1. **Purpose**

The quantitative determination of Thrombin Time in human citrated Plasma

1. **Principle**
   1. STA**®** -Thrombin, [Cat # 00611 (2ml)], is the Thrombin Time reagent for STA**®** Analyzers. In the presence of a predetermined quantity of thrombin, plasma will consistently clot in a finite time unless there is abnormal thrombin formation. The time of Clot formation is measured on the STA-Compact Max®. The STA-Compact Max® 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 Max®. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time.
   2. Prolongation of the thrombin time indicates:
      1. An abnormality of fibrinogen
         1. Qualitative; dysfibrinogenemia
         2. Quantitative congenital: hypofibrinogenemia or afibrinogenemia
         3. Quantitative acquired: DIC, fibrinolysis, liver disease
      2. The presence of antithrombins
         1. Therapeutic: heparin, hirudin, argatroban
         2. Abnormal: FDP, myelomas
2. **SPECIMEN**
   1. Collection Tubes
      1. Becton Dickinson (B‑D) #366415 blue top tube containing 0.5 ml buffered 3.2% sodium citrate in a sterile, silicone‑coated tube.
         1. Approximate draw 4.5 ml blood ±10% to achieve a 9:1 blood to anticoagulant ratio.
      2. Becton Dickinson (B‑D) #36308 pediatric blue top tube containing 0.2 ml buffered 3.2% sodium citrate in a sterile, silicone coated tube.
         1. Approximate draw 2.7 ml ±10% to achieve a 9:1 blood to anticoagulant ratio.
   2. Establishing Proper Tube Fill
      1. A minimum and maximum draw tube demographic is kept at the coagulation station against which each specimen is to be compared. Any specimen not falling within acceptable range is to be rejected (see **HEM10-001**,Rejection of Hematology Specimens Procedure).
         1. "Short draw" tubes provide insufficient blood for the amount of anticoagulant and lead to prolonged results.
         2. Overfilled tubes are unacceptable because insufficient anti-coagulation may occur, especially in severely anemic patients.
   3. Specimen handling
      1. Stability
         1. Coagulation testing is optimally performed within two (2) hours, but no longer than twenty-four hours, following collection. Once a specimen is uncapped, stability is (4) hours following collection.
      2. Centrifugation
         1. Centrifuge for 7 minutes at 5,500 rpm OR 10 minutes at 3,500 rpm (within 30 minutes of collection).This speed or centrifuge will yield platelet poor plasma.
         2. Centrifuge STATs for 6 minutes at 4500 rpm using the EBA centrifuge. This speed or centrifuge will yield platelet poor plasma.
      3. Removal of Plasma
         1. Using a plastic transfer pipette, transfer the plasma into a plastic tube and cap. Transcribe the patient's name, medical record number or DOB, accession number, and date unto the plastic tube. Place tube in the freezer in Blood Bank. This is to be followed for samples not processed within 24 hours of collection. Samples may be frozen for 2 weeks; do rapid thaw and process within 1 hour.
      4. Checking for Hemolysis and visible clots
         1. Check plasma for visible clots and hemolysis; the presence of visibly pink plasma indicates RBC destruction, which may have occurred in vivo prior to or during or after filling the collection tube. (The presence of hemolysis strongly suggests the possibility of in vitro clots.) Append "HEM" to the result (hemolyzed).
      5. Sample Storage
         1. Room Temperature
            1. Centrifuged samples can be left up to 24 hours at room temperature.
      6. Freezing
         1. If the sample must be frozen and tested later, quick freezing of the plasma in small aliquots at ‑70oC is desirable to prevent formation of ice particles.
            1. **NOTE**: Frozen plasma should be rapidly thawed at 37oC before testing. (Factor VII and factor XI activities may increase with storage when frozen.)
3. **Equipment and Materials**
   1. Equipment
      1. MLA or Volumetric pipette
      2. Centrifuge
   2. Materials
      1. Tips for volumetric pipette
      2. Cuvettes
      3. Printer paper
      4. Waste container
      5. Specimen Cup
      6. Transfer Pipette
      7. Reagent Grade water
4. **Instrumentation**
   1. Identification

|  |  |  |  |
| --- | --- | --- | --- |
| **Facility** | **Name** | **Serial #** | **“Live” Date** |
| **EMCP** | Compact Max 1 | SN5071531 | 3-15-2016 |
| Compact Max 2 | SN5071537 | 3-15-2016 |
| **EMC-EP** | Compact Max 3 | SN5061483 | 3-15-2016 |
| Start 4 | B932 | 3-15-2016 |

* 1. Parts information and Physical Requirements
     1. Refer to instrument reference manual
  2. Routine Maintenance
     1. Refer to The Stago operators manual regarding all daily, weekly, and as needed maintenance.
        1. Record all maintenance on Stago Compact Maintenance form HEM40-012/HEM40-013/HEM40-014/HEM40-015/HEM40-016 Form B Stago Maintenance.
  3. Emptying of waste
     1. Liquid Waste
        1. The Stago does not contain Sodium Azide and can be discarded in a dirty sink. The instrument utilizes emptied rinse containers as its liquid waste chamber. When replacing the rinse bottle also remove the waste container and dump the liquid waste down the drain of a dirty sink. The rinse bottle being replaced is now used to collect future liquid waste.
     2. Cuvettes
        1. When the cuvette waste is full remove the bag from inside the analyzer and dispose of in the sharps container. Replace the yellow biohazard liner of the cuvette waste container.

### STANDARDS AND CONTROLS

* 1. A new range is established for each new lot of control material by repetitive analysis on both instruments for one month, during which the manufacturer’s range is used.
  2. STA**®** Coag N + ABN **(Cat. No 00676)**
     1. Reconstitution
        1. Reconstitute each vial of control with exactly 1mL of distilled water.
        2. Allow the material to stand at room temperature for 30 minutes.
        3. Swirl each vial gently before use.
     2. Storage and Stability
        1. Unreconstituted vials are stable until the expiration date listed on the box label when stored at 2-8 oC.
        2. Once reconstituted both levels of controls are stable for 8 hours and is to be kept on board the analyzer.
     3. Loading on the analyzer
        1. Click Products then Loading Products or click the  icon to request the product drawer.
        2. Scan the barcode on the reagent bottle and press Enter **⮠**
        3. Select  and click confirm to close the drawer.
     4. Running and Reviewing QC
        1. Qc can be ordered from the Quality Control Menu
        2. All controls are monitored automatically by the Compact Max.
           1. Any controls outside the ± 2SD range will result in audible and visual alarms.
        3. Results can be reviewed in the individual QC files.
        4. All controls must be resulted on form HEM40-012/HEM40-013/HEM40-014/HEM40-015/HEM40-016 Form A Daily Qc on Stago, and in the Lab Information System.
        5. Control results are automatically filed on the STA Compact Max QC file
        6. All results for a 24 hour period will be converted to a “mean” value on the first run after midnight.
           1. This mean is used in the statistical data and is plotted on the Levi- Jennings chart as a daily mean.
        7. Print all the QC data points prior to the first run after midnight.
           1. Prior to midnight when the analyzer is not running any test s click the  icon or select Quality Controls and the Windows Methodologies List appears.
           2. Double click the **TT2** test and click the QC Tables icon 
           3. Click the  icon.

Printout dialogue box will appear. Select print, then click confirm.

* + - * 1. Click the  icon to return to the QC Graph.
        2. Click **Next Level** and repeat process for other levels.

1. **Reagents**
   1. All new reagent package inserts are reviewed by the Supervisor, Lead Technologist, or designee and compared with the current lot numbers to ensure no changes have been made. The current package insert is signed and dated with the date the new reagents are put into use.
   2. Reagent 1: STA**®** -Thrombin: Contains titrated calcium thrombin (Human) approximately 1.5 NIH units/ml, freeze-dried. Reconstitute each vial with 10.0 ml (Cat. No. 00669) or 2.0 ml (Cat. No. 00611) of reagent grade water. Let stand 30 minutes at room temperature. Add a reducer (10 ml size only) and replace the perforated plastic cap on the vial. Swirl gently
      1. Stability after opening: 15 days on the STA Compact Max® Analyzer.
   3. Reagent 2: STA® DESORB U [Cat. No. 0975]: is a decontaminating solution (contains KOH < 1%) for use with the STA**®** line of instruments.
      1. Install a new STA**®** - maxi reducer (REF 00801) and the perforated cap on a freshly opened bottle before loading in the reagent drawer.
   4. STA**®** - Cleaner Solution [Cat. No. 0973]: is a washing aqueous solution used on the STA**®** line of instruments. Sufficient STA**®** - Cleaner Solution must be loaded to operate the analyzer.
   5. Load Reagents
      1. Click Products, then loading products or click the  icon to open the product drawer.
      2. Scan the barcode on the reagent bottle and press Enter **⮠**
         1. Place the reagent into a stirring position in the product drawer
      3. Select  and click confirm to close the drawer
   6. Parts information and Physical Requirements
      1. Refer to instrument reference manual
2. **Procedure**
   1. **Running Quality Control**
      1. Click theicon or click Quality Controls and the Methodologies list appears.
      2. Select the check box for TT2 and click the  icon.
      3. Type the access code and click Confirm.
      4. A yellow triangle  is displayed to the right of the methodology showing the selected Quality Control is running.
   2. **Running Patient Samples.**
      1. Click Patient Analyses
      2. Click loading samples or the  icon.
      3. After the drawer opens, identify the type of specimen by clicking the box
         1. micro-sample
         2. Stat (urgent) by clicking the box.
      4. Identify the sample by scanning the bar-code or manually entering the ID using the keyboard.
         1. Place the specimen into the drawer.
            1. In AUTO MODE, the STA CompactMax® will automatically order the test(s) selected in the AUTO MODE profile.
            2. In MANUAL MODE, the operator must order the test(s) from the Selection menu, or from the Recorded Profile(s). Double click each methodology or Profile, click **Confirm**.
         2. As soon as the sample drawer closes, the ANALYSIS STATUS screen will appear.
            1. If there is not enough reagent(s) to run the test(s), the deficient reagent(s) and the amount of reagent necessary to complete the testing will appear in red.
            2. This deficiency will BLOCK the SAMPLE PIPETTING and the icon will appear on the bottom of the screen. When you receive this error you need add the necessary reagent(s).

Respond to the error “New Tests are Delayed- Reactivate?” with N or NO.

Add required reagents to the drawer and reactivate test(s).

* + - 1. All patient results are displayed on the **TEST PANEL** screen and automatically print out and transmit if selected on the **SYSTEM** menu.
      2. For results in question that need operator intervention, cursor to the identification number in the **TEST PANEL** screen and double click.
         1. This will display the **PATIENT REPORT FORM** screen for the specific sample. Follow the options on the bottom of the screen (i.e. Confirm, Re-run, Delete, Add test). Click the  icon to save the changes.
         2. Click the icon to return to the **TEST PANEL** screen.

1. **CALCULATIONS**
   1. No calculations are required for the Thrombin Time.
2. **QUALITY ASSURANCE**
   1. Tolerance Limits of Controls
      1. Acceptable limits for STAGO are calculated monthly via Stago Clarity peer comparison program.
      2. All control ranges are monitored by the STAGO. If any controls are outside the SD range, the STAGO will audibly and visually alarm the operator by FAILING.
      3. Otherwise, the results can be found in the QC files. Control results are automatically filed in the STAG QC LOG.
      4. QC is also monitored in Cerner via ARE. Data points must be entered into the LIS, using ARE function. If a data point fails criteria, QC must be rerun and corrective action documented on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form B**.
   2. Out of Tolerance Controls
      1. When control time values fall outside of set limits:
         1. Repeat to verify.
         2. Recheck all instrument operations and volume of reagents onboard and ensure sufficient volume of sample, and confirm expiration time and date.
         3. Open and reconstitute a new vial of control material, making sure the Protocol water and not saline is used.
         4. If step #3 is still out of tolerance, notify supervisor and call Biomedical Engineering.
         5. Write comment in Action Log (form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form B**).
   3. Tolerance Limits For Acceptable Performance ‑‑ Patient Samples
      1. Any Thrombin Time over 240 seconds or less than 13 seconds should be checked for clots then repeated for confirmation.
   4. Tolerance Limits of Temperature Controlled Areas
      1. Reagent Cooling System
      2. Maintain reagents at 25oC or lower. Instruments will Auto Stop when temperatures of controlled areas are out of range.
   5. Frequency of Assay
      1. Each Shift:
      2. The Normal and Abnormal Control must be assayed at least every 8 hours when patient testing is performed.
   6. Quality Control Program
      1. New Lot of Controls
         1. When a new lot of control is put in use utilize the assayed range over twenty runs to establish and or confirm assayed range.
      2. Failed QC
         1. Failed QC on the STAGO will cause a FAILED flag on the printout alerting staff of a problem which should result in a repeat of the failed control. If failed QC is acceptable on the basis of the Cerner range, still repeat failed control(s) on the analyzer. If controls continue to fail alert Lead Tech or Supervisor.
      3. Controls in Cerner
         1. Controls are released into LIS QC files for Westgard rules. All QC is released the same as a patient result in Cerner and **requires** technical review prior to release.
   7. INSTRUMENT CORRELATION
      1. The four STAGO instruments are correlated semiannually.
         1. Three patient samples are to be processed for correlation on each instrument and recorded on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form C** Semi-Annual Coagulation Instrument Correlations.
            1. TT’s are to correlate +/- 8%.
   8. Verification of platelet poor plasma
      1. The actual platelet concentration of the normally spun plasma used for coagulation testing is counted semiannually or when centrifuge is changed to confirm that it is platelet-poor.
         1. Obtain plasma from spun blue top tube.
         2. Run plasma on the Hematology analyzer.
         3. Print out and record on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form D** Centrifuge Platelet Concentration Correlation.
         4. The platelet count of this platelet poor plasma must be less than 10,000
         5. Notify supervisor if outside acceptable limits and discontinue use of centrifuge.
3. PROCEDURE NOTES

A. LIMITATIONS OF THE PROCEDURE

Prolongation of the Thrombin Time indicates an abnormal fibrin formation. This abnormal formation can be found with dysfibrinogenemia, afibrinogenemia, and hypofibrinogenemia, either congenital or acquired, i.e. DIC, fibrinolysis, liver disease. 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.

* 1. STAT Specimens
     1. All stat specimens are to be run and released within an hour of receipt. All stroke specimens are to be run and released within a half hour of receipt.
  2. Hematocrit greater than 55%
     1. When a patient has a hematocrit greater than 55%, the 9:1 ratio of blood to anticoagulant (3.2% sodium citrate) is not adequate and will result in inaccurate PT, APTT, and Fibrinogens. A specially prepared tube, based on the patient's hematocrit, must be used.
        1. Implementation
        2. According to the following formula, calculate the amount of 3.2% sodium citrate that is required:

See equation on page 11

Amount of citrate = 0.00185 x amt. blood to be drawn x (100 - Hematocrit)

**Below is an example of the calculation when a 2.7 ml tube is to be drawn**

Patient's hematocrit = 60%

You wish to draw 2.7 ml of blood

Amt. citrate = 0.00185 x 2.7 x (100-60)

Amt. citrate = 0.00185 x 2.7 x 40

Amt. citrate = 0.199 ml

* + 1. Open several blue top tubes and pool the sodium citrate in a small, plastic, capped tube.
    2. Pipette the required amount of sodium citrate into an empty red top tube (do not use a gel tube).
    3. Patient's blood must be **drawn by syringe** and the exact amount of blood used in the calculations must be added to the tube containing the measured amount of citrate.
    4. When this tube is received in Hematology Laboratory, handle as any other specimen for testing.
    5. Append the footnote “HCT” (HCT > 55%, SPECIAL TUBE REQUIRED) to the result.

1. **EXPECTED VALUES**
   1. Normal Range: 14.6-16.6 seconds
2. **REPORTING RESULTS**
   1. Worksheet
      1. AEMC Stago
   2. Reportable Range:
      1. : 13.0-240 seconds

Numerical results outside of the reportable ranges are to be resulted as less than (<) or greater than (>) the values listed above the reportable range. Any results above the linearity (<Min or >Max) must repeated to confirm results and checked for clots.

* 1. Release of results
     1. Results will print from the analyzer once completed.
     2. Review printout for any errors before resulting.
     3. Results are entered in function ARE (Accession Result Entry), enter Accession number from print out and worksheet AEMC Stago and click ENTER.
     4. Review results against print out. If results do not cross over, enter results from print out for each test performed, review and VERIFY.

1. **CALIBRATION**
   1. No calibration of the system is necessary for performing the Thrombin Time
2. BACK‑UP INSTRUMENTATION AND/OR METHODOLOGY
3. EMC has two Stago instruments, which serve as backups for each other.
4. EP has one Stago Compact Max instrument and One Start 4, , which serve as backups for each other
5. In the event of a total system shutdown or instrumentation failure in which ECMP Stago Compacts are out of service, specimens must be transported to another facility (i.e. ECM-EP or Montgomery).
6. In the event of a total system shutdown or instrumentation failure in which ECM-EP Stago analyzers are out of service specimens must be transported to another facility (i.e. ECMP or Montgomery).
7. **LIMITATIONS** 
   1. The presence of antithrombins will affect the results of the Thrombin Time. These include therapeutic heparin and hirudin.
   2. Abnormally high FDPs may also affect the results.
   3. Do not test samples that may have been contaminated by heparin (in collection tubes, syringes, etc.
   4. Thrombin Time should first be performed before any other specific assays are attempted, when a prolongation of the overall test (PT, APTT) cannot be explained.
8. **NOTES**
   1. **New lot of Thrombin Reagent**: With each new lot of Thrombin Reagent, the operator must enter the reference time (mean) before the STA Compact Max® will allow QC to be run. The mean or ***Reference Time*** is entered in the **Calibration** menu**.** Click the  icon. The **Calibration Methodologies List** screen appears. Double click **TT** from the list of tests. Select **Modify Parameters** and a window requesting the access code will appear. Type the access code and then click **Confirm.** The cursor is positioned over the **Reference Time** field to modify. When the modification has been made, click **Confirm**. Click the icon to return to the **Calibration – Methodologies List** screen. A green triangle  is displayed on the right of the TT test showing the calibration is valid.

**REFERENCES**

1. STA**®** - Liatest ® D-Dimer, Immuno – Turbidometric Assay of D-Dimer by STA® Analyzers. Package insert 23296 - Revised August 2012.
2. STA**®** Liatest**®** ControlⓃ+Ⓟ (Cat. No. 00526): Control Plasmas of Immuno-Turbidimetric Assays of vWF and D-Dimer on the STA® Analyzers. Package insert 23189 – revised July 2011.
3. STA**® -** Owren-Koller Buffer (Cat. No. 00360)Buffer Solution for CoagulationTesting. Package insert 23070 06 – April 2012.
4. STA - Desorb U (Cat. No. 0975) Decontamination solution for STA**®** analyzer systems. Package insert #26265 – revised June 2011.
5. STA Compact Max® Operators Manual. 0931946 October 2012
6. A.J.Moss, et. al., “Thrombogenic Factors and Recurrent Coronary Events”. Circulation. May 18, 1999. pp. 2517-2522
7. Woodhams B *et al*. Stability of Coagulation Proteins in Frozen Plasma. *Blood Coag Fibrinol.* 2001;12(4):229-236.
8. Clinical and Laboratory Standards Institute (CLSI). Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline- Fifth Edition. H21-A5 vol. 28 No. 5 or latest revision.
9. Clinical Laboratory Standards Institute. Collection and Processing of Blood Specimens for Testing Plasma Based Coagulation Assays and Molecular Hemostasis Assays:  Approved Guideline. Fifth Edition.  Wayne, PA: Clinical Laboratory Standards Institute; 2008. Document H3 – A6 or latest revision.
10. Clinical Laboratory Standards Institute (CLSI): Evaluation of Stability of *In Vitro* Diagnostic Reagents: Approved Guideline Wayne, PA: Clinical Laboratory Standards Institute; 2009. Document EP 25-A
11. Clinical Laboratory Standards Institute: Protocols for Determination of Limits of Detection and Limits of Quantitation: Approved Guideline Wayne, PA: Clinical Laboratory Standards Institute; 2004. Document EP 17-A
12. Clinical Laboratory Standards Institute: Interference Testing in Clinical Chemistry: Approved Guideline. Second Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2005. Document EP 7-A2.

*For additional information, please refer to the manufacturer’s package insert.*

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**Approval signatures:**

|  |  |  |
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| 3-4-2016 | Vanessa Rawlings  Elkins Park Supervisor |  |
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## *Review History*

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| **Date**  **Reviewed** | **Reviewed By** | **Revisions** |
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