UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

Effective Date: 08-16-18
Date Reviewed/ Date Revised: 08-15-18

PROTHROMBIN TIME

Sta Compact Max

I. PRINCIPLE

The **STA® - Neoplastine® CI Plus** Plus kit provides reagents for the determination of the prothrombin time (PT) in plasma. The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of the patient's plasma and to compare it with that of a normal standard. The test measures, as a whole, the activity of the coagulation factor II (prothrombin), factor V (proaccelerin), factor VII (proconvertin), factor X (Stuart factor) and factor I (fibrinogen).

II. CLINICAL SIGNIFICANCE

A prolonged PT has been observed in the following clinical states:

A. congenital or acquired deficiencies of factor II, V, VII, X or fibrinogen
B. liver failure (cirrhosis, hepatitis)
C. treatments with vitamin K antagonists
D. hypovitaminosis K: nutritional intake deficiency, disorders in absorption or metabolism of vitamin K (hemorrhagic disease of the newborn, cholestasis, treatment with antibiotics)
E. fibrinolysis
F. DIC

The PT is commonly used for monitoring vitamin K antagonist therapy (3) because of its sensitivity to variations in the concentration of the vitamin-K dependent factors II, VII and X. Consequently, the comparability of results of this test is essential for finding the therapeutic range.

It is well known that the PT value of a plasma may vary according to the origin of the thromboplastin reagent and to the instrument used to measure it. In this system the PT ratio is converted into the International Normalized Ratio (INR). The INR value corresponds to the value of the ratio of the patient's PT to that of the standard PT raised to the ISI (International Sensitivity Index) power of the thromboplastin used:

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

The use of the INR is recommended for the assessment of the vitamin K antagonist therapy in patients

III. SPECIMEN:

A. 2.7 ml or 1.8 ml Blue Top Tube. Mix nine parts of freshly collected blood with one part of 0.11 mol/L (3.2%) sodium citrate anticoagulant. Invert the tube gently three or four times immediately after venipuncture to ensure proper mixing of blood and anticoagulant.

- If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 mL saline and the first 5 mL of blood or six dead space volumes of the catheter discarded.

B. Samples received from patients who have hematocrit values above 55% must have the volume of citrate in the blue top tube adjusted. See STA Compact Max Start Up Operating Procedure for calculations.
C. Specimens that are clotted, collected in the wrong tube, have visible hemolysis or have less than a 90% fill or >10% overfill are rejected.
D. It is unacceptable to combine the contents from separate under-filled sodium citrate tubes.

E. Handling/ Storage Conditions:
1. The whole blood specimen is checked for clot formation by gently inversion and observation.
2. Centrifuge the capped blood specimen as soon as possible after collection for 2 minutes at 7200 RPM (3500 x g) in the Stat Spin Express 3 or S/P ® Brand Stat-60.
   a. Testing requires produce platelet-poor plasma (platelet count $<10x10^9/L$).
   b. The plasma may remain on the packed cells if testing immediately or separated if freezing.
   c. To separate plasma, use a plastic transfer pipette; remove the plasma to a properly labeled polypropylene/plastic tube until ready to test.
3. If testing is not complete within acceptable time for specimen stability (see below), the plasma must be removed to a polypropylene/ plastic tube and frozen. A frost-free freezer should not be used. Frozen plasma samples must be rapidly thawed at 37°C while gently mixing and tested immediately after thawing.
4. If testing is delayed, the sample may be held for 24 hours at room temperature.
5. Specimens should be stored at room temperature after testing. Once removed from the analyzer, caps must be removed if additional testing is ordered

IV. REAGENTS

A. Neoplastine STA® - Neoplastine® CI Plus kit
1. Reagent 1: STA® - Neoplastine® CI Plus , lyophilized thromboplastin prepared from fresh rabbit cerebral tissue. The ISI value of STA® - Neoplastine® CI Plus, correlated with a secondary standard of the RBT (rabbit brain thromboplastin) by instruments of the STA® line, is indicated on the Assay Value insert provided in the box.
   The STA® - Neoplastine® CI Plus reagent contains a specific heparin inhibitor. Any prolongation of the prothrombin time is, therefore, related to a real deficiency of factor II, V, VII, X and/or fibrinogen (see Limitations).
2. Reagent 2: solvent containing calcium 10-ml per vial.
3. Preparation: Transfer the entire contents of one vial of Reagent 2 into one vial of Reagent 1 of the same kit. Allow the reconstituted reagent to stand at room temperature (18-25 °C) for 30 minutes. Swirl the Reagent 1 vial gently to obtain a homogeneous suspension. Then, add a stirring-bar to the vial, place a new STA®- Maxi Reducer and install the perforated cap
4. Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C.

a. Once reconstituted, Reagent 1 is stable: 48 hours on board in a stir position of the product drawer or 8 days at 2-8°C.
b. Before loading onto analyzer, add stir-bar, STA® - Reducer, and put the perforated plastic cap in place
c. Do not freeze.

B. STA® - Coag Control N + ABN Plus kit: provides a normal plasma and an abnormal plasma intended for the quality control of the following tests on analyzers of the STA® brand name suitable with these reagents: prothrombin time (PT), activated partial thromboplastin time (APTT)
1. Reagent 1: STA® - Coag Control N, citrated normal human plasma, lyophilized.
2. Reagent 2: STA® - Coag Control ABN, citrated abnormal human lyophilized.
3. Preparation: Reconstitute each vial of Reagent 1 or 2 with exactly 2 ml of fresh reagent grade Nerl water. Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Then, mix by turning the vial upside down, 3-4 times, to obtain a homogeneous solution.
4. Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.
5. Once reconstituted, Reagents 1 and 2 remain stable for 24 hours on STA Compact®.

C. STA – DESORB U is a decontaminating solution for use with the STA Compact®. It is designed as an integral part of the STA® analyzer system.
1. Preparation: Install a new STA® - maxi Reducer) and the perforated cap on a freshly opened bottle of STA® - Desorb U before loading it into the analyzer.
N.B.: a fine white sediment may be observed in the bottom of the bottle; this has no effect on the performance of the product.
2. Storage: The reagent in intact bottles is stable until the expiration date indicated on the box label, when stored at 2-8 °C and protected from light.
Once opened, the STA® - Desorb U with STA® - maxi Reducer and perforated cap in place, is stable for 5 days on board STA Compact®
3. The STA® - Desorb U reagent contains KOH, a corrosive chemical at the concentration provided (< 1 %).
Danger:
a. Causes severe skin burns and eye damage.
b. Wear protective gloves/protective clothing/eye protection/face protection.
c. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
d. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
e. Discard all unused leftover STA® - Desorb U in the liquid waste container provided on the analyzer.

CAUTION:
1. Store all reagents for this test at 2-8 °C until loaded on the analyzer.

2. For in vitro diagnostic use only.
3. The stirring-bar used in the reagent vial should never be the source of contamination.
4. Decontaminate stirring-bars once a week according to the following procedure:
   - Immerse the bars in a vial of STA® - Desorb U and let them soak for 5 minutes with constant magnetic stirring;
   - Transfer the bars from the STA® - Desorb U vial to a vial of distilled water and let them soak for another 5 minutes with constant movement; repeat this rinsing step with another vial of distilled water;
   - Finally, remove the stirring-bars from the distilled water vial and dry them carefully to remove all traces of moisture.
   - Prior to adding them to reagent vials rinse the bars with distilled water and dry them carefully to remove all traces of moisture before adding them to reagent vials.

D. INSTRUMENTATION/EQUIPMENT

A. STA Compact®
B. Stirring-bar
C. STA® - mini Reducer or STA® - maxi Reducer
D. Cuvette roll – 1000
E. Distilled Water
F. Pipettes & tips

E. CALIBRATION

A. The ISI value for the Prothrombin time must be the value indicated on the insert included in the STA® line product. The operator must check the ISI value before leaving the menu if there has been a lot change, a software update, or any other major change. An incorrect ISI value can lead to inaccurate INR (International Normalization Ratio) results.
B. Entering or Modifying the ISI ratio and/ or the reference time (geometric mean): Refer to STA Compact Max Start Up Operating Procedure or Reference Manual for further information.
C. With a change in Neoplastine lot, a geometric (reference) mean must be entered before the Compact Max will allow the QC to run. The Diagnostic Stago Technical Service Representation calculates this value after the side-by-side evaluation of the new and old reagents is complete. See New Lot Implementation Procedure.
   1. Select Calibration or click the calibration icon
   2. Double click PT
   3. Select Run Controls
   4. Enter the Geometric Mean (Reference Mean)
   5. Click Confirm

F. QUALITY CONTROL

A. It is necessary to run controls in order to ensure accuracy and reproducibility of the results. Two different levels of control should be used.
B. Daily QC Schedule for the N and Abn Plus controls: Check product status→After morning run, perform daily maintenance.

1. Quality control for PT and APTT is automatically run when the STA Compact Max® has to perform an analysis using that methodology and when the time since the last control exceeds 8 hours. The time period is defined in the METHODOLOGIES screens
2. 09:00- 09:30 Make up new QC and any reagents needed
3. 09:30-10:00 Load QC material and Manually run QC
   a. Reconstituted Coag Control Plus N+ABN is stable onboard the Sta Compact Max for 24 hours
4. 17:30-18:00 Manually run QC if sample are not being run
5. 01:30-02:00 Manually run QC if sample are not being run
6. QC will automatically run when testing switches to a new vial of reagent.

C. If Quality Control is not within acceptable range, check that all the components of the test system are functioning correctly, i.e., reagents, assay conditions, etc.
   1. Rerun out-of-range control material. If still out of range, reconstitute a new vial of control and run.
   2. Verify reagent performance. Use new reagents if suspect.
   3. Document out of range QC on Instrument Action Log along with all steps taken to identify and correct the problem.
   4. Do not run patients samples or turn out patient results until the QC is within Acceptable limits
   5. If a patient sample is run with either QC out of range or not done, the analyzer will flag the result with an alarm, "Quality control: out of range or not done". Rerun patient sample once QC is within range

D. See STA Compact Max Start Up Operating Procedure or Reference Manual for further information.

G. PROCEDURE:

A. Refer to STA Compact Max Start Up Operating Procedure for the analyzer before running patient and QC specimens at the start of each shift.
B. There are two modes to load samples.
   1. Automatic Mode:
      a. Instrument is connected to a LIS and can download testing
      b. Load sample onto instrument and the tests will auto-populate with test ordered in LIS
   2. Manual Mode used for LIS downtime or Interface downtime:
      a. Instrument is either not linked to a LIS, or sample is not ordered in the LIS
      b. Load sample onto instrument, then select the test(s) to be performed

H. CALCULATIONS:

A. The International Normalized Ratio (INR) is calculated by STA Compact® and when INR is selected as a reporting unit in Methodologies.

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

Geometric (Reference) Mean = Found on PT Calibration screen

ISI (Specific to Lot) = PT Calibration screen or in Package insert

B. An Assay Value insert with a barcode is provided in the box. This barcode contains the following information: lot number, kit code number, reagent code number, expiration date, calibration values and ISI value. The ISI appears in the Calibration screen for the PT.
C. To enter or change the ISI ratio: see Start Up procedure calibration section.

I. **REPORTING RESULTS:**

Report results using interface/manual result entry in the LIS system.
Reference Ranges for UPH-Pekin for Protimes: 11.7 - 14.6 seconds
Reference Ranges for UPH-Pekin for INR: 0.85 - 1.13 seconds

A. Procedure for Abnormal Results:
Critical Value: PT ≥ 4.5 INR

B. Analytical Measurement Range (Linearity): 10-120 seconds.
1. Below AMR turn out as <10 seconds with an INR <0.7
2. Above AMR turn out as >120 seconds with an INR >17.0

J. PROCEDURAL NOTES

A. August 2018: Geometric Mean = 13.1, ISI = 1.28
B. New lot: With each new lot of STA® - Neoplastine® CI Plus, the operator must enter the geometric mean time (Reference Time) as described in CALIBRATION section of the StartUp procedure, before the analyzer will allow QC to be run.
C. The PT reference range and geometric mean are validated with each change of PT reagent lot number. See New Lot Implementation Procedure.
D. RISK OF INCORRECT RESULTS-The ISI value for the Prothrombin time must be the value indicated on the insert included in the STA® line product. The operator must check the ISI value before leaving the menu if there has been a lot change, a software update, or any other major change. An incorrect ISI value can lead to inaccurate INR (International Normalization Ratio) results.

K. LIMITATIONS OF THE PROCEDURE

A. Sample: The slightest coagulation (micro-clots) will induce considerable shortening of the times measured (autocatalytic activation of all the factors) whereas extensive coagulation will prolong the clotting times because of consumption of factors and fibrinogen. Do not keep plasmas at 2-8 °C because in this temperature range the factor VII may be activated by the kallikrein system (2).
B. Anticoagulant: Maintain the correct anticoagulant/blood sample volume ratio of 1:9. If there is any considerable variation in hematocrit, modify the quantity of anticoagulant accordingly. See Start Up Procedure.
C. Heparins: The STA® - Neoplastine® CI Plus 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.
D. Thrombin Inhibitors: Thrombin inhibitors (e.g., hirudin, argatroban...) present in the sample to be tested may lead to a prolonged prothrombin time for this sample.

E. Vitamin K antagonists:Vitamin K antagonists will depress plasma levels of factors II (prothrombin), VII (proconvertin), X (Stuart factor) and IX (antihemophilic factor B).For the assessment of the vitamin K antagonist therapy, refer to the current recommendations.

L. REFERENCES

A. STA Compact Max® Reference Manual June 2016.
B. STA Compact Max® User Guide November 2015.
C. STA Compact Max® Software version 109.08.01.00
D. STA-Neoplastin CI Plus® package insert. Revised January 2016.

POLICY CREATION :		***Date:***
Author:	***Kelly Hall, MLS (ASCP)***	***08-08-2018***
Medical Director:	***Kathryn O. Kramer, M.D.***	***08-14-2018***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
8-14-18	Kathryn O. Kramer MD	
SECTION MEDICAL DIRECTOR		
	N/A	

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date

Reviewed By:

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date