TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	5/5/2016
Department:	Core	**Due Date:**	5/19/2016
		Implementation:	**Refer to lab alert**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Fibrinogen SGAH / WAH.G05 v5

Thrombin Time WAH.G06 v4

Description of change(s):

Both SOPs

Section	Reason
3.2	Change whole blood to Plt poor plasma, update stability, add over-filled tubes as unacceptable
5	Add explanation for STA QC and Bio-Rad QC. Add STA QC info.
6.1, 6.2	Update to Bio-Rad QC
6.2	Add instruction for loading onboard
6.7	Add TEa criteria and QC submitted to Bio-Rad on monthly
8.1	Specify temperature checks every shift

These revised SOPs will be implemented based on old QC inventory:

SG = 5/5/16

WAH = 5/9/16

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

Approved draft for training (version 5)

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.		

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

Form revised 10/31/02

TABLE OF CONTENTS

1. TEST INFORMATION

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

Synonyms/Abbreviations
FIB

Department
Coagulation

Form revised 10/31/02

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	If hematocrit >55%, refer to appendices A and B for collection instructions.
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	PLT Poor Plasma (sodium citrate)
-Other Acceptable	None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum	2.7 mL (9:1 blood to anticoagulant) in a 2.7 ml tube
- Minimum	2.4 mL (9:1 blood to anticoagulant) in a 2.7 ml tube
- Optimum	1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
- Minimum	1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.

Criteria		
Stability & Storage Requirements	Room Temperature:	4 hours ~~(opened, vaccum broken)~~ ~~72 hours (unopened, vaccum intact)~~
	Refrigerated:	4 hours (PLT Poor Plasma)
	Frozen plasma: -20C or colder	2 weeks (PLT Poor Plasma)
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, under-filled or over-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 Safety Data Sheet (SDS) 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	Millipore or NERL Thermo Scientific (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 Safety Data Sheet (SDS) 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 reagent grade 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	2-8°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	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

5.1 Calibration Procedure

The pre-calibrated Fibrinogen values are identical for all the vials of each lot.
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 Stago Fibrinogen controls are run and tested.

The calibration curve is considered verified for the new reagent lot when both the STA®-Coag Control N + ABN (*purchased as needed*) and the Biorad QC are within acceptable range. Once the new reagent lot is verified, Biorad QC will be used to monitor assay/instrument performance. The acceptable STA®-Coag Control N + ABN range is supplied by Stago. Biorad QC ranges must fall within the acceptable

range which is established utilizing the peer group data in combination with our current/historic analytic performance.

5.2 Controls Used for Calibration (New Lot Conversion) ONLY

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

5.3 Control Preparations and Storage

Control	**STA®-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.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
Lyphochek® Coagulation Control Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 744, 745 & 746

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	**Lyphochek Coagulation Control Levels 1, 2 & 3**
Preparation	Reconstitute each vial with exactly 1 mL of Reagent Grade water. Replace the stopper and allow this product to stand for 10 – 15 minutes swirling occasionally. Before sampling, gently swirl the vial several times to ensure homogeneity.
Storage	2 - 8° C

Stability	This product will be stable until expiration date when stored unopened at 2 - 8° C. After reconstitution, this product will be stable for 48 hours when stored onboard Stagos.

QC Loading Instructions	
1	When QC is thawed and it is ready for use, obtain 3 micro adapters and glass micro-cups. Label each glass micro-cup with the QC lot number, open date and time, and expiration date and time. Pour each QC vial into the matching glass micro-cup.
2	From Home Page select **Loading** – **Products** – Press **F8 (Micro volume)**
3	Use the following IDs: Level 1: **CC1** Level 2: **CC2** Level 3: **CC3**
4	Use the following Names: Level 1: **COAG-1** Level 2: **COAG-2** Level 3: **COAG-3**
5	For volume type: **1.00** mL
6	For stability type: **48** hours
7	Use the original QC vial for lot number

1. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.
2. 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.
3. To print all the QC data points for the Fibrinogen test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** ↵ Cursor to the FIB test and press **Enter** ↵to view the Levy-Jennings chart. Press **F1** to view the results in tabular form. Press **F6, select Execute then** press **Enter** ↵to print the individual values under current controls. Press ESC key to exit (back to graph). Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.

6.3 Frequency

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

Controls are run after any maintenance is performed on the analyzer.

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 Unity Real Time and can be viewed in that program. Any out-of-range QC results will be flagged.
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

Each result is reviewed for error messages. Refer to the STA® Compact system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it 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; utilize published TEA for acceptability criteria.
- 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.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- 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	Verify instrument temperatures every shift and record on the maintenance sheet. If the Needle number 3, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patient samples 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 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.

8.2	Analytical Procedure
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. The instrument reports results as a whole number.

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

IF the result is …	THEN…
< 100	Repeat, report with comment "REP"
> 800	Repeat, report with comment "REP"
For any of the above situations, be sure the specimen is not under-filled or over-filled, and then check the Hematocrit (HCT) result. If the HCT is greater than 55%, refer to appendices A and B for special tube preparation.	

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 – 400 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 *y* 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. No. 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. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Hemolysis, Icteria and Lipemia Interference (Lab policy)
5. Repeat Testing Requirements (Lab policy)
6. Critical Values (Lab policy)
7. STA Compact Operating Instructions, Coagulation procedure
8. Verification of Platelet Poor Plasma, Coagulation procedure
9. Current package insert for STA® Fibrinogen

17. REFERENCES

1. Diagnostic Stago Fibrinogen package insert: Revised 03/2015.
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 11/2013.
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 05/2014.
5. Clauss A,"Rapid Physiological Coagulation Method for the Determination of Fibrinogen [German], "*Acta Haematol*, 1957,17:237-46.
6. Quest Diagnostics Nichols Institute in Chantilly, VA. SOP ID QDHE716 Version 3.1, Coagulation Specimen Collection and Handling in 3.2% Sodium Citrate Blue Topped Tubes.
7. Package Insert, Lyphochek Coagulation Control, Bio-Rad Laboratories, revised 01/2016.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G002.005		
000	10/31/11	10.5	Revise MMin to > 1800 mg/dl and MMax to <60 mg/dl; add special notes	C Reidenauer	C Reidenauer
000	10/31/11	15	Update to standard wording	L Barrett	C Reidenauer
000	10/31/11	17	Add reference 5	C Reidenauer	C Reidenauer
001	10/19/12	3.2	Delete frozen storage	C Reidenauer	R SanLuis
001	10/19/12	4.1	Remove Millipore water	L Barrett	R SanLuis
002	6/3/14	3.1	Add reference to Appendices	A Chini	R SanLuis
002	6/3/14	3.2	Update tube volumes, add opened container storage	A Chini	R SanLuis
002	6/3/14	4.2	Change storage temp and prep for buffer	A Chini	R SanLuis
002	6/3/14	6.2	Add step to print QC	A Chini	R SanLuis
002	6/3/14	10.5	Add instruction for Hct >55	A Chini	R SanLuis
002	6/3/14	11.1	Change upper limit form 500 to 400	A Chini	R SanLuis
002	6/3/14	16	Update titles	L Barrett	R SanLuis
002	6/3/14	17	Add references 6	A Chini	R SanLuis
002	6/3/14	19	Add Appendix A and B	A Chini	R SanLuis
002	6/3/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
3	4/2/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
4	4/22/16	3.2	Change whole blood to Plt poor plasma, update stability, add over-filled tubes as unacceptable	A Chini	R SanLuis

4	4/22/16	5	Add explanation for STA QC and Bio-Rad QC. Add STA QC info.	A Chini	R SanLuis
4	4/22/16	6.1, 6.2	Update to Bio-Rad QC	A Chini	R SanLuis
4	4/22/16	6.2	Add instruction for loading onboard	A Chini	R SanLuis
4	4/22/16	6.5	Update review patient data criteria	A Chini	R SanLuis
4	4/22/16	6.7	Add TEa criteria and QC submitted to Bio-Rad on monthly	A Chini	R SanLuis
4	4/22/16	8.1	Specify temperature checks every shift	A Chini	R SanLuis
4	4/22/16	17	Add Bio-Rad QC	A Chini	R SanLuis

19. ADDENDA

A. Instructions for Preparing Collection Tube for Hematocrit >55%
B. Phlebotomist Instructions for Blood Collection

Approved draft for training (version 4)

Technical SOP

Title	**Thrombin Time**	
Prepared by	Ashkan Chini	Date: 4/8/2011
Owner	Robert SanLuis	Date: 6/3/2014

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

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

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Thrombin Time	Clot based assay / STA® Compact	TT

Synonyms/Abbreviations
TT

Department
Coagulation

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

In the presence of a predetermined quantity of thrombin, normal plasma will consistently clot in a finite time unless there is abnormal thrombin formation. The time of clot formation is measured on the STA® Compact. The STA® Compact is a fully automated coagulation instrument, which uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette with the reagents and plasma is monitored by the STA® Compact. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time.

The thrombin time is a rapid and simple test designed for the assessment of fibrin formation. The thrombin time remains normal in deficiencies of Factor XIII (fibrin stabilizing factor). Thrombin time should first be performed before any other specific assays are attempted, when a prolongation of the overall tests (PT, APTT) cannot be explained.

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	If hematocrit >55%, refer to appendices A and B for collection instructions.
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	PLT Poor Plasma (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum **- Minimum** **- Optimum** **- Minimum**	2.7 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 2.4 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.

Criteria	
Stability & Storage Requirements	Room Temperature: 4 hours 2 hours (if on heparin therapy) Refrigerated: 4 hours (PLT Poor Plasma) Frozen plasma: 2 weeks (PLT Poor Plasma) -20C or colder
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, under-filled or over-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 Safety Data Sheet (SDS) for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Thrombin	Diagnostic Stago (REF 00669)
Pure Reagent Grade water	Millipore or NERL Thermo Scientific (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 Safety Data Sheet (SDS) 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	**Thrombin**
Container	Manufacturer supplied vial
Storage	2-8°C
Stability	Stable until expiration date indicated on the box label. Once reconstituted, with the perforated cap in place, the reagent is stable for 7 days on the STA® Compact analyzer.
Preparation	Reconstitute the vial of Reagent with exactly 10 mL of Reagent Grade water. Allow the reconstituted material to stand at room temperature (18-25°C) for 30 minutes. Then, swirl the vial gently before use.

Reagent 2	**NERL Reagent Grade water**
Container	Manufacturer supplied vial
Storage	Room temperature
Stability	Stable 30 days after opening.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

5.1 Calibration Procedure

The pre-calibrated Thrombin Time values are identical for all the vials of each lot. 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 Stago Thrombin Time controls are run and tested.

The calibration curve is considered verified for the new reagent lot when both the STA®-Coag Control N + ABN (*purchased as needed*) and the Biorad QC are within acceptable range. Once the new reagent lot is verified, Biorad QC will be used to monitor assay/instrument performance. The acceptable STA®-Coag Control N + ABN range is supplied by Stago. Biorad QC ranges must fall within the acceptable range which is established utilizing the peer group data in combination with our current/historic analytic performance.

5.2 Controls Used for Calibration (New Lot Conversion) ONLY

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

5.3 Control Preparations and Storage

Control	**STA®-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.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
Lyphochek® Coagulation Control Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 744, 745 & 746

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	**Lyphochek Coagulation Control Levels 1, 2 & 3**
Preparation	Reconstitute each vial with exactly 1 mL of Reagent Grade water. Replace the stopper and allow this product to stand for 10 – 15 minutes swirling occasionally. Before sampling, gently swirl the vial several times to ensure homogeneity.
Storage	2 - 8° C
Stability	This product will be stable until expiration date when stored unopened at 2 - 8° C. After reconstitution, this product will be stable for 48 hours when stored onboard Stagos.

QC Loading Instructions	
1	When QC is thawed and it is ready for use, obtain 3 micro adapters and glass micro-cups. Label each glass micro-cup with the QC lot number, open date and time, and expiration date and time. Pour each QC vial into the matching glass micro-cup.

QC Loading Instructions	
2	From Home Page select **Loading – Products** – Press **F8 (Micro volume)**
3	Use the following IDs: Level 1: **CC1** Level 2: **CC2** Level 3: **CC3**
4	Use the following Names: Level 1: **COAG-1** Level 2: **COAG-2** Level 3: **COAG-3**
5	For volume type: **1.00** mL
6	For stability type: **48** hours
7	Use the original QC vial for lot number

1. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.
2. 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.
3. To print all the QC data points for the TT test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** ↵ Cursor to the TT test and press **Enter** ↵ to view the Levy-Jennings chart. Press **F1** to view the results in tabular form. Press **F6, select Execute then** press **Enter** ↵to print the individual values under current controls. Press ESC key to exit (back to graph). Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.

6.3 Frequency

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

Controls are run after any maintenance is performed on the analyzer.

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 Unity Real Time and can be viewed in that program. Any out-of-range QC results will be flagged.
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 QC/QA SOP "QC Responsibilities" 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

Each result is reviewed for error messages. Refer to the STA® Compact system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it 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; utilize published TEA for acceptability criteria.
- 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.

Quest Diagnostics
Site: Washington Adventist Hospital
Title: **Thrombin Time**

Form revised 10/31/02

- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- 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

- Glass micro containers
- Plastic transfer pipettes
- Plastic micro cups
- STA Reducer

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	Verify instrument temperatures every shift and record on the maintenance sheet. If the Needle number 3, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patient samples 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 TT test. Select TT 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 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

No calculations are required for the Thrombin Time.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

Results are reported out in seconds as a whole number.

10.3 Units of Measure

Seconds

10.4 Clinically Reportable Range (CRR)

13 - 120 seconds

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. If result is still < Mmin, report as: < 13 seconds, REP
> Mmax	Repeat, check for clots. If result is still > Mmax report as: >120 seconds, REP
For any of the above situations, be sure the specimen is not under-filled or over-filled, then check the Hematocrit (HCT) result. If the HCT is greater than 55%, refer to appendices A and B for special tube preparation.	

11. EXPECTED VALUES

11.1 Reference Ranges

15 - 20 seconds

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Heparinized samples will yield prolonged thrombin times. Thrombin times are included in lupus anticoagulant profiles to rule out or confirm presence of heparin in the patient sample, which will affect APTT-based test results.

Prolongation of the thrombin time indicates:

- An abnormality of fibrinogen, which may be qualitative (dysfibrinogenemia) or quantitative (severe hypofibrinogenemia; congenital afibrinogenemia; or acquired hypofibrinogenemia, which includes DIC, fibrinolysis, and liver diseases).
- The presence of antithrombins, which may be therapeutic (heparin, hirudin, argatroban) or abnormal (FDP – which appears during myelomas, rheumatoid arthritis, etc.).

13. PROCEDURE NOTES

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

After reconstitution, make sure there are no bubbles in the bottle. If there are any bubbles, mix the reagent with a wooden stick to disperse. The Thrombin Time should be performed first before any other specific assays are attempted, when a prolongation of the PT and APTT cannot be explained.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

13 – 120 seconds

14.2 Precision

Different plasmas were used for the intra assay and inter assay reproducibility studies on the STA® Compact.

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
n	21	21	10	10
mean (seconds)	19.1	32.2	17.9	33.4
SD (seconds)	0.53	0.55	0.29	1.09
CV (%)	2.8	1.7	1.6	3.3

14.3 Interfering Substances

The presence of antithrombins will affect the results of the Thrombin Time. These include therapeutic heparin and hirudin. Abnormally high FDPs may also affect the results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

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. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Hemolysis, Icteria and Lipemia Interference (Lab policy)
5. Repeat Testing Requirements (Lab policy)
6. STA Compact Operating Instructions, Coagulation procedure
7. Verification of Platelet Poor Plasma, Coagulation procedure
8. Current package insert for STA® Thrombin.

17. REFERENCES

1. Diagnostic Stago Thrombin package insert: Revised 09/2014.
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 11/2013.
3. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.
4. Reagents for STA® Compact Line, Reconstitution and Handling Information, revised 02/20/2009.
5. Package Insert, Lyphochek Coagulation Control, Bio-Rad Laboratories, revised 01/2016.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G006.004		
000	6/12/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	6/12/12	6.3	Add QC performed after maintenance	J. Buss	J. Buss, RSL
000	6/12/12	15	Update to standard wording	L. Barrett	J. Buss, RSL
001	6/3/14		Update owner	L Barrett	R SanLuis
001	6/3/14	3.1	Add reference to Appendices	A. Chini	R SanLuis
001	6/3/14	3.2	Update tube volumes, remove frozen temp. stability	A. Chini	R SanLuis
001	6/3/14	4.2	Remove reconstitution for a 2 mL reagent vial	A. Chini	R SanLuis
001	6/3/14	6.2	Add directions to print QC results	A. Chini	R SanLuis
001	6/3/14	10.5	Add instruction for Hct >55 and reference to appendices A and B	A. Chini	R SanLuis
001	6/3/14	16	Update titles	L Barrett	R SanLuis
001	6/3/14	17	Add reference 4	A Chini	R SanLuis
001	6/3/14	19	Add Appendix A and B	A Chini	R SanLuis
001	6/3/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
2	4/2/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
3	4/22/16	2	Correct typo for word 'clot'	A Chini	R SanLuis
3	4/22/16	3.2	Change whole blood to Plt poor plasma, update stability, add over-filled tubes as unacceptable	A Chini	R SanLuis
3	4/22/16	5	Add explanation for STA QC and Bio-Rad QC. Add STA QC info.	A Chini	R SanLuis
3	4/22/16	6.1, 6.2	Update to Bio-Rad QC	A Chini	R SanLuis
3	4/22/16	6.2	Add instruction for loading onboard	A Chini	R SanLuis
3	4/22/16	6.5	Update review patient data criteria	A Chini	R SanLuis
3	4/22/16	6.7	Add TEa criteria and QC submitted to Bio-Rad on monthly	A Chini	R SanLuis
3	4/22/16	8.1	Specify temperature checks every shift	A Chini	R SanLuis
3	4/22/16	17	Add Bio-Rad QC	A Chini	R SanLuis

19. ADDENDA

A. Instructions for Preparing Collection Tube for Hematocrit >55%
B. Phlebotomist Instructions for Blood Collection