STA® - NeoPTimal

Quantitative determination of Prothrombin Time (PT)

STA® - NeoPTimal ⑤

- Kit Containing:
  – 6 x 5-ml Vials of Reagent 1 (STA® - NeoPTimal ⑤)
  – 6 x 5-ml Vials of Reagent 2 (Solvent)

(REF 01163)

STA® - NeoPTimal ⑩

- Kit Containing:
  – 12 x 10-ml Vials of Reagent 1 (STA® - NeoPTimal ⑩)
  – 12 x 10-ml Vials of Reagent 2 (Solvent)

(REF 01164)

STA® - NeoPTimal ⑳

- Kit Containing:
  – 12 x 20-ml Vials of Reagent 1 (STA® - NeoPTimal ⑳)
  – 12 x 20-ml Vials of Reagent 2 (Solvent)

(REF 01165) IVD CE

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1/ INTENDED USE

The STA® - NeoPTimal kits provide thromboplastin reagents from rabbit brain extract, for the quantitative determination, in human citrated plasma (3.2 % sodium citrate), of prothrombin time (PT) on STA-R® family, STA Compact® family and STA Satellite® family instruments. STA® - NeoPTimal is a coagulation screening test intended to be used by professional laboratory personnel for the evaluation of the extrinsic coagulation pathway and the monitoring of **oral vitamin K antagonist therapy using the International Normalized Ratio (INR)**.

2/ SUMMARY AND EXPLANATION

The prothrombin time is a coagulation screening test.

- A prolonged PT is observed (11) in various clinical situations such as:
  – deficiencies of factor II, V, VII, X or fibrinogen (3)
  – liver failure (cirrhosis, hepatitis) (10)
  – treatments with vitamin K antagonists (VKA) (5)
  – DIC (3).
- PT is also used for INR calculation in patients receiving vitamin K antagonists therapy (4, 15). 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:

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

The ISI value of a given thromboplastin is determined by testing normal plasmas and VKA-treated patient plasmas with that thromboplastin and with the International Reference Preparation for thromboplastin (2).

3/ TEST PRINCIPLE

The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of the patient's plasma sample 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).

4/ KIT REAGENTS

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, ISI value and calibration values for result in percentages.

- **Reagent 1**: STA® - NeoPTimal, lyophilized thromboplastin prepared from rabbit brain extract. The ISI value of STA® - NeoPTimal, correlated with a secondary standard of the RBT (rabbit brain thromboplastin) for the instruments of the STA® line, is indicated on the Assay Value insert provided in the box. The ISI value for STA® - NeoPTimal is near 1.0.

  The STA® - NeoPTimal 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 section 11).
- **Reagent 2**: solvent containing calcium, 5-ml, 10-ml or 20-ml per vial according to the packaging size.

Reagent 2 contains nickel sulfate hexahydrate. At the concentration provided (< 0.1 %), this reagent is classified as sensitising.
Warning
May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection.
IF ON SKIN: Wash with plenty of soap and water.

Reagent 2 contains sodium azide (< 1 g/l) as a preservative.
Reagents containing sodium azide should be discarded with care to prevent the formation of explosive metallic azides. If waste materials are dumped into sinks, use copious quantities of water to flush plumbing thoroughly.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
Some reagents provided in these kits contain materials of human and/or animal origin. Whenever human plasma is required for the preparation of these reagents approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.

5/ 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.
  In the U.S.: Caution: Federal law restricts this device to sale by or on the order of a physician.
  Take care to mix Reagent 1 vials with Reagent 2 vials from the same lot. 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.

6/ SPECIMEN COLLECTION AND TREATMENT

Sample collection must be in conformity with the recommendations for haemostasis tests.

- Blood (9 vol.) is collected in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.) [in the USA follow CLSI guidelines GP41-A6 (12) and H21-A5 (14)].
- Centrifugation: 15 minutes at 2000-2500 g.
- Plasma storage: 24 hours at 20 ± 5 °C (14).
  Do not store plasma at 2-8 °C (1).

7/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Transfer the entire contents of one vial of Reagent 2 (R2) into one vial of Reagent 1 (R1) of the same lot. Allow the reconstituted reagent to stand at room temperature (18-25 °C) for 30 minutes. **Then, shake very vigorously 10 seconds the Reagent 1 vial to obtain a homogeneous suspension**. Then, add a stirring-bar (REF 27425) to the vial (STA® - NeoPTimal ⑤, ⑩ or ⑳), place a new STA® - Reducer (REF 00797 for STA® - NeoPTimal ⑤ or REF 00801 for STA® - NeoPTimal ⑩) and install the perforated cap (STA® - NeoPTimal ⑤ or ⑩).
- **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, STA® - NeoPTimal ⑤ is stable:
  – with the stirring-bar, STA® - Reducer and perforated plastic cap in place:
    - 48 hours on STA-R® and STA Compact® families
    - 4 days on STA Satellite® family
  – in its original capped vial (remove the STA® - Reducer): 8 days at 2-8 °C.

  Once reconstituted, STA® - NeoPTimal ⑩ is stable:
  – with the stirring-bar, STA® - Reducer and perforated plastic cap in place:
    - 48 hours on STA-R® and STA Compact® families
    - 4 days on STA Satellite® family.

  Once reconstituted, STA® - NeoPTimal ⑳ is stable:
  – with the stirring-bar:
    - 48 hours on STA-R® and STA Compact® families.

  Do not freeze.

NB: 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.

8/ REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - Owren-Koller** (REF 00360).
- **STA® - System Control N + P** (REF 00678), **STA® - Coag Control N + P** (REF 00679), **STA® - Coag Control (N + ABN) PLUS** (REF 00677), **STA® - Routine QC 2 ml*** (REF 00554) or **STA® - Routine QC P PLUS*** (REF 00714): normal and abnormal levels.
- STA-R® or STA Compact® families (STA® - NeoPTimal ⑤, ⑩ or ⑳).
- STA Satellite® family (STA® - NeoPTimal ⑤ or ⑩).
- STA® - mini Reducer (REF 00797) for STA® - NeoPTimal ⑤.
- STA® - maxi Reducer (REF 00801) for STA® - NeoPTimal ⑩.
- Stirring-bar (REF 27425) for STA® - NeoPTimal ⑤, ⑩ or ⑳.
- Common clinical laboratory equipment and materials.

* Not available in the US.

9/ PROCEDURE

9.1. Calibration
The pre-calibrated PT values are identical for all the vials of each lot. To enter the calibration data on the analyzer, scan the barcode printed on the Assay Value insert across the instrument barcode reader. The calibration data will be validated for the lot being used once the two PT control levels have been determined.
The calibration curve for PT can be examined on the screen of the analyzer in the "Calibration" menu (see the Reference Manual).

9.2. Patients' Plasmas
Patients' plasmas are tested undiluted. They are loaded in the instrument (see the Reference Manual of the analyzer model).
Then select the test(s) to be performed.

9.3. Quality Control
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.

9.4. Assay
Refer to the "Standardized Operating Procedures" of the instrument for full details on how to proceed from this point.
The instrument starts the assay as soon as sample loading is completed.

10/ RESULTS

The PT value of the plasmas being tested is displayed, in the unit selected by the operator (seconds, INR, %, ratio), in the "Test Panel/Test Status" screen of the instrument (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 boxes. 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, integrity of the plasmas being tested, etc. If necessary, repeat the tests.

11/ LIMITATIONS

- **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.
- **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.
- **Heparins**
  The STA® - NeoPTimal test is insensitive to unfractionated heparin levels up to 1.0 IU/ml and to low molecular weight heparin levels up to 1.5 IU anti-Xa/ml. These tests were performed according to the CLSI guideline EP07-A2 (9).
- **Thrombin and Factor Xa Inhibitors**
  These inhibitors present in the sample to be tested may lead to a prolonged prothrombin time (6, 8).

12/ REFERENCE INTERVAL

Normal values vary from one laboratory to the next, depending on reagents, instrumentation and technique. So, each laboratory must determine its own expected values based on technique and instrumentation in use.
If PT results are expressed in percentage of normal activity, normal expected values should be greater than 70 % (19). Values greater than 100 % have no pathological significance.
For example, 125 human plasma presumed normal have been tested with the STA® - NeoPTimal on STA-R®, STA Compact® and STA Satellite®. The mean PT defined according to the CLSI EP28-A3c (20) is 13.5 seconds ± 1.8 seconds.

13/ VITAMIN K ANTAGONIST THERAPY

- 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.

14/ PERFORMANCE CHARACTERISTICS

Different samples were used for the repeatability and within-laboratory precision studies. Results obtained with STA® - NeoPTimal by STA-R® are shown below. Precision studies were performed according to the CLSI guideline document EP05-A3 (17).

	Repeatability		Within-laboratory precision	
Sample	Sample 1	Sample 2	Sample 1	Sample 2
n	80	80	80	80
$\bar{X}$ (s)	15.0	28.0	15.0	28.0
SD (s)	0.12	0.36	0.19	0.57
CV (%)	0.8	1.3	1.2	2.0

15/ METHOD COMPARISON

A correlation study on 403 samples has been carried out with the STA® - NeoPTimal and the Thromborel® S from Siemens. Results obtained are the following: r = 0.98, slope = 1.09, y intercept = -0.01.

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Significant changes are indicated by dotted lines in the margin.

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