PT ON THE STA-R EVOLUTION

Purpose

This document describes the procedure for the performance of the PT assay on the Sta-R Evolution analyzer.

Principle

STA® - Neoplastine® CI 5 & 10 ml (Cat. # 0605 & Cat. # 0666) and STA® - Neoplastine® CI PLUS 5 & 10 ml (Cat # 0606 & Cat. # 0667) are reagents used for Prothrombin times (PT) and extrinsic factor assays on the STA-R Evolution®. A mixture of thromboplastin is added to citrated plasma and the time for clot formation is determined. The STA-R Evolution ® is a fully automated coagulation instrument which uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette containing the thromboplastin and plasma is monitored by the STA-R Evolution®. When the oscillation of the steel ball is slowed by clot formation, the sensor determines the time in seconds. The PT is a basic coagulation screening test for the activity of the extrinsic pathway (factors I (fibrinogen), II, V, VII, and X).

A prolonged PT has been observed in the following clinical disease states: congenital or acquired deficiencies of Factor II, V, VII, X, or fibrinogen. The PT may also be prolonged in liver disease, treatment with vitamin K antagonists, disorders of metabolism of vitamin K, fibrinolysis, and DIC.

The PT 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 PT results with different thromboplastin reagent and instruments, it is recommended (by the World Health Organization) that PT results be converted to an International Normalized Ratio (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:

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

The ISI value of a thromboplastin reagent is determined by performing PTs on normal plasmas and coumadin-treated patient plasmas with the thromboplastin reagent and the WHO reference thromboplastin and plotting the corresponding linear regression. The slope of this regression curve of the matched pairs is the ISI that is assigned for the thromboplastin by the manufacturer. The ISI value for STA® - Neoplastine® CI 5 & 10 ml and STA® - Neoplastine® CI PLUS 5 & 10 ml is determined using a secondary reference standard of rabbit brain thromboplastin on STA® analyzers. The resulting ISI value is provided by the manufacturer for each lot of reagent and is also contained within the barcode of the reagent for use on the STA® line of Stago analyzers. The geometric mean is calculated from the data collected for the PT reference range at the customer site.

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Sample Preparation and Requirements

1. Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Follow CLSI guidelines H3 – A6 and H21 – A5, or latest revision.
2. Centrifugation: 5 minutes at 5100 g
3. Plasma Storage: 8 hours at 20 ± 5 °C
   Do not store at 2-8^{0}C
4. Unacceptable Specimens: Samples that are short, over draws, clotted or hemolyzed may yield incorrect results.

Equipment

- Centrifuge
- Pipettes
- Pipette tips
- Reagent Grade Water
- Cuvette roll (Cat. No. 38669)
- Magnetic Stir Bars (Cat. No. 27425)
- STA Reducer (Cat. No. 00801)
- STA – R® Evolution

Reagents

1. Reagent 1: STA® - Neoplastine® CI [Cat. # 0605 or Cat. # 666] or STA® - Neoplastine® CI Plus [Cat. # 606 or Cat. # 667]: Lyophilized thromboplastin prepared from fresh rabbit cerebral tissue. The reagent contains a specific inhibitor of heparin; thus, times obtained will not be affected by heparin at therapeutic levels. Transfer the entire contents of one vial of Reagent 2 into one vial of Reagent 1 of the same lot. Let sit 30 minutes at room temperature. Swirl gently. Add a Magnetic Stir Bar (Cat. No. 27425) to the vial, place STA® – Reducer Cat. # 00797 (for 5 mL vials) or Cat. No. 00801 (for 10-15 mL vials) and replace the perforated plastic cap. Request the product drawer to open by clicking the  icon and clicking on the OPEN tab. Barcode the reagent and place it into a stirring position in the reagent drawer (R2).
   Reconstituted stability:
   - with the Magnetic Stir Bar, STA® - Reducer and perforated plastic cap in place: 48 hours for 5 ml (Ref 00606 & 00605) and 10 ml(Ref 00667 & 00666) vials when loaded on the STA-R Evolution®.
   - in its original capped vial: 8 days at 2 – 8 ^{O} C for **5 ml vial ONLY** (00605 & 00606). Allow refrigerated reagent aliquot to stand at room temperature for 30 minutes before use.

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2. Reagent 2: 5 or 10 ml solvent. Ready to use.
3. STA® - Coag Control N+ABN PLUS (Cat. No. 00677): citrated control plasmas normal and abnormal levels; or STA® - both freeze-dried. Reconstitute each vial with 2.0 ml reagent grade water. Let sit 30 minutes at room temperature. Swirl gently. Reconstituted stability on the STA-R Evolution® is 24 hours.
4. STA – DESORB U is a decontaminating solution for use with the STA® line of instruments. Install a new STA® - maxi reducer (REF 00801) and the perforated cap on a freshly opened bottle before loading in the reagent drawer. The analyzer will train "BLOCK SAMPLE PIPETTING" when there is not sufficient STA – DESORB U to run requested testing. Stability is 5 days on the STA® - Compact® / STA® - Compact® CT.
5. STA® - Cleaner Solution is a washing aqueous solution used on the STA® line of instruments. Sufficient STA® - Cleaner Solution must be loaded to operate the analyzer.

Calibration

No calibration of the system is necessary for performing a PT if results are reported in seconds.

If PT-INRs are reported by the STA–R® Evolution, a lot specific ISI value and geometric mean or *Reference Time* must be entered into the instrument. The ISI is automatically entered into the analyzer when the new lot of reagent is first bar coded and loaded onto the instrument.

1. Entering or Modifying the ISI value

   - Click the  icon
   - Double-click the PT test abbreviation
   - Click **Edit the Ref. Time and ISI**
   - Click the ISI field to enter data
   - Confirm by clicking **Validate**
   - The ISI in the instrument should match the ISI listed on the barcode sheet that accompanies each box of Neoplastine reagent; it is always lot number specific.

With each new lot of PT reagent, the geometric mean or *Reference Time* mus be manually entered into the analyzer in order for the correct calculation of the INR. Validation of the normal range geometric mean must be confirmed onsit with each new lot of reagent.

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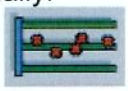
2. Entering or Modifying the *Reference Time* (geometric mean)

- Click the icon
- Locate the test whose Reference time must be modified
- Double-click the test abbreviation
- Click **Edit the Ref. Time and ISI**
- Click the Reference time field to enter the geometric mean
- Confirm by clicking **Validate**

Quality Control

1. STA® - Coag Control N+ABN PLUS (Cat. No. 00677): After the reconstitution period, request the products drawer to open by clicking the  icon and selecting Open tab. Barcode the controls and place them in the product drawer (R0). Scan the barcode sheet for each new lot of controls.
2. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu. To order QC manually:



- Click the icon
- Click the PT test abbreviation
- Click the **Control Level #** tab (#corresponds to the number of the level)

- Click the button
- Confirm by clicking **OK**
- Repeat for each level, or hold down the "**Ctrl**" key to select multiple tests
- When ordering QC for multiple tests all QC levels are ordered

3. All control ranges are monitored automatically by the STA–R® Evolution. Control results are automatically filed in the STA–R® Evolution QC file. All results for a 24-hour period will be averaged 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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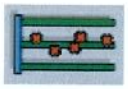
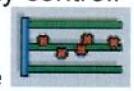
4. To print all the QC data points for the PT test used to calculate the mean for a specific day or month, perform the following procedure.

   - Click the  icon.
   - Locate the PT test and click the abbreviation
   - Click the **Control Level #** tab (#corresponds to the number of the level)
   - Click the date or month on the Levy-Jennings chart
   - Click the  icon
   - Then select the appropriate button 

   - Confirm by clicking **Validate**

Additionally, QC can be printed for a specific date range or in Levy Jennings format.

Procedure

1. Refer to START-UP procedure for STA–R® Evolution before running patient specimens on the STA–R® Evolution at the start of each shift.
2. Request quality control.
   - Click the  icon
   - Click the test abbreviation
   - Click the **Control Level #** tab (#corresponds to the number of the level)
   - Click the  button
   - Confirm by clicking **OK**
3. Load patients' samples:

   Barcode labels with LIS downloading of tests

   - Click the  icon
   - Click the **Loading** tab
   - Click Downloading to display the **Flagged** symbol
   - Place the previously centrifuged tubes in the rack(s)
   - Place the rack(s) in the rack tray

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- Place the rack tray in the instrument: the tubes will be automatically loaded,
  their barcode labels read and the job list will be fed to the host computer for
  each tube. The tests will be automatically carried out by the STA–R® Evolution.

Barcode labels with automatic profile

- Click the  icon
- If necessary, specify the automatic profile
- Click the **Loading** tab
- Click **Add automatic profile** to display the **Flagged** symbol
- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded, their barcode labels read and the test profile will be added to each patient's identification. The tests will be automatically carried out by the STA–R® Evolution.

Barcode label and manual test selection

- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded
  and their barcode labels read
- Add the test(s): either via the Test panel or via the Patient File Acquisition
  screen. (refer to manual for adding a test)

Barcode and Stat

- Click  icon.
- Place previously centrifuged **Stat** tube(s) in the rack
- Make sure loader is ready to accept Stat rack (screen in green)
- Slide rack into loader area
- STA–R® Evolution will automatically read barcode label: test information will be determined as previously mentioned.

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4. Reagent Forecast

   - Click the  icon.
   - Loaded patient samples with accession numbers and tests will appear on screen
   - If a product is missing or not sufficient to run tests, the   or  icon will blink at the bottom of the Test Panel screen.
   - Click the  icon.
   - Click the **Forecast** tab
   - Reagent missing will be listed in **RED**
   - Load necessary product and the tests will resume

5. All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected.

6. Operator intervention of sample(s)

   Re-Run Result

   - Double click result in question
   - Click **Rerun**
   - Confirm by clicking on 

   Add (insert) Test to Sample

   - Scroll on the file identity (accession number…)
   - Double click test box to be added for accession number
   - Confirm by clicking on 

   Change Priority of Loaded Sample

   - Double click on the file identity (accession number…)
   - Click STAT icon

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Calculations

1. All instrument measurements and calculations are performed by the STA–R Evolution®. Results are printed in a report form.

2. The International Normalized Ratio (INR) is automatically calculated by the STA–R Evolution® when INR is selected as a reporting unit in the test setup.

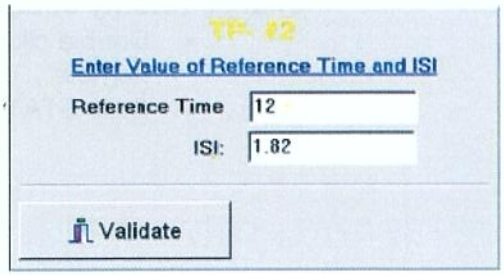
$$INR = \left(\frac{Patient's\ PT}{Geometric\ Mean\ PT} \right)^{ISI}$$

The INR is (see formula) used to standardize PT values performed throughout the world to the World Health Organization (WHO) reference thromboplastin.

3. The International Sensitivity Index (ISI) is the value determined by the manufacturer for a specific lot number of thromboplastin as compared to the WHO thromboplastin standard. It is used as an exponential power for calculating INR by the laboratory performing a PT using that specific thromboplastin lot number with a specific instrument. It is stored in the

CALIBRATION Screen for PT.

- Click the  icon
- Select the appropriate calibration (#1 or #2)
- Double click the PT test
- Click **Edit the Ref. Time and ISI**
- Click ISI field, modify
- Click Validate



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4. The PT Geometric Mean (*Reference Time)* is the statistically calculated value based on the reference population for PT in seconds for the reagent. It must be manually entered in the CALIBRATION screen for PT as previously described for the ISI.
5. Patient's PT is the measured PT in seconds for the reagent.

Reference Range

Secs	12.0 – 15.4
INR	0.8 – 1.2

Reporting Results

1. The results for a PT are reported in seconds to the nearest 0.1 sec.
   (Example: 14.1 sec).
2. The INR is reported to the 0.01 number. (Example: 1.63).

Notes

1. **New lot of Thromboplastin**: With each new lot of thromboplastin, the operator must enter the geometric mean time (*Reference Time)* as described in CALIBRATION, before the STA–R Evolution® will allow QC to be run.
2. The PT reference range and geometric mean are validated with each change of PT reagent lot number.

Limitations of Procedure

1. Many commonly administered drugs affect the results obtained in PT testing. (Example: coumadin and heparin and direct thrombin inhibitors).
2. STA® - Neoplastine® CI and CI+, both contain a specific inhibitor of heparin. The test is insensitive to unfractionated heparin levels up to 1 IU/ml and to low molecular weight heparin levels up to 1.5 anti-Xa IU/ml.

References

1. STA® - Neoplastine® CI 5 ml (Cat. # 0605) or STA® - Neoplastine® CI 10 ml (Cat. # 0666), or
   STA® - Neoplastine® CI PLUS, 5 ml (Cat # 00606) & STA® - Neoplastine® CI PLUS (Cat. # 0667) are used for Determination of Prothrombin Time (PT) by STA® Analyzers. Package insert for use in PT determinations.
   STA® - Neoplastine® CI package insert 26332 06-Revised November 2011
   STA® - Neoplastine® CI PLUS package insert 26336 08-Revised June 2012.

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2. STA® - Coag Control N+ABN Plus (Cat. No. 00677): citrated control plasmas normal and abnormal levels package insert – December 2017
3. STA - Desorb U (Cat. No. 0975) Decontamination solution for STA analyzer systems. Package insert #26265 – revised June 2011.
4. STA® Evolution Operator's Manual.
5. Woodhams B *et al.* Stability of Coagulation Proteins in Frozen Plasma. *Blood Coag Fibrinol.* 2001;12(4):229-236.
6. Clinical Laboratory Standards Institute. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Guideline. Sixth Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2008. Document H3-A6.
7. Clinical Laboratory Standards Institute. Collection, Transport 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 vol. 28 No. 5.

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HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Area Lab Manager/ Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
NEW		M.Acosta 07-24-14	J. Wolf 07-24-14	S. Wirio MD 07-29-14	08-12-14
Major	• New STA® - Coag Control N+ABN Plus (Cat. No. 00677)	11/15/18	11/21/18	11/21/18	11/21/18