STA® - CEPHASCREEN®

Determination of the Activated Partial Thromboplastin Time (APTT)

STA® - CEPHASCREEN® ④

- Kit Containing 12 x 4-ml Vials

(REF 00308)

STA® - CEPHASCREEN® ⑩

- Kit Containing 12 x 10-ml Vials

(REF 00310) IVD CE

January 2014 *English 2*

1/ INTENDED USE

The **STA® - Cephascreen®** kits provide reagents for the determination of the activated partial thromboplastin time (APTT) in plasma according to Langdell R.D. *et al.* (1) and Larrieu M.J., Weilland C. (3) by analyzers of the STA® line suitable with this reagent.

2/ SUMMARY AND EXPLANATION

- The activated partial thromboplastin time (APTT) is a general coagulation screening test of the coagulation factors XII, XI, IX, VIII, X, V, II and fibrinogen. The determination of the APTT is also useful to monitor heparin therapy with unfractionated heparin (UFH).
- A prolongation of the APTT is encountered in the following situations (7):
  - Congenital Deficiencies
    - ◊ If the prothrombin time (PT) is normal, the following factors may be deficient:
      - factor VIII (STA® - Deficient VIII, REF 00725)
      - factor IX (STA® - Deficient IX, REF 00724)
      - factor XI (STA® - Deficient XI, REF 00723)
      - factor XII (STA® - ImmunoDef XII, REF 00315).
    - ◊ If all these factors are normal, a deficiency in HMW kininogen (Fitzgerald factor) should be considered.
  - Acquired Deficiencies and Abnormal Conditions
    - ◊ Liver diseases
    - ◊ Consumptive coagulopathy
    - ◊ Fibrinolysis
    - ◊ Circulating anticoagulants (LA type or circulating anticoagulant against a factor)
    - ◊ During heparin or vitamin K antagonist therapy
    - ◊ Treatments with thrombin inhibitors (e.g., hirudin, argatroban...).

3/ TEST PRINCIPLE

The APTT involves the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) and a factor XII activator (polyphenolic component).
The APTT explores the coagulation factors XII, XI, IX, VIII, X, V, II and I except the platelets.

4/ KIT REAGENT

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 and expiration date.

STA® - Cephascreen®: reagent containing cephalin (platelet substitute), prepared from rabbit cerebral tissues (2) and a polyphenolic activator (US patent n°: 7,208,266) in a buffered medium, 4-ml vials (REF 00308) or 10-ml vials (REF 00310).

This reagent contains a 3:1 mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. At the concentration provided (< 0.06 %), this mixture 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.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
The reagent provided in these kits contains materials of human and/or animal origin. Whenever human plasma is required for the preparation of this reagent, 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. This reagent is to be used only by certified medical laboratory personnel authorized by the laboratory.
The STA® - Cephascreen® kits are designed for use with analyzers of the STA® line suitable with this reagent. Read the Reference Manual of the analyzer model carefully before starting.
In the USA, wherever appropriate, observe CLIA-88 requirements.
Exercise great care in the handling of this reagent and of patient samples. The disposal of waste materials must be carried out according to current local regulations.

6/ SPECIMEN COLLECTION AND TREATMENT

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

- **Sampling**
  Collect blood (9 vol.) in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.) in a non-wettable tube (plastic or siliconized glass) or use a CTAD tube, which is a specially designed sample collection tube to prevent heparin inactivation (6). [In the USA follow CLSI guideline documents H21-A5 (11) and H3-A6 (10)].
- **Centrifugation**
  Centrifuge blood samples for 15 minutes at 2000-2500 g.
- **Storage**
  Plasmas remain stable for 4 hours at 20 ± 5 °C (9). If on heparin therapy, plasmas obtained with conventional citrate anticoagulant may be stored at 20 ± 5 °C for up to 2 hours; those plasmas collected with CTAD tube can be kept at 20 ± 5 °C for up to 4 hours.

7/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Before use, allow the reagent to stand at room temperature (18-25 °C) for 30 minutes. Then, shake **very vigorously** or vortex (at maximum speed, 3-5 seconds) the vial to obtain a homogeneous suspension. Then, install a new STA® - Reducer (REF 00797 or 00801) in the vial and the perforated plastic cap on top. The reagent is ready for 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 homogenized and opened the reagent, with STA® - Reducer and perforated cap in place, remains stable for:
  – 7 days (REF 00308) or 10 days (REF 00310) on STA-R® and STA Compact®
  – 8 days (REF 00308) or 10 days (REF 00310) on STA Satellite®.
  Do not freeze.

8/ REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - $CaCl_2$ 0.025 M** (REF 00367).
- **STA® - Coag Control N + P** (REF 00679), **STA® - System Control N + P** (REF 00678), **STA® - Coag Control (N + ABN) PLUS** (REF 00677) or **STA® - Routine QC 2 ml** (REF 00554): control plasmas, normal and abnormal levels.
- STA-R®, STA Compact® or STA Satellite®.
- STA® - mini Reducer REF 00797 (STA® - Cephascreen® ④) or STA® - maxi Reducer REF 00801 (STA® - Cephascreen® ⑩).
- Common clinical laboratory equipment and materials.

9/ PROCEDURE

Compare the patient's APTT with the reference APTT control in use in the laboratory. When using a normal human plasma pool (plasmas serologically tested), ensure that the plasmas are collected from healthy individuals, either male or female, aged between 18 and 55, not taking any medication and giving blood voluntarily.

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

9.2. 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 controls and scan the information contained in the barcodes printed in the Assay Value insert to the instrument. They are used undiluted.

9.3. Assay
Refer to the "Standardized Operating Procedures" of the instrument for full details on how to proceed from this point.
The APTT determination of the plasmas to be tested is automatically carried out by the analyzer as soon as the samples have been loaded.

10/ RESULTS

The APTT value of the plasmas being tested is displayed, in the unit selected by the operator, in the "Test Status/Test Panel" 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 in 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, integrity of the plasmas being tested, etc. If necessary, repeat the tests.

11/ LIMITATIONS

- STA® - Cephascreen® is usually insensitive to prekallikrein deficiencies. It is reported in the literature that prekallikrein deficient homozygous patients do not manifest any particular haemorrhagic events (8).
- When monitoring heparin therapy, any release of platelet factor 4 (PF4) which is a potent inhibitor of heparin, represents a major source of error.
  – Do not collect blood in glass, which might cause this release; collect blood in plastic, siliconized glass or CTAD tubes.
  – Perform centrifugation within 1 hour after sample collection if the blood was collected in conventional citrate anticoagulant and within 4 hours if the blood was collected with CTAD tubes.

12/ REFERENCE INTERVAL

Normal values may vary depending on local conditions (types of population...). Therefore, it is necessary that each laboratory establish its own normal ranges and acceptable control values for their particular local patient population. In general, values are considered normal if they fall within the range of: mean ± 2 standard deviations ($\bar{X}$ ± 2 SD) (5).
For example, 357 presumed normal human plasmas were tested with STA® - Cephascreen® on the STA® analyzer, the observed mean time was 29.2 seconds with a standard deviation of 2.8 seconds.

The APTT is statistically lengthened in young subjects. By contrast, shorter times were found in older populations (4).

13/ PERFORMANCE CHARACTERISTICS

Different samples were used for the intra-assay and inter-assay reproducibility studies on STA®. Results obtained with STA® - Cephascreen® are shown below:

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
n	21	21	10	10
$\bar{X}$ (s)	29.8	47.2	29.8	48.0
SD (s)	0.19	0.40	0.42	0.44
CV (%)	0.6	0.8	1.4	0.9

14/ ALTERNATIVE PROTOCOL

The chapters 1, 2, 3, 4, 5, 6 and 11 above are still valid if the assay is to be performed by means other than the analyzers of the STA® line.

14.1. Reagent Preparation and Storage
Before use, allow the reagent to stand at room temperature (18-25 °C) for 30 minutes. Then, shake **very vigorously** or vortex (at maximum speed, 3-5 seconds) the vial to obtain a homogeneous suspension (do not add neither STA® - Reducer, nor perforated plastic cap).
Once homogenized and opened the reagent is stable 24 hours at 20 ± 5 °C and 14 days at 2-8 °C, the vial being capped after used.

14.2. Reagents and Equipment Required but not Provided
- **STA® - $CaCl_2$ 0.025 M** (REF 00367).
- **Coag Control N + P** (REF 00621) or **System Control N + P** (REF 00617): control plasmas, normal and abnormal levels.
- Instrument such as ST art®.
- Common clinical laboratory equipment and materials.

14.3. Patients' Plasmas and Controls
The plasmas to be tested and controls are used undiluted.

14.4. Protocol
Compare the patient's APTT with the reference APTT control in use in the laboratory. Keep STA® - Cephascreen® reagent at room temperature (18-25 °C) before use. Follow the instrument manufacturer's instructions for APTT determination. For instance:

In a prewarmed cuvette (37 °C):	
• Undiluted plasma (patient's or control)	1 vol.
• STA® - Cephascreen®	1 vol.
• Incubate at 37 °C for	4 min
• Starting the stop-watch, dispense STA® - $CaCl_2$ 0.025 M prewarmed at 37 °C	1 vol.
Note the clotting time (seconds).	

14.5. Results
Note the clotting time (seconds) of the patient's plasma and that of the reference normal plasma. 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 in the Assay Value Insert 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, integrity of the plasmas being tested, etc. If necessary, repeat the test-run.

14.6. Reference Interval
For example, 30 presumed normal human plasmas were tested with STA® - Cephascreen® on ST art®. The observed mean time was 28.7 seconds with a standard deviation of 2.5 seconds.

14.7. Performance Characteristics
Different samples were used for the intra-assay and inter-assay reproducibility studies on ST art®. Results obtained with STA® - Cephascreen® are shown below:

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	Sample a	Sample b	Sample c	Sample d
n	24	24	10	10
$\bar{X}$ (s)	29.8	50.5	30.8	49.9
SD (s)	0.26	0.50	0.45	0.59
CV (%)	0.9	1.0	1.4	1.2

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Significant changes are indicated by dotted lines in the margin.

DIAGNOSTICA STAGO S.A.S.
9 rue des Frères Chausson
92600 Asnières sur Seine (France)
+33 (0)1 46 88 20 20
webmaster@stago.com

English