procedure for STA® Compact before running patient specimens on the STA® Compact at the start of each shift.
2	Request quality control. Through MAIN MENU under CALIB. /CONTROL select QUALITY CONTROL and press **Enter** ↵. Cursor to the FIB test. Select FIB by pressing **F1** and then **F10.** Type in your Access Code to run the QC.
3	Load patients' samples: Access the sample drawer(s) through the MAIN MENU, under LOADING, Select Sample, press **Enter** ↵. After the drawer opens, identify the type of specimen, such as micro sample (press **F8**), or stat (press **F12**). Identify the sample by bar coding or manually entering on the keyboard the patient identification number and then placing the specimen into the drawer.
4	In MANUAL MODE, the operator must order the test(s) from the Selection menu or from the Recorded Profile/s Cursor to the test and press **Enter** ↵to select. When all tests are ordered, press F10 to save.
5	In AUTO MODE, the STA®/STA® Compact will automatically order the test(s) selected in the AUTO MODE profile.
6	If TELELOADING is selected as the AUTO MODE profile, the STA®/STA® Compact will query the host computer and download the test(s) as well as assign the status (i.e. stat).
7	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 depletion. This depletion of reagent 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

8.2	Analytical Procedure
	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?
8	All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the system status menu.
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).

9. CALCULATIONS

The STA® Compact automatically converts the results in seconds from a standard curve (log-log) to mg/dL. The assay uses a dilution of 1:20 sample plasma to buffer. The STA® System automatically dilutes this sample to a 1:8 dilution on samples with a concentration <150 mg/dL or a 1:40 dilution if the value is >900 mg/dL. If the auto redilute feature is necessary the results are displayed on the Screen in Blue numerals, instead of the normal Black numerals.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

No rounding is necessary.

10.3 Units of Measure

mg/dL

10.4 Clinically Reportable Range (CRR)

60 - 1800 mg/dL

10.5 Repeat Criteria and Resulting

The printout from the STA Compact is reviewed for repeat criteria and samples are repeated if needed. Results will be transmitted to the LIS and released using the OEM function.

IF the result is …	THEN…
< Mmin	Repeat, check for clots before reporting results as: >1800 mg/dL, REP
>Mmax	Repeat, check for clots before reporting results as: <60 mg/dL, REP
< 100	Repeat, report with comment "REP"
> 800	Repeat, report with comment "REP"

Definitions:

<Mmin: The shortest time limit below which no result will be given. In the case of Fibrinogen this means the value is greater than 1800 mg/dl

>Mmax: The longest time limit above which no result will be given. In the case of Fibrinogen this means the value is less than than 60 mg/dl

SPECIAL NOTES RELATED TO FIBRINOGEN RESULTS:

- A >Mmax for the result for Fibrinogen means the Fibrinogen value is **extremely low**.
- A <Mmin result for Fibrinogen means the Fibrinogen value is **extremely high**.
- See Note # 1 in section 13. It is possible to have a >Mmax or <Mmin. Result after the instrument does the auto redilutes.

11. EXPECTED VALUES

11.1 Reference Ranges

200 – 500 mg/dL

11.2 Critical Values

< 100 mg/dL
> 800 mg/dL

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

An increase of fibrinogen level is found in cases of diabetics, inflammatory syndromes, obesity, and pregnancy. A decrease of the fibrinogen is observed in DIC and fibrinogenolysis. Furthermore, fibrinogen seems to be involved in the pathogenicity of the thrombotic cardiovascular events. Fibrinogen is composed of six chains: two alpha, two beta and two gamma chains. Thrombin (factor IIa) breaks up the fibrinogen molecule to split out two fibrinopeptide fragments from the A*α* chain and two fibrinopeptide fragments from the Bβ chain. The fibrin monomers that are produced from these reactions then aggregate to form fibrin, 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. This binding is the origin of the D-Dimer, the degradation product that is specific of fibrin.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

1. The STA uses electro-mechanical clot detection test, therefore lipemia and icterus do not interfere with the fibrinogen result. These findings should be reported with the results.
2. When the STA® Compact redilutes a patient sample at a more appropriate dilution (as pre-determined in Test Set-up) the results in the TEST PANEL screen which appear in Blue numerals have already been corrected by the STA® Compact for the dilutional difference.
3. Patients receiving thrombolytic therapy will have a rapid drop in the plasma Fibrinogen level and these samples **MUST** be collected with an anticoagulant containing a plasmin inhibitor such as Aprotinin, Cat # 0820, to determine an accurate Fibrinogen result.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

150 – 900 mg/dL

14.2 Precision

Different plasmas were used for reproducibility studies with the STA Fibrinogen Results obtained on the STA analyzer are shown in the package insert.

14.3 Linearity

The package insert states that the working range of the reagent on the STACompact® System instrument is 150-900 mg/dL. This is at the normal dilution (1:20) which, the instrument uses to assay samples. The linearity range on the STA® System instrument is 60-1800 mg/dL (see the bar-coded Calibration Curve) due to the different dilutions used for the auto redilution:

1:8 if < 150 mg/dl and 1:40 if > 900 mg/dL. For extremely high Fibrinogen samples a higher dilution can be set up as a dependent test.

14.4 Interfering Substances

1. 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 of 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 130 µg/mL increasingly inhibit the thrombin clotting rate assay. High levels of paraproteins may interfere with the polymerization of fibrin monomers.
2. 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.
3. 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.

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. QC Responsibilities and Review
3. Laboratory Safety Manual
4. Material Safety Data Sheets (MSDS)

5. Repeat Testing Requirements (Lab policy)
6. Critical Values (Lab policy)
7. Verification of Platelet Poor Plasma, Coagulation procedure
8. Current package insert for STA® Fibrinogen

17. REFERENCES

1. Diagnostic Stago Fibrinogen package insert: Revised November 2008.
2. STA®-Coag Control N + ABN (REF 00676): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA®, Revised December 2009.
3. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.
4. Diagnostic Stago Owren – Koller buffer solution for coagulation tests, revised November 2009.
5. Clauss A,"Rapid Physiological Coagulation Method for the Determination of Fibrinogen [German], *"Acta Haematol,* 1957,17:237-46.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G002.005		
000	10/31/2011	10.5	Revise MMin to > 1800 mg/dl and MMax to <60 mg/dl; add special notes	C Reidenauer	C Reidenauer
000	10/31/2011	15	Update to standard wording	L Barrett	C Reidenauer
000	10/31/2011	17	Add reference 5	C Reidenauer	C Reidenauer
001	10/19/2012	3.2	Delete frozen storage	C Reidenauer	R SanLuis
001	10/19/2012	4.1	Remove Millipore water	L Barrett	R SanLuis

19. ADDENDA

None