Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/3/2012
Department:	Coag	**Due Date:**	7/31/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Prothrombin Time (PT) and INR	**GEC.G03.001**	**SGAH.G03.001**	**WAH.G03.001**
D-Dimer	**GEC.G04.001**	**SGAH.G04.001**	**WAH.G04.001**
Thrombin Time			**WAH.G06.001**

Description of change(s):

Section	Reason
4.1	Remove Millipore water (*only NERL water is used to prepare controls*)
6.3	Add QC performed after maintenance

Other changes to D-Dimer SOP

Section	Reason
2.0	Update to match package insert
3.2	Add frozen temperature
4.2	D-DI reagent open stability edited

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

Approved draft for training all sites (version 001)

Technical SOP

Title	**Prothrombin Time (PT) and INR**	
Prepared by	Ashkan Chini	Date: 3/10/2011
Owner	Robert SanLuis, Jean Buss	Date: 3/10/2011

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

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

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1. TEST INFORMATION

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

Synonyms/Abbreviations
Prothrombin, PT/INR

Department
Coagulation

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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:

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

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	Hematocrit of >55% or < 20%: Recollect the specimen using the correct volume of anticoagulant determined by the following formula: 100 – HCT equals the volume of anticoagulant (60 x 0.5) to mix with blood for a total of 5.0 ml
Other	N/A

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3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Whole Blood (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum **- Minimum**	4.5 mL (9:1 blood to anticoagulant) in 5ml tube 2.7 mL whole blood (9:1 blood to anticoagulant) in 3.0 mL tube
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
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	NERL Diagnostics (Cat. No. 0015)

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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.

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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.

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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.

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- 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.

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8.1	Instrument Set-up Protocol
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).
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.

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

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.
>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

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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)

SGAH only: If patient location is SDS or PAT, then call INR >1.3

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.

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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.

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- 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. Verification of Platelet Poor Plasma, Coagulation procedure
8. Current package insert for STA® Neoplastine CL Plus Prothrombin Time

17. REFERENCES

1. Diagnostic Stago Neoplastine CL Plus package insert: Revised March 2009.
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.

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

19. ADDENDA

None

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Technical SOP

Title	**D-Dimer**
Prepared by	Ashkan Chini Date: 4/7/2011
Owner	Robert SanLuis, Jean Buss Date: 4/7/2011

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

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

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1. TEST INFORMATION

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

Synonyms/Abbreviations
D - Dimer

Department
Coagulation

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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	Hematocrit of >55% or < 20%: Recollect the specimen using the correct volume of anticoagulant determined by the following formula: $\frac{100 - HCT}{(60 \times 0.5)}$ equals the volume of anticoagulant to mix with blood for a total of 5.0 ml
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**	4.5 mL (9:1 blood to anticoagulant) in 5ml tube 2.7 mL whole blood (9:1 blood to anticoagulant) in 3.0 mL tube
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.

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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

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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	2-25°C
Stability	The buffer solution in intact bottles is stable until the expiration date indicated on the box label. After opening it remains stable for 3 days.
Preparation	If the buffer solution is refrigerated, allow it to stand at room temperature (18-25°C) for 30 minutes before use.

Reagent 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.

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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 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.

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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.

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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

- 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.

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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).
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.

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8.2	Analytical Procedure
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.

10.3 Units of Measure

µg/ml FEU

10.4 Clinically Reportable Range (CRR)

0.22 – 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

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> 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

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

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1. The detection threshold of the STA® Liatest® D-Dimer on the STA® Compact is 0.22 µg/ml FEU. The printout limits are pre-set at 0.22 – 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)

0.22 – 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. FDP concentrations 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 5 g/l); bilirubin (up to 200 mg/l); unfractionated heparin (up to 2 IU/ml0; LMWH (up to 2 anti-Xa IU/ml)

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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. Repeat Testing Requirements (Lab policy)
5. Critical Values (Lab policy)
6. Verification of Platelet Poor Plasma, Coagulation procedure
7. 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 November 2005.
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 December 2004.
4. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.

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5. Diagnostic Stago STA® Owren-Koller Buffer Solution for Coagulation Tests. Revised: November 2009.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G003.006		
000	06/11/12	2.0	Update to match package insert	J.Buss	J. Buss, RSL
000	06/11/12	3.2	Add frozen temperature	J.Buss	J. Buss, RSL
000	06/11/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	06/11/12	4.2	D-DI reagent open stability edited	J.Buss	J. Buss, RSL
000	06/11/12	6.3	Add QC performed after maintenance	J.Buss	J. Buss, RSL
000	06/11/12	15	Update to standard wording	L. Barrett	J. Buss, RSL

19. ADDENDA

None

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Technical SOP

Title	**Thrombin Time**	
Prepared by	Ashkan Chini	Date: 4/8/2011
Owner	Robert SanLuis, Jean Buss	Date: 4/8/2011

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

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

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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

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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	Hematocrit of >55% or < 20%: Recollect the specimen using the correct volume of anticoagulant determined by the following formula: $\frac{100 - HCT}{(60 \times 0.5)}$ equals the volume of anticoagulant to mix with blood for a total of 5.0 ml
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**	4.5 mL (9:1 blood to anticoagulant) in 5ml tube 2.7 mL whole blood (9:1 blood to anticoagulant) in 3.0 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.

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Criteria		
Stability & Storage Requirements	Room Temperature: (20 ± 5° C)	Plasma is stable for 8 hours. If on heparin therapy, plasmas remain stable for 2 hours.
	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
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.

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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 for the 10 mL size and 8 hrs for the 2 mL size on the STA® Compact analyzer.
Preparation	Reconstitute the vial of Reagent with exactly 10 mL of Reagent Grade water (2 mL for the 2 mL size vial). 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

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	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. **New lot of Thrombin Reagent**: With each new lot of Thrombin Reagent, the operator must enter the reference time before the STA® Compact will allow QC to be run. Through the MAIN MENU select CAL/CONTROL. Select CALIBRATION, press **Enter** ↵ Cursor to the TT test and press **Enter** ↵ Press ESC key for options. Select MODIFY REFERENCE TIME/RANGE, press **Enter** ↵ Type in your Geometric Mean Time and save with **F10**.

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)

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Step	Action
	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 for review and signature.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

STA® Compact – Analyzer

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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.
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.

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8.2	Analytical Procedure
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

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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

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

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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.

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- 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. Verification of Platelet Poor Plasma, Coagulation procedure
8. Current package insert for STA® Thrombin.

17. REFERENCES

1. Diagnostic Stago Thrombin package insert: Revised September 2004.
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.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G006.004		
000	06/12/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	06/12/12	6.3	Add QC performed after maintenance	J. Buss	J. Buss, RSL
000	06/12/12	15	Update to standard wording	L. Barrett	J. Buss, RSL

19. ADDENDA

None

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