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Determination of Prothrombin Time (PT) on STA® Analyzers

Purpose

This procedure provides instructions for the prothrombin time (PT) testing with STA-R® Max and/or STA Compact®Max.

Principle

The STA® - Neoplastine® CI Plus (REF 00606 or REF 00667) Plus kit provides reagents for the determination of the prothrombin time (PT) in plasma with STA-R® Max, STA Compact® Max, and STA Satellite®. This procedure has been assigned to the moderate complexity category per CLIA 1988 – CDC Analyte Code 4922; CDC Test System Codes 4677 and 4875.

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 PT clotting time measures the integrity of the extrinsic pathway coagulation factor VII (7, proconvertin), and common pathway coagulation factors V (5, proaccelerin), X (10, Stuart factor), II (2, prothrombin) and I (1, fibrinogen).

A prolongation of the PT is encountered in the following clinical situations:

PT typically prolonged more than APTT (activated partial thromboplastin time):	Both APTT and PT typically prolonged:

Congenital and acquired deficiencies:
• Factor VII deficiency, vitamin K-dependent
• Factor V deficiency
• Factor X deficiency, vitamin K-dependent
• Factor II deficiency, vitamin K-dependent
• Factor I deficiency
 	
Acquired causes:
• Anticoagulation therapy with vitamin K antagonists (warfarin)
• Hypovitaminosis K: nutritional deficiency, fat malabsorption, cholestasis, hemorrhagic disease of the newborn, antibiotic use
• Circulating inhibitor
  ○ lupus anticoagulant-hypoprothrombinemia syndrome (LAHS): deficiency of factor II and history of lupus anticoagulant or antiphospholipid syndrome
  ○ specific inhibitor (e.g., factor VII inhibitor)
	• Liver diseases – most coagulation factors are made in the liver
	• Consumptive coagulopathy (e.g., DIC, disseminated intravascular coagulation)
	• Fibrinolysis

The PT is commonly used for monitoring vitamin K antagonist therapy 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.

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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. A solution for standardization adopted by the World Health Organization is a "system of international reference standards for thromboplastins permitting the definition of an international scale for the intensity of anticoagulant therapy" . 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:

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

- The **ISI** value of a given thromboplastin is determined by testing normal plasmas and warfarin-treated patient plasmas with that thromboplastin and with the International Reference Preparation for thromboplastin. The PT values obtained with the two thromboplastins are plotted on log-log graph paper, and the orthogonal regression line is drawn. The slope of this line multiplied by the ISI value of the reference thromboplastin represents the ISI value of the thromboplastin of interest.
- **Geometric Mean PT (Reference Time)** is a statistically calculated value based on the reference population for PT in seconds for the reagent. The PT reference range and geometric mean must be validated and confirmed onsite with each change of PT reagent lot number.
- **Patient's PT** is the measured PT in seconds for the reagent.

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

Scope

This procedure is to be performed by a trained Clinical Laboratory Scientist (CLS) or Medical Laboratory Technician (MLT).

Specimen sources

Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Follow CLSI guideline documents GP41 ED7 and H21-A5. No other anticoagulant is acceptable.

Specimen collection, transport, storage

Sample collection must be in conformity with the recommendations for hemostasis tests. See procedure for "Specimen Requirements for Coagulation Studies."

Specimen rejection

Unacceptable Specimens: Samples that are short, overdraws, clotted or hemolyzed may yield incorrect results.

Equipment

- STA-R® Max and/or STA Compact® Max
- Stirring-bar (REF 27425)
- STA® - mini Reducer (REF 00797) or STA® - maxi Reducer (REF 00801)

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- Cuvette roll – 1000 (REF 38669)
- Centrifuge
- Deionized Water
- Pipettes & tips

Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

1) **STA® - Neoplastine® CI Plus kit** (REF 00606 or REF 00667)

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).

Reagent 2: solvent containing calcium, 5-ml per vial (REF 00606) or 10-ml per vial (REF 00667).

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.

Preparation: Transfer the entire contents of one vial of Reagent 2 (R2) into one vial of Reagent 1 (R1) 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 (REF 27425) to the vial, place a new STA®- Reducer REF 00797 (STA® - Néoplastine® C Plus 5 ml) or REF 00801 (STA® - Neoplastine® CI Plus 10 ml) and install the perforated cap.

Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.

Once reconstituted, Reagent 1 is stable:

- add the stir-bar, STA® - Reducer and put the perforated plastic cap in place: 48 hours on STA-R® Max and STA Compact® Max
- in its original capped vial (remove the STA® - Reducer): 8 days at 2-8 °C (REF 00606 – 5 ml vial).

Do not freeze.

When Reagent 1 is ready for use, load into the instrument according to the recommendations of the Reference Manual of the analyzer model. The vial position in the instrument is the following:

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Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

- on STA-R® Max model, place in a stir position in the R2 area of the product drawer
- on STA Compact® Max model, place in a stir position of the product drawer

NOTE: Considering the numerous combinations of storage conditions (partly on board, partly at 2-8 °C), 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.

In case of storage at 2-8 °C, allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use.

CAUTION: Store reagent kit at 2-8 °C. For in vitro diagnostic use only. These reagents are to be used only by certified medical laboratory personnel authorized by the laboratory.
The STA® - Neoplastine® CI Plus kits are designed for use with analyzers of the STA® line suitable with these reagents. Read the Reference Manual of the analyzer model carefully before starting.
Exercise great care in the handling of these reagents and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

The stirring-bar used in the reagent vial should never be the source of contamination. To ensure that stirring-bars are contamination-free, rinse the bars with distilled water and dry them carefully to remove all traces of moisture before adding them to reagent vials. In addition, decontaminate stirring-bars once a week according to the following procedure:

- immerse the bars in a vial of STA® - Desorb U (REF 00975) and let them soak for 5 minutes with constant magnetic stirring;
- use tweezers to 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 magnetic stirring; 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.

2) **STA – DESORB U** (REF 00975): is a decontaminating solution for use with the STA-R® and/or STA Compact®. It is designed as an integral part of the STA® analyzer system.

STA® - Desorb U: solution containing potassium hydroxide (KOH < 1 %).

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Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

Preparation: Install a new STA® - maxi Reducer (REF 00801) and the perforated cap on a freshly opened bottle of STA® - Desorb U before loading it into the analyzer.

NOTE: a fine white sediment may be observed in the bottom of the bottle; this has no effect on the performance of the product.

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-R® Max and STA Compact® Max

When STA® - Desorb U is ready for use (with STA® - maxi Reducer and perforated plastic cap in place), load it into the instrument according to the recommendations of the Reference Manual of the analyzer model. The vial position in the instrument is the following:

- on STA-R® Max model, place one bottle in the R0, R1 and R2 areas of the product drawer
- on STA Compact® Max model, place one bottle in the product drawer

The instrument will use STA® - Desorb U automatically (see the Reference Manual).

CAUTION: Store at 2-8 °C. For in vitro diagnostic use only. This solution is to be used only by certified medical laboratory personnel authorized by the laboratory.

The STA® - Desorb U reagent contains KOH, a corrosive chemical at the concentration provided (< 1 %).

Danger:

- **Causes severe skin burns and eye damage.**
- **Wear protective gloves/protective clothing/eye protection/face protection.**
- **IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.**
- **IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.**

The STA® - Desorb U must be used with the instruments of the STA® line.

Read the Reference Manual of the analyzer model carefully before using this solution. Discard all unused leftover STA® - Desorb U in the liquid waste container provided on the analyzer. See the Reference Manual of the analyzer model.

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Exercise great care in the handling of these reagents and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

3) **STA® – Coag Control N + ABN PLUS (REF 00677), Medical Center Labs:** kit containing assayed normal and abnormal plasmas intended for the quality control of the following quantitative tests on STA-R® Max and STA Compact® Max analyzers: prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, thrombin time (TT), and antithrombin (AT).

Reagent 1: STA® - Coag Control N PLUS, citrated normal human plasma, lyophilized.
Reagent 2: STA® - Coag Control ABN PLUS, citrated abnormal human plasma, lyophilized.

An Assay Value insert with two barcodes, one for each control level, is provided in the box. Each barcode contains the following information: lot number, kit code number, reagent code number, expiration date and parameter values determined with analyzers of the STA® line for the relevant lot.

Preparation: Reconstitute each vial of Reagent 1 or 2 with exactly 2 ml of distilled 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.

Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C. Once reconstituted, Reagents 1 and 2 remain stable in their original vials for 24 hours on the STA-R® Max and STA Compact® Max.

When Reagents 1 and 2 are ready for use, load them into the instrument according to the recommendations of the Reference Manual of the analyzer model. The vial position in the instrument is the following:

- on STA-R® Max model, place the control vials in the R0 area of the product drawer
- on STA Compact® Max model, place the control vials in one of the positions 1 to 18 or 35 to 38 of the product drawer.

STA® - Coag Control PLUS N and ABN PLUS are automatically used by the instrument - the STA-R® Max, STA Compact® Max. (see the Reference Manual).

CAUTION: Store at 2-8 °C. For in vitro diagnostic use only. These reagents are to be used by certified medical laboratory personnel only. Read the Reference Manual of the analyzer model carefully before starting.

Determination of Prothrombin Time (PT) on STA® Analyzers,
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Exercise great care in the handling of these reagents and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

4) **STA® - System Control N + P kit** (REF 00678), **Regional Reference Lab:** provides a normal plasma and an abnormal plasma intended for use as two control levels for the following assays performed on analyzers of the STA® line suitable with these reagents:

Reagent 1: STA® - System Control N, citrated normal human plasma, lyophilized.

STA® - System Control N is for the following tests: prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Clauss' method), thrombin time (TT), Reptilase® time, factors II, V, VII, VIII, IX, X, XI and XII, antithrombin (AT), protein C, protein S, plasminogen and antiplasmin.

Reagent 2: STA® - System Control P, citrated abnormal human plasma, lyophilized.

STA® - System Control P is for the tests as described above except thrombin time (TT) and Reptilase® time.

Two Assay Value inserts are provided in the box, one for each control level. Each barcode contains the following information: lot number, kit code number, reagent code number, expiration date and parameter values determined with analyzers of the STA® line for the relevant lot

Preparation: Reconstitute each vial of Reagent 1 or Reagent 2 with exactly 1 ml of distilled water. Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Then, swirl the vial gently before use

Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.

Once reconstituted, both controls remain stable for 8 hours on board STA-R®Max, and STA Compact® Max. However, in view of the lability of factor VIII, both control values for factor VIII are valid only for 4 hours after reconstitution.

When the Reagents 1 and 2 are ready for use, load them into the instrument according to the recommendations of the Reference Manual of the analyzer model. The vial position in the instrument is the following:

- on STA-R® Max model, place the control vials in the R0 area of the product drawer
- on STA Compact® Max model, place the control vials in one of the positions 1 to 18 or 35 to 38 of the product drawer

Determination of Prothrombin Time (PT) on STA® Analyzers,
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Reagents
1-2): ALL
3): Med Cntr
4): Regional Reference Lab

The STA® - System Control N and P are automatically used by the instrument (see the Reference Manual).

CAUTION: Store at 2-8 °C. For in vitro diagnostic use only. These reagents are to be used only by certified medical laboratory personnel authorized by the laboratory. Read the Reference Manual of the analyzer model carefully before starting. Exercise great care in the handling of these reagents and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

Quality Control
1): Med Cntr
2): Regional Ref Lab

1) **STA – Coag Control N + ABN PLUS (REF 00677): Med Center Labs**, or
2) **STA®- System Control N+P (Ref 00678): Regional Reference Lab.**

It is necessary to run controls in order to ensure accuracy and reproducibility of the results. Two different levels of control should be used. Prepare the control reagents and scan the information contained in the barcode printed on their respective Assay Value insert to the instrument. They are used undiluted.

Refer to Start-Up procedure for further information.

Procedure

Refer to Start-Up procedure for the analyzer before running patient specimens at the start of each shift.

Method Performance Specifications

Analytical measurement range (reportable range) for PT: 8 - 150 seconds
Analytical measurement range (reportable range) for INR: 0.5-10

Calculations/ Calibration

A. Automated software calculation for the INR:

The INR is automatically calculated by the STA-R® when INR is selected as a reporting unit in Methodologies.

To avoid incorrect calculations for the INR, the **ISI** and geometric **mean PT** values must be reviewed and updated in the instrument software calibration settings with each reagent lot change, software update, or any other major test change (e.g., instrument change, establishment of new reference range).

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

- The **ISI** is assigned by the manufacturer with the PT reagent (**STA® - Néoplastine® CI Plus**) package insert (Assay Value) provided in the box. The operator must update the ISI value in the Calibration screen from the instrument software menu for the PT test whenever there is a change in reagent lot. See START-UP procedure calibration section.

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- The geometric **mean normal PT** must be calculated with each new lot change of PT reagent. CLSI standard H47-A2 specifies the geometric mean for computing the mean normal PT for the INR , and cautions against the arithmetic mean.

    1. Run 20 - 40 normal patient samples with the new reagent, or use a commercial normal donor set for coagulation studies, such as Precision Biologic's CRY check Normal Donor Set (Cat.# CCNS-10) and follow the manufacturer's instructions for handling and storage of donor sets.
    2. Calculate the geometric mean of the normal PT values. The geometric mean is a special type of average that multiples all the numbers together and then takes the n^{th} root of the numbers. Microsoft Excel can be used to compute the geometric mean. In an Excel worksheet, enter the PT results from all tested samples. Enter the following formula in a new cell to calculate for the geometric mean PT: " =GEOMEAN(*select PT values for all samples tested*)"
    3. Enter the geometric mean in the instrument software as described in the STARTUP procedure calibration section.

B. Manual calculation for the INR

A manual calculation for the INR may be performed to verify the calculations performed automatically by the instrument. Follow the steps below to manually calculate the INR for the PT result. The calculations can be performed in Microsoft Excel for convenience.

1. Obtain the result of the patient PT test in seconds.
2. Calculate the ratio of the patient result to the geometric **mean normal PT** using the following formula (geometric mean PT is described in the prior section):
    - Ratio = Patient PT / Mean Normal PT
3. Use this calculated ratio and the assigned ISI value from the PT reagent package insert (Assay Value) to determine the INR with this formula:
    - $INR = (Ratio)^{ISI}$
    - Calculation in Excel looks like "=(*Ratio*)^(*ISI*)"
4. Example:
   Patient PT result (seconds) = 18.2
   Geometric mean normal PT (seconds) = 13.0
   ISI = 1.26

   Ratio = 18.2 / 13.0 = 1.4
   $INR = 1.4^{1.26} = 1.5$

NOTE: The PT reagent package insert (Assay Value) should be filed in the laboratory with any other documentation and worksheets that support the test validation.

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Reference Interval

PT test orders at KPSC:

	Reference Range	Critical Value
PT	12 – 15.4 seconds	
INR	0.8-1.2	> 5

The INR therapeutic goal is defined for each patient in the Problem List in HealthConnect(the electronic medical record), by the provider and the pharmacist when the patient is being monitored for anticoagulation purposes.

Reporting Results

The PT value of the plasmas being tested is displayed, in the "Test Panel/Test Status" screen of the analyzer (see the Reference Manual). The result is to be interpreted according to the patient's clinical and biological states.

Ensure that the values obtained for the controls are within the ranges stated on the Assay Value inserts provided in the control box. If the control values are outside the stated ranges, check all components of the test system to ensure that all are functioning correctly, i.e., assay conditions, reagents.

Manual review of the sample is recommended for the following:

- When the PT or INR result is close to the lower limit of the analytical range (e.g., 0.7 or less for INR), carefully inspect the original patient tube for problems. If no problems causing rejection are identified, repeat testing and release result if repeat is also low.
- If PT or INR exceeds the analytical range, carefully inspect the original sample to assess that the sample is plasma (versus serum). Release result if sample is satisfactory.

Refer to "Approved KP SCAL Coagulation Autoverification Rules" for details on regional autoverification rule settings.

Limitations

Storage temperature: Do not keep plasmas at 2-8 °C because in this temperature range the factor VII may be activated by the kallikrein system.

Heparins: The STA® - Néoplastine® 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.

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.

Vitamin K antagonists: Vitamin K antagonists (e.g., warfarin) will depress the activity of factors II, VII, IX, and X, and cause prolongation of the PT clotting time and increase the INR.

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Non-Controlled Documents / References

The following non-controlled documents support this procedure:

1. CLSI Document GP41: "Collection of diagnostic venous blood specimens; approved standard". Seventh edition, April 2017.

2. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". Fifth edition, 28, May, 2008.

3. CLSI Document H47-A2: "One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplasin Time (APTT) Test; approved guideline". Second Edition, May 2008.

3. STA® - Neoplastine® CI PLUS, 5 ml (REF 00606) & STA® - Neoplastine® CI PLUS 10 ml (REF 00667) used for Determination of Prothrombin Time (PT) on STA Analyzers package insert May 2019.

4. STA® - Coag Control N+ABN PLUS (REF 0677): Control Plasmas for Assays of Coagulation Parameters on STA® Analyzers package insert March 2018.

5. STA® - System Control N+P (REF 00678): Control Plasmas for Assays of Coagulation Parameters on STA® Analyzers package insert October 2017.

6. STA - Desorb U (REF 0975) Decontamination solution for STA® analyzer systems. Package insert June 2019.

Controlled Documents

Approved KP SCAL Coagulation Autoverification Rules, 05-2019 version
Specimen Requirements for Coagulation Studies