STA® - C.K. PREST® ⑤

Determination of the Kaolin-Activated Partial Thromboplastin Time (APTT)

- Kit Containing:
  – 6 x 5-ml Vials of Reagent 1 (STA® - C.K. Prest® ⑤)
  – 6 x 5-ml Vials of Reagent 2 (Activator)

(REF 00597) CE

March 2008 *English 2*

1/ INTENDED USE

The **STA® - C.K. Prest® ⑤** kit provides reagents for the determination of the kaolin-activated partial thromboplastin time (APTT), according to Langdell R.D. *et al.* (1) and Larrieu M.J., Weilland C. (3) by analyzers of the STA® line suitable with these reagents. (In the USA this procedure has been assigned to the moderate complexity category per CLIA 1988 - CDC Analyte Code 0409 ; CDC Test System Codes 4677 and 4875).

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.
- A prolongation of the APTT is encountered in the following situations (8):
  – 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® - Deficient XII, REF 00722).

    ◊ 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 (kaolin).
The APTT explores the coagulation factors XII, XI, IX, VIII, X, V, II and I except the platelets.

4/ KIT REAGENTS

A bar-code insert is provided in the box. This bar-code contains the following information: lot number, kit code number, reagent code number and expiration date.

- **Reagent 1**: cephalin (platelet substitute), prepared from rabbit cerebral tissues according to Bell and Alton (2), lyophilized.
- **Reagent 2**: 5-ml vial, buffered suspension of kaolin (5 mg kaolin per ml).

The 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 this kit 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

- For *in vitro* diagnostic use only. Store at 2-8 °C. These reagents are to be used by certified medical laboratory personnel only. The disposal of waste materials must be carried out according to current local regulations.
  The STA® - C.K. Prest® ⑤ kit is 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; refer to the "Warnings" chapter of the Reference Manual.
  In the USA, wherever appropriate, observe CLIA-88 requirements.
- The stirring-bar that is to be added to 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 Desorb solution 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 collections must be in conformity with the recommendations for haemostasis tests.

- Collect blood (9 vol.) in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.). Use sample collection tubes made of plastic or siliconized glass. [In the USA follow CLSI guidelines H21-A5 (12) and H3-A5 (11)].
  When monitoring heparin therapy, use preferably CTAD tubes available from Becton Dickinson, specially designed sample collection tube to prevent heparin inactivation (6).
- Centrifuge blood samples for 15 minutes at 2000-2500 g. Collect the plasmas in plastic tubes.
- Plasmas remain stable for 4 hours at 20 ± 5 °C (10).
  If on heparin therapy, plasmas remain stable for 2 hours at 20 ± 5 °C when collected with citrate anticoagulant and for 4 hours at 20 ± 5 °C when collected with CTAD tubes.

7/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Shake a vial of Reagent 2 (R2) well and transfer its entire contents into a vial of Reagent 1 of the same kit. Allow the reconstituted Reagent 1 to stand at room temperature (18-25 °C) for 30 minutes. Swirl the Reagent 1 vial gently to obtain a homogeneous suspension. Add a stirring-bar (REF 26674) to the vial and install the perforated plastic cap on it. Then, place the reagent in the instrument and after 20 additional minutes for stabilization/mixing, 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 reconstituted, the reagent is stable:
  – with the stirring-bar (intermittent stirring) and perforated cap in place:
    - 24 hours on STA-R®
    - 48 hours on STA Compact®
  – in its original capped vial: 7 days at 2-8 °C.
  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® - $CaCl_2$ 0.025 M** (REF 00367).
- **STA® - Coag Control N + P** (REF 00679), **STA® - Coag Control N + ABN*** (REF 00676), **STA® - Coag Control (N + ABN) PLUS**** (REF 00677) or **STA® - System Control N + P** (REF 00678): control plasmas, normal and abnormal levels.
- Analyzer of the STA® line suitable with these reagents.
- Stirring-bar (REF 26674).
- Common clinical laboratory equipment and materials (centrifuge...).

* Available in the United States only.

** Not available for market in the United States.

9/ PROCEDURE

Refer to the appropriate chapters of the Reference Manual, particularly those for loading and quality control.
Compare the patient's APTT with the reference APTT control in use in the laboratory.

9.1. 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.

9.2. 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. Use STA® - Coag Control N + P/ABN or STA® - Coag Control (N + ABN) PLUS or STA® - System Control N + P. Prepare the controls and scan the information contained in the bar-code printed in their respective bar-code inserts 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 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

- The STA® - C.K. Prest® is usually insensitive to prekallikrein deficiencies. It is reported in the literature that prekallikrein deficient homozygous patients do not manifest any particular haemorrhagic events (9).
- 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/ EXPECTED VALUES

Normal values may vary depending on local conditions. 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 normal human plasmas were tested with the STA® - C.K. Prest® ⑤ on the STA® analyzer. The observed mean time was 29.6 seconds with a standard deviation of 2.4 seconds.

The APTT is statistically lengthened in new-born babies (7). By contrast, shortened times are found in older populations (4).

13/ PERFORMANCE CHARACTERISTICS

Different samples were used for the intra-assay and inter-assay reproducibility studies on the STA®. Results obtained with STA® - C.K. Prest® 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)	31.6	45.9	32.2	67.0
DS (s)	0.19	0.34	1.13	1.74
CV (%)	0.6	0.7	3.5	2.6

REFERENCES

1. LANGDELL R.D., WAGNER R.H., BRINKHOUS K.M.:
   "Effect of antihemophilic factor on one-stage clotting tests". J. Lab. Clin. Med., **41**, 637-647, 1953.
2. BELL W.N., ALTON H.G.:
   "A brain extract as a substitute for platelet suspensions in the thromboplastin generation test". Nature, **174**, 880-881, 1954.
3. LARRIEU M.J., WEILLAND C.:
   "Utilisation de la "céphaline" dans les tests de coagulation". Nouv. Rev. Fr. Hématol., **12**, 2, 199-210, 1957.
4. CAWKWELL R.D.:
   "Patient's age and the activated partial thromboplastin time test". Thromb. Haemostasis, **39**, 780-781, 1978.
5. LEVIN HILLMAN C.R., LUSHER J.M.:
   "Determining the sensitivity of coagulation screening reagents: a simplified method". Lab. Med., **13**, 3, 162-165, 1982.
6. CONTANT G., GOUAULT-HEILMANN M., MARTINOLI J.L.:
   "Heparin inactivation during blood storage: its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole - C.T.A.D. mixture". Thromb. Res., **31**, 365-374, 1983.
7. ANDREW M., PAES B., MILNER R., JOHNSTON M., MITCHELL L., TOLLEFSEN D.M., POWERS P.:
   "Development of the human coagulation system in the full-term infant". Blood, **70**, 1, 165-172, 1987.
8. SAMAMA M., CONARD J., HORELLOU M.H., LECOMPTE T.:
   "Physiologie et exploration de l'hémostase". Paris: Doin, 152-153, 1990.
9. BORG J.Y.:
   "Déficits constitutionnels en facteur de la coagulation en dehors de l'hémophilie" in "Manuel d'hémostase", J. Sampol, D. Arnoux, B. Boutière, Paris: Elsevier, 359-377, 1995.
10. "Etude des différents paramètres intervenant dans les variables préanalytiques (revue de la littérature)". Sang Thromb. Vaiss., **10**, 5-18, 1998.
11. NCCLS Document H3-A5:
    "Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard". Fifth edition, **23**, 32, 2003.
12. 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**, 5, 2008.

Significant changes are indicated by dotted lines in the margin.

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

 English