Thrombin Time

Purpose

This procedure provides instructions for performing the Thrombin Time assay.

Scope

This procedure is intended for Clinical Laboratory Scientists (CLS) and Medical Laboratory Technicians (MLT) who are trained and competent in performing the Thrombin Time assay.

Principle

- The Thrombin Time is a rapid and simple test designed for the assessment of fibrin formation.
- In the presence of a predetermined quantity of thrombin, normal plasma will consistently clot in a finite time.
- Thrombin reagent contains 1.5 NIH unit/mL calcium thrombin (human).
- Reconstituted calcium thrombin is added to test plasma and time of clot formation is measured on the STA-R Evolution and Compact automated coagulation analyzers.
- Automated instrumentation uses an electromagnetic mechanical clot detection system.

Clinical Significance

- The Thrombin Time remains normal in deficiencies of factor XIII (fibrin stabilizing factor).
- Thrombin Time should first be performed before any another specific assays are attempted, when a prolongation of the overall tests (PT, APTT) cannot be explained.
- The Thrombin Time is prolonged when fibrinogen is decreased (e.g. acquired hypofibrinogenemia from disseminated intravascular coagulation or liver disease, congenital afibrinogenemia) or dysfunctional (hereditary or acquired), or when a thrombin inhibitor is present (e.g., heparin, hirudin, argatroban, dabigatran, etc.). Thrombin Time is not affected by warfarin therapy.

Specimen Collection and Transport

Follow CLSI H21-A3 guidelines and the laboratory's specimen collection, handling and transport policies pertaining to coagulation samples.

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Thrombin Time, Continued

Specimen Handling

Centrifuge specimen at 2500 g/3000 rpm for 10 minutes.

Specimen Storage

Store test plasma frozen at -20 ºC for up to two weeks or at -70 ºC for up to six months.

Specimen Rejection

Reject specimen under any of the following conditions:

- Clotted
- Grossly hemolyzed
- Specimen volume less than 85%
- Any condition that do not meet the stability and handling guidelines

Materials and Equipment

The following table lists the necessary materials and equipment needed for this assay.

Notes:

- Reagents, standards and controls are for in vitro diagnostic use only. Avoid contact and inhalation. Normal precautions exercised in handling laboratory reagent should be followed.
- Refer to Material Safety Data Sheet(s) for any updated risk, chemical hazard or safety information.

Equipment	• STA line analyzers • Water bath 37 ºC
Reagents	• Deionized Water • STA – Thrombin 2 (ref 00611 or STA – Thrombin 10 (ref 00669) • Normal Control • Abnormal Control. • Desorb U (ref 00975) – Stago • Cleaner Solution (ref 00973) - Stago
Supplies	• Pipettes • Pipette tips • STA Cuvettes (ref 38669) – Stago • STA Microtainer (ref 00741) – Stago • STA Microcups for reagent (ref 00802) – Stago • Teflon Syringe Tip and O-Ring Kit (ref 27530) - Stago

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Thrombin Time, Continued

Reagents

The following reagents are required to perform this procedure.

Reagent	Preparation	Storage and Stability
Deionized Water	Ready for use	May be stored at room temperature. Use within 30 days of opening.
STA Thrombin 2 (Diagnostica Stago, ref 00611) OR STA Thrombin 10 (Diagnostica Stago, ref 00669)	Reconstitute vial with 2 or 10 ml of reagent grade water depending on reagent used. Let stand at room temperature for 30 minutes. Mix by swirling vial without creating bubbles.	Once reconstituted, the reagent of both STA-Thrombin 2 or Sta-Thrombin 10 remains stable for 2 days at 20 +/- 5 ºC and 7 days at 2-8 ºC in its original vial (without placing maxi reducer or the perforated cap on vial).
Normal Control	Reconstitute and prepare based on manufacturer's guidelines	Store based on manufacturer's guidelines.
Abnormal Control	Reconstitute and prepare based on manufacturer's guidelines	Store based on manufacturer's guidelines.

Safety Precautions

- All human samples should be handled and disposed of as if they were potentially infectious.
- All laboratory employees are expected to maintain a safe working environment and an injury-free workplace.
- Laboratory employees are responsible for their own safety and the safety of others and adhering to all departmental safety policies and procedures.
- Refer to the safety manual for general safety requirements.

Quality Control

Corrective action(s) must be taken when tolerance limits are exceeded.

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Thrombin Time, Continued

Procedure

- Follow the steps below to perform this procedure.
- Refer to Procedure for Operating Stago Automated Coagulation Analyzers for more detail.

Step	Action
1	Check specimens for clot formation, hemolysis and proper labeling.
2	Open product/reagent drawer and load controls and reagent according to the following scheme: **Reconstituted Product** / **Load** STA Thrombin 2 or STA Thrombin 10 — Scan barcode and place into R2 area of reagent drawer. Normal Control — Scan barcode and place into R0 area of reagent drawer. Abnormal Control — Scan barcode and place into R0 area of reagent drawer.
3	Place patient samples onto rack and then load rack onto tray.
4	Load tray onto loading/unloading area of instrument. Analyzer will automatically run bar-coded patient samples after running control. ***Note:*** To manually initiate quality control run, refer to instrument operator's manual.

Reporting of Results

Follow these steps to review the results table.

Step	Action
1	Check the control results to verify if they are within the acceptable ranges in order to accept or reject a run.
2	Report results in whole numbers. ***Notes:*** • Any result that is < 13 sec will translate into < 13 sec in the LIS. • Any result that is > 150 sec will translate into >150 sec in the LIS.
3	Report result in the Laboratory Information System according to the LIS procedure '**Resulting in Cerner GenLab: Manual Entry**' (Laboratory Informatics Procedure posted in LabNet).

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Reference Ranges

Reference Range	14 – 19 sec
Clinical Reportable Range	13 – 150 sec
Limit of Detection	150 sec

Carryover

There is no carryover in the Thrombin Time Assay.

Interpretation / Results / Alert Values

There is no interpretation or alert value for the Thrombin Time assay.

Procedure Notes and Limitations

- No calibration curve or calculations are required for Thrombin Time.
- Results for the Thrombin Time are reported out in the nearest whole second.
- Prolongation of the Thrombin Time indicates abnormal fibrin formation.
- The abnormal formation can be found with dysfibrinogenemia, afibrinogenemia, and hypofibrinogenemia, either congenital or acquired, i.e. DIC, fibrinolysis, liver disease…).
- The presence of antithrombins such as therapeutic heparins, hirudin, argatroban and dabigatran will prolong Thrombin Time.
- Abnormally high FDPs will prolong Thrombin Time.

Non-Controlled Documents

The following non-controlled documents support this procedure.

- Determination of the Thrombin Time by STA Analyzers. Package insert. Diagnostica Stago. 2011
- Reference Manual-STA-R Evolution Automated Coagulation Analyzer. March 2011
- Normal and Abnormal Control Inserts
- Reference Manual-STA-R Compact Automated Coagulation Analyzer

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Thrombin Time, Continued

Controlled Documents

The following controlled documents support this procedure.

Title
Specimen Criteria and Preparation for Coagulation Studies
Procedure for Operating Stago Automated Coagulation Analyzers
Resulting in Cerner GenLab: Manual Entry (Laboratory Informatics Procedure, posted in LabNet)

Thrombin Time

Reviewed and approved by (for Medical Center Area Approval Only):

SIGNATURE	DATE
Name:_____ **Operations Director, Area Laboratory**	
Name:_____ **CLIA Laboratory Director**	

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HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Quality Systems Leader/Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
New					

Signature Manifest

Document Number: SCPMG-PPP-0089 **Revision:** 01

Title: Procedure for Thrombin Time

All dates and times are in Pacific Standard Time.

Thrombin Time

New Document or Change Request

Name/Signature	Title	Date	Meaning/Reason
Vincent Dizon (I713793) Mary Anne Umekubo (K076412)	ASST DIR	09 Mar 2015, 10:34:19 AM	Approved

Collaboration

Name/Signature	Title	Date	Meaning/Reason
Mary Anne Umekubo (K076412)	ASST DIR	09 Mar 2015, 11:29:19 AM	Complete & Quit
Vincent Dizon (I713793)	Director of Lab Services, Chem	09 Mar 2015, 04:12:07 PM	Complete

Initial Approval

Name/Signature	Title	Date	Meaning/Reason
Ji Yeon Kim (B727360)	Physician-In-Charge, Chem Svcs	19 Mar 2015, 10:26:30 AM	Approved

Final Approval

Name/Signature	Title	Date	Meaning/Reason
Darryl Palmer-Toy (T188420)	RRL MEDICAL DIRECTOR	26 Mar 2015, 03:54:42 PM	Approved

Set Effective Date

Name/Signature	Title	Date	Meaning/Reason
Vincent Dizon (I713793)	Director of Lab Services, Chem	26 Mar 2015, 04:03:58 PM	Approved

Notify Trainers

Name/Signature	Title	Date	Meaning/Reason
Vincent Dizon (I713793)	Director of Lab Services, Chem	26 Mar 2015, 04:03:58 PM	Email Sent