TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/29/2014
Department:	Core - Coag	**Due Date:**	8/19/2014
		Implementation:	**8/19/2014**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Coagulation SOPs

Prothrombin Time (PT) and INR	SGAH.G03.2	WAH.G03.2	GEC.G03.2
D Dimer	SGAH.G04.2	WAH.G04.2	GEC.G04.2
Fibrinogen	SGAH.G05.3	WAH.G05.3	
Thrombin Time		WAH.G06.2	

Description of change(s):

All of the above have the following changes

Section	Description
3.1	Add reference to Appendices
3.2	Update tube volumes, add opened container storage (PT & Fibrinogen)
6.2	Add step to print QC
10.5	Add instruction for Hct >55 and reference to appendices A and B
19	Add Appendix A and B (these are identical to what was added to PTT sop)

Other changes to these individual SOPs -

Ddimer

4.2	Change storage temp and prep for buffer
10.4	Change CRR lower value
13, 14.1	Change lower value of analytical range
14.3	Update to match package insert

PT

11.3	Remove calling PAT and SDS

Fibrinogen

4.2	Change storage temp and prep for buffer
11.1	Change upper limit from 500 to 400

Thrombin time

4.2	Remove reconstitution for a 2 mL reagent vial

These revised SOPs will be implemented on August 19, 2014

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

Technical SOP

Title	**D-Dimer**	
Prepared by	Ashkan Chini	Date: 4/7/2011
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**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
D - Dimer Quantitative	Immunoturbidometric / STA® Compact	DDIMER

Synonyms/Abbreviations
D - Dimer

Department
Coagulation

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

This assay is based on the change in turbidity of a microparticle suspension that is measured by photometry. A suspension of latex microparticles, coated by covalent bonding with monoclonal antibodies specific for D-dimer, is mixed with the test plasma whose D-dimer level is to be assayed. An antigen-antibody reaction takes place, leading to an agglutination of the latex microparticles which induces an increase in turbidity of the reaction medium. This increase in turbidity is reflected by an increase in absorbance, the latter being measured photometrically. The increase in absorbance is a function of the D-dimer level present in the test sample.

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	Whole Blood (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.
Stability & Storage Requirements	Room Temperature: 8 hours (20 ± 5° C)
	Refrigerated: Not recommended
	Frozen plasma: 1 month at -20 C.

Form revised 10/31/02

Criteria	
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 - LIATEST® D – DI: Buffer & Latex	Diagnostic Stago (REF 00515)
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 & 2	**STA - LIATEST® D – DI : Buffer and Latex**
Container	Manufacturer supplied vial
Storage	2-8°C

Stability	Unopened reagents are stable until expiration date indicated on the box label. With the STA-mini Reducer and perforated cap in place the stability of Reagents 1 and 2 after opening and in their original vials is 15 days on the Stago.
Preparation	Allow the reagents 1 and 2 to stand at room temperature (18-25°C) for 15 minutes before use. Mix the reagents by gentle swirling of the vials without creating any bubbles. Then place the perforated cap on each vial.

Reagent 3	**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 4	**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. The kit reagents are pre-calibrated: this 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 Liatest D-Dimer reagent, the STA® Compact will request the operator to scan the bar code printed on the bar code insert across the STA® Compact bar codes reader.
   - The calibration curve will be validated for the lot being used once the two Liatest D-Dimer controls 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 screen:
   - Through the MAIN MENU under CALIB/CONTROL select CALIBRATION.
   - Move the cursor to D-Dimer and press **Enter** ↵. Curve will appear on STA® Compact screen.

4. Print calibration curve:
   - While examining the curve on the STA® Compact screen, press ESC key for options.
   - Select print Option **Enter** ↵ Select Execute **Enter** ↵
   - The curve will print along with the information on all reagents and control lot numbers. Also included are control results and ranges.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
STA® Coag control N + P	Diagnostic Stago (REF 00526)

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 Controls N + P**
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.

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.
4. To print all the QC data points for the D-Dimer test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** ↵ Cursor to the D-Dimer 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

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.

Both 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 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 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

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 Lead Technologist 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 – brass adaptors
- Plastic micro cups
- STA Mini-Reducer
- Plastic transfer pipettes

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 D-Dimer test. Select D-Dimer 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).

8.2	Analytical Procedure
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	If the patients' results fall outside the assay reportable range **4.0 µg/mL** the instrument will automatically do a 1:5 dilution on the samples in question. This auto dilution will let the instrument report results up to **20.0 µg/mL**.
9	All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the system status menu.
10	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

1. The STA® Compact automatically plots the results in delta OD off of a standard curve and converts the results to µg/ml FEU.

2. The assay uses the sample undiluted. If the result is greater than the reportable range, 4.0, a dependent test with a 1:5 dilution will be ordered to take the reportable range to 20.0. The STA® Compact automatically corrects the result for the dilution change.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

No rounding is necessary. The instrument reports results up to two decimal points.

10.3 Units of Measure

µg/ml FEU

10.4 Clinically Reportable Range (CRR)

0.27 – 20.0 µg/ml FEU

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 <0.22 *u*g/ml FEU, REP
> Mmax	Check for clots, repeat using the D-Di 1:5 test
If D-Di 1:5 is quantifiable	Report the result with comment REP
If D-Di 1:5 is > Mmax	Repeat. Report the result as > 20.0 *u*g/ml FEU, 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.	

Note: All patient results are reported with the following comment:
Less than ≤0.50 ug/mL FEU = Negative
Greater than >0.50 ug/mL FEU = Positive
Positive results are non-specific and are seen in a variety of conditions including DVT, pulmonary embolism, recent surgery, cancer, trauma and pregnancy. Values greater than 0.50 ug/mL FEU may also be seen in otherwise healthy patients >70 years of age.

11. EXPECTED VALUES

11.1 Reference Ranges

≤ 0.5 µg/ml FEU

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

D-Dimer quantitative assay detects the presence of Disseminated Intravascular Coagulation (DIC). In DIC the fibrinolytic system is activated and therefore the D-Dimer level increases. D-Dimer assays can help in the diagnosis of DIC in these cases. It is established that a normal D-Dimer level is an important factor to rule out the diagnosis of deep venous thromboses (DVT) or pulmonary embolisms (PE). The decrease of D-Dimer levels during heparin therapy for a DVT allows the monitoring of evolution and prognosis of the thrombosis. This decrease reflects the quality of the endogenic thrombolysis. The D-Dimer level increases during the activation states of coagulation because they induce the production of thrombin which is followed by the formation of fibrin and leads to fibrinolysis, the latter being most frequently reactive. The D-Dimer level thus increases following coagulation activation.

Increased levels of D-Dimer have been reported in post-operative period, cancers, cirrhosis, and hemorrhages.

13. PROCEDURE NOTES

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

1. The detection threshold of the STA® Liatest® D-Dimer on the STA® Compact is 0.27 µg/ml FEU. The printout limits are pre-set at 0.27 – 4.00 µg/ml FEU. When a dependent test is set-up to extend the reportable range of the main test, the print out limit should be extended to 20.00 µg/ml FEU.
2. The STA® Liatest® D-Dimer results are expressed in FEU, Fibrinogen Equivalent Units. By definition, an FEU is the quantity of fibrinogen initially present that leads to the observed level of D-Dimer. In general, the actual quantity of D-Dimer is approximately half of an FEU.
3. A >Mmax result on the primary assay dilution (1:1 dilution) indicates a result that is greater than 4.00 µg/ml FEU. In this case the analyzer will automatically do a 1:5 dilution to obtain the result.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.27 – 4.00 µg/ml FEU

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)	0.29	2.71	0.32	2.78
SD (seconds)	0.04	0.08	0.05	0.14

14.3 Interfering Substances

1. Cloudy plasmas may lead to an under-estimation of the D-Dimer level. Ensure that the absorbance value at 540 nm of the plasma diluted 1:6 with STA® - Owren-Koller buffer is < 0.35.
2. Concentration of Fibrinogen Degradation Products greater than 15 µg/ml may lead to an over-estimation of the D-Dimer level.
3. The presence of rheumatoid factor at a level greater than 50 IU/ml may lead to an over-estimation of the D-Dimer level.
4. The STA® Liatest® D-Dimer is insensitive to fibrinogen and the E fragment. A low cross-reactivity is observed with the D fragment.
5. The STA® Liatest® D-Dimer is insensitive to the following substances: hemoglobin (up to 2 g/l); conjugated bilirubin (up to 290 mg/L); unconjugated bilirubin (up to 200 mg/L); unfractionated heparin (up to 2 IU/mL; LMWH (up to 2 anti-Xa IU/ml)

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. Material Safety Data Sheets (MSDS)
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® LIATEST D-DIMER

17. REFERENCES

1. van der Graaf F, et. al., Exclusion of Deep Venous Thrombosis with D-Dimer Testing, Thromb Haemost. 2000;83:191-198
2. Diagnostic Stago STA® LIATEST D-DIMER package insert: Revised August 2012.
3. STA®-Coag Control N + P (REF 00526): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA®, Revised July 2011.
4. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.
5. Diagnostic Stago STA® Owren-Koller Buffer Solution for Coagulation Tests. Revised: April 2012.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G003.006		
000	6/11/12	2.0	Update to match package insert	J.Buss	J. Buss, RSL
000	6/11/12	3.2	Add frozen temperature	J.Buss	J. Buss, RSL
000	6/11/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	6/11/12	4.2	D-DI reagent open stability edited	J.Buss	J. Buss, RSL
000	6/11/12	6.3	Add QC performed after maintenance	J.Buss	J. Buss, RSL
000	6/11/12	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	A Chini	R SanLuis
001	6/3/14	4.2	Change storage temp and prep for buffer	A Chini	R SanLuis
001	6/3/14	6.2	Add step to print QC	A Chini	R SanLuis

001	6/3/14	10.4	Change CRR lower value	A Chini	R SanLuis
001	6/3/14	10.5	Add instruction for Hct >55	A Chini	R SanLuis
001	6/3/14	13, 14.1	Change lower value of analytical range	A Chini	R SanLuis
001	6/3/14	14.3	Update to match package insert	A Chini	R SanLuis
001	6/3/14	16	Update titles	L Barrett	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

19. ADDENDA

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

Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.

13. Release the tourniquet. Ask the patient to open hand.
14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code.
22. Transport specimen to the Lab.

Technical SOP

Title	**Prothrombin Time (PT) and INR**	
Prepared by	Ashkan Chini	Date: 3/10/2011
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**

Quest Diagnostics Nichols Institute
Site: Shady Grove Adventist Hospital
Title: **Prothrombin Time (PT) and INR**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Prothrombin Time / INR	Clot based assay / STA® Compact	PTA

Synonyms/Abbreviations
Prothrombin, PT/INR

Department
Coagulation

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

STA®-Neoplastine CI PLUS is used for Prothrombin times, and extrinsic Factor Assays on the STA ® Compact. A mixture of thromboplastin is added to citrated plasma and the time of clot formation is determined. 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 thromboplastin 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 in seconds. The prothrombin time is a basic coagulation-screening test for the assessment of congenital and acquired deficiencies of the extrinsic pathway (factors II, V, VII, X). The prothrombin time can be prolonged in certain clinical states, i.e. warfarin therapy, intestinal reabsorption disorders, liver failure, fibrinolysis and DIC.

The prothrombin time is also used to monitor warfarin therapy because of its sensitivity to variations in the concentration of the Vitamin-K dependent factors II, VII and X. Because of the variations in the prothrombin time results with different thromboplastins and instruments, it is recommended that the prothrombin time results be converted to an INR. The INR corresponds to the value of the ratio of the patient's PT and the geometric mean PT of the normal reference population raised to the ISI (International Sensitivity Index) power:

$$\text{INR} = \left(\frac{\textit{Patient's PT}}{\textit{Geometric Mean PT}} \right)^{\text{ISI}}$$

The ISI value of a given thromboplastin is determined by performing PT's on normal plasmas and coumadin-treated patient plasmas with the given thromboplastin and the WHO (World Health Organization) reference thromboplastin. The slope of this regression curve of the matched pairs is the ISI for the thromboplastin. The ISI of the WHO reference thromboplastin is 1.0.

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**	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** **- 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.
Stability & Storage Requirements	Room Temperature: 4 hours (opened, vacuum broken) (20 ± 5° C) 48 hours (unopened, vacuum intact) Refrigerated: Not recommended Frozen plasma: 1 month
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
NEOPLASTINE® CI Plus	Diagnostic Stago (REF 00667)
Pure Reagent Grade water	Millipore or 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.

Kit Component contains reagent 1 & 2	
Reagent 1	**STA® Neoplastine CI Plus**
Storage	2-8° C
Stability	Stable until expiration date on the vial. Once reconstituted: • It remains stable in its original capped vial without the stirring-bar, STA®-Reducer, for 8 days at 2-8° C. • It remains stable with the stirring-bar, STA®-Reducer and perforated plastic cap in place, for 48 hours on STA® Compact.
Preparation	Transfer the entire contents of one vial of Reagent 2 into one vial of Reagent 1 of the same lot. Allow the reconstituted reagent to stand at room temperature (18-25° C) for 30 minutes. Swirl gently. Add a stirring bar to the vial and place the STA® Reducer and install the perforated plastic caps. Request the product drawer to open through MAIN MENU under LOADING and bar code the reagent. Place the reagent into a stirring position in the product drawer on the STA® Compact.

Reagent 2	**10 ml Solvent**
Container	Manufacturer supplied vial.
Storage	2-8°C
Stability	Stable until expiration date indicated on the box label.
Preparation	Ready to 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

No calibration of the system is necessary for performing a PT.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
STA® Coag control	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 Controls N and 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 an 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.
4. To print all the QC data points for the PT test, perform the following procedure prior to midnight. From the MAIN MENU under CAL/CONTROL select QUALITY CONTROL. Cursor to the PT test and press **Enter** ↵to view the Levy Jennings chart. Press **F1** to view the results in tabular form. Press **F6** and 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

Both 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.

Both 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 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 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

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 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

- Magnetic Stirbars
- Cuvette Roll – Diagnostic Stago
- STA – brass adaptors
- STA – Reducer
- STA - Cleaner solution
- Plastic micro cups
- Plastic transfer pipettes

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 PT (or PT+) test. Select PT (or PT+) 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).

8.2	Analytical Procedure
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

The INR is calculated by the STA Compact and transmitted to the LIS computer.

$$\text{INR} = \left(\frac{Patient's\ PT}{Geometric\ \ Mean\ \ PT} \right)^{\text{ISI}}$$

The INR is automatically calculated by the STA® Compact. The ISI is furnished by the manufacturer in the package insert and is stored in the CALIBRATION page for PT (or PT+) along with the geometric mean (reference time).

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

Results are reported out in seconds to the nearest 0.1 sec.

10.3 Units of Measure

Seconds

10.4 Clinically Reportable Range (CRR)

10 - 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…
INR > 3.9 seconds	Check for clots, but there is no need to repeat. 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.
>Mmax	Repeat, check for clots. If result is still > Mmax, report with >120 and INR is reported as >16.2
< Mmin	Repeat, check for clots, most likely this sample clotted during collection. If no clots are detected and the repeat matches, Report as < 10. Report the INR < 0.7.

Note: All patient results are resulted with the following INR comment - The American College of Chest Physicians/National Heart/Lung Institute has recommended MODERATE INTENSITY ANTICOAGULATION REGIMEN INR 2.0-3.0 for most indications with the exception of patients with MECHANICAL PROSTHETIC HEART VALVES AND RECURRENT EMBOLISM for whom the recommended range is INR 2.0-3.5.

11. EXPECTED VALUES

11.1 Reference Ranges

PT 12.5 – 14.8 seconds

11.2 Critical Values

INR ≥ 4.0

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

The Prothrombin Time (PT) is a basic coagulation-screening test that is useful in the assessment of congenital and acquired extrinsic coagulation pathway deficiencies involving factors II, V, VII and X. The International Normalized Ratio (INR) is a means of standardizing therapeutic range interpretation of the PT. The use of the INR is limited to the assessment of PT in patients on oral anticoagulant therapy.

If a PT monitored factor deficiency is present, immediate and incubated mixing studies will correct to normal. If an inhibitor is present, the immediate result **may** correct, but the incubated result will be abnormal.

13. PROCEDURE NOTES

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

The STA uses electromechanical clot detection for the Protime. Lipemia and icterus do not interfere with PT result. These findings should be reported with the PT value. The STA® is programmed to detect the prothrombin times from 10 seconds to 120 seconds. Prothrombin Times that clot in less than 10 seconds will yield a <Vmin result and Prothrombin Times that do not clot in 120 seconds yield a >Vmax result.

New lot of Thromboplastin: With each new lot of thromboplastin, a patient geometric mean time must be established. The operator must enter that geometric mean time before the STA®/STA® Compact will allow QC to be run. Through the MAIN MENU select CALIB/CONTROL. Select **CALIBRATION**, press **Enter** ↵. Cursor to the PT (or PT+) test and press **Enter** ↵ to select. Press ESC key for options. Select MODIFY REFERENCE TIME/RANGE, press **Enter** ↵Type in the Geometric Mean Time and save with **F10**. This screen also stores the ISI value, as downloaded from the reagent bar code sheet.

INR Verification: The INR calculation will be manually verified with each new reagent lot number and with the semi-annual instrument-to-instrument comparison.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

10 – 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)	13.6	22.7	15.1	29.4
SD (seconds)	0.10	0.12	0.22	0.46
CV (%)	0.7	0.5	1.5	1.6

14.3 Interfering Substances

PT results will not be affected by levels of unfractionated heparins up to 1 IU/mL and by LMWH up to 1.5 anti-Xa IU/mL.

Many commonly administered drugs affect the results obtained in PT testing. (Example: coumadin and heparin).

STA® Neoplastine CI Plus, contain a specific inhibitor of heparin. Therefore, only levels of heparin outside of the therapeutic range will affect the PT results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

The stirring-bar used in the reagent vial should never be the source of contamination. To ensure that stirring-bars are contamination-free, rinse the bars with distilled water and dry them carefully to remove all traces of moisture before adding them to reagent vials.

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. Material Safety Data Sheets (MSDS)
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® Neoplastine CL Plus Prothrombin Time

17. REFERENCES

1. Diagnostic Stago Neoplastine CL Plus package insert: Revised June 2012.
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. 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.
5. Reagents for STA® Compact Line, Reconstitution and Handling Information, revised 02/20/2009.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G004.005		
000	06/08/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	06/08/12	6.3	Add QC performed after maintenance	J. Buss	J. Buss, RSL
000	06/08/12	15	Update to standard wording	L. Barrett	J. Buss, RSL
001	6/3/14		Update owner	L Barrett	R SanLuis
001	6/3/14	1	Update order code	A Chini	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, add opened container storage	A Chini	R SanLuis
001	6/3/14	11.3	Remove calling PAT and SDS	L Barrett	R SanLuis
001	6/3/14	10.5	Add reference to Appendices	A Chini	R SanLuis
001	6/3/14	16	Update titles	L Barrett	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

19. ADDENDA

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

Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.

13. Release the tourniquet. Ask the patient to open hand.
14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code. Transport specimen to the Lab.

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**

Quest Diagnostics Nichols Institute
Site: Shady Grove Adventist Hospital
Title: **Fibrinogen**

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 **-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** **- 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: (20 ± 5° C)	4 hours (opened, vaccum broken) 72 hours (unopened, vaccum intact)
	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	2-8°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

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.

4. 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 Fibrinogen 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

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.

Both controls are run after any maintenance 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 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).

8.2	Analytical Procedure
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

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
< 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, 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 (changed from 200 – 500)

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. 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. 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. 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 July 2012.
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 April 2012.
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.

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

19. ADDENDA

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

Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.

13. Release the tourniquet. Ask the patient to open hand.
14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code.
22. Transport specimen to the Lab.

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**

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

TABLE OF CONTENTS

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 lot 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**	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** **- 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: (20 ± 5° C)	8 hours 2 hours (if on heparin therapy)
	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
Thrombin	Diagnostic Stago (REF 00669)
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	**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

No calibration of the system is necessary for performing a Thrombin Time.

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 Controls 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 an 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.
4. 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

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.

Both controls are run after any maintenance 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 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.

Step	Action
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

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

- 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	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 TT test. Select TT by pressing **F1** and then **F10.** Type in your Access Code to run the QC.

8.2	Analytical Procedure
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. Material Safety Data Sheets (MSDS)
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 September 2013.
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. Reagents for STA® Compact Line, Reconstitution and Handling Information, revised 02/20/2009.

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

19. ADDENDA

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

Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Form revised 10/31/02

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL

Form revised 10/31/02

Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP ‘Patient Identification’, Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient’s consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient’s arm in a downward position to prevent reflux of ‘backflow’ of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.

13. Release the tourniquet. Ask the patient to open hand.
14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code.
22. Transport specimen to the Lab.