APTT ON THE STA-R EVOLUTION

Purpose

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

Principle

STA® - PTT A 5 ml, (Cat # 0595), provides reagent for the determination of the activated partial thromboplastin time. It involves the recalcification time of plasma in the presence of a standardized amount of platelet substitute (cephalin) and a specific activator (silica). The time of clot formation is measured on the STA–R® Evolution. 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 with 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 APTT is a general coagulation screening test for the activity of the intrinsic pathway (factors XII, XI, IX, VIII, X, V, II, and fibrinogen). It is also the test most often used to monitor heparin therapy.

A prolongation of the APTT may be seen in the following deficiencies; Factors VIII, IX, XI, XII, prekallikrein and high molecular weight kininogen. It may also be abnormal in liver disease, DIC, circulating anticoagulants (such as LA, or a specific factor inhibitor) during heparin or oral anticoagulant therapy or when treated with direct thrombin inhibitors (such as hirudin, argatroban, etc).

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
   Within 1 hour after sample collection if the blood was collected in conventional citrate anticoagulant
3. Plasma Storage: 4 hours at 20 ±5^{0}C
4. Unacceptable Specimens: Samples that are short, over draws, clotted or hemolyzed may yield incorrect results.

Equipment, and Supplies

- Centrifuge
- Reagent Grade Water
- Pipettes
- Cuvette roll 1000 cuvettes (Cat. No. 38669)
- Pipette tips
- STA–R®/STA-R Evolution®

APTT ON THE STA-R EVOLUTION

Reagents

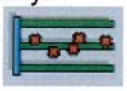
1. Reagent 1: STA® - PTT A 5 ml [Cat. No. 0595]: Lyophilized reagent containing cephalin prepared from rabbit cerebral tissues and a particulate activator (silica) in a buffered medium. Reconstitute each vial with 5.0 ml of reagent grade water. Let sit 30 minutes at room temperature. Mix vigorously by turning the vial upside down, 5 – 10 times or vortex on low for 5 seconds. Remove rubber stopper and replace the perforated plastic cap. Request the products drawer to open by clicking the  icon and selecting load products. Barcode the reagent and place in the **R1**section of the drawer.
   Reconstituted stability:
   - 24 hours with the perforated cap in place on the analyzer
   - In its original capped vial: 7 days at 2 – 8O C. Allow the refrigerated reagent aliquot to stand at room temperature for 30 minutes before use.
   - Considering the numerous combinations of storage conditions (partly on board, partly at 2-8C), each laboratory should establish its own stability durations according to its practices. These durations should not exceed the above mentioned figures which have been determined under controlled conditions.
2. Reagent 2: 0.025 M $CaCl_2$ (Cat. No. 00367): Ready to use. If refrigerated, keep at room temperature for 30 minutes before use. Request the products drawer to open by clicking the above icon, select load products, barcode the reagent and place in the **R2** section of the drawer. Stability is 72 hours on the STA–R® Evolution.
3. STA® - Coag Control N + ABN PLUS (Cat. No.00677): citrated control plasmas, normal and abnormal levels - both freeze-dried. Reconstitute each vial with 2ml 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 (Cat. No. 0975): is a decontaminating solution (contains KOH < 1%) 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 state "BLOCK SAMPLE

APTT ON THE STA-R EVOLUTION

PIPETTING" when there is not sufficient STA® – DESORB U to run requested testing. Stability is 5 days on STA–R® Evolution.

5. 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.

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 PTT 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.
4. To print all the QC data points for the APTT test used to calculate the mean for a specific day or month, perform the following procedure.
   - Click the  icon.
   - Locate the APTT test and click the abbreviation

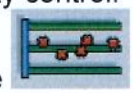
APTT ON THE STA-R EVOLUTION

- 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
   - 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.

APTT ON 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)

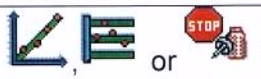
Barcode and Stat

- 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.

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

APTT ON THE STA-R EVOLUTION

 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. For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and double click. This will display the FILE PROCESSING screen. Follow the options on the left-hand side of the screen (i.e. F3 - rerun test).

Reference Range

25 to 37 seconds

Reporting Results

The results for an APTT are reported out in seconds to the nearest 0.1 sec

(Example: 31.1 sec).

Limitations of Procedure

1 Many commonly administered drugs affect the results obtained in APTT testing.

(Example: coumadin and heparin).

2. When monitoring heparin therapy, any release of platelet factor 4 (PF4) which is a potent inhibitor of heparin, represents a major source of error.

References

1. STA® – PTT A⑤: Determination of Activated Partial Thromboplastin Time (APTT) Package insert for use in APTT determinations. 24528 - Revised June 2012..
2. STA® - Coag Control N+ABN PLUS (Cat. No. 00677): citrated control plasmas normal and abnormal levels;Package insert December 2017.
3. STA–R® Evolution Operators Manual.
4. Woodhams B *et al.* Stability of Coagulation Proteins in Frozen Plasma. *Blood Coag Fibrinol.* 2001; 12(4):229-236.
5. Clinical and Laboratory Standards Institute (CLSI). Collection,

APTT ON THE STA-R EVOLUTION

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.

6. 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.

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Continued on next page

APTT ON THE STA-R EVOLUTION

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