PTT Automate

Determination of Activated Partial Thromboplastin Time (APTT)

PTT Automate ⑤

- Kit Containing 12 x 5-ml Vials for Approx. 600 Tests

(REF 00480)

PTT Automate ⑩

- Kit Containing 12 x 10-ml Vials for Approx. 1200 Tests

(REF 00482) IVD CE

May 2014 *English 2*

1/ INTENDED USE

The **PTT Automate** kit is intended for the determination of the activated partial thromboplastin time (APTT) according to Langdell R.D. *et al.* (1) and Larrieu M.J., Weilland C. (3).

2/ SUMMARY AND EXPLANATION

- The activated partial thromboplastin time (APTT) is a general coagulation screening test of the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II and I).
- 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® - Deficient XII, REF 00722).

    ◊ If all these factors are normal, a deficiency in the following should be considered:
    - prekallikrein (Fletcher factor)
    - HMW kininogen (Fitzgerald factor).

  – Acquired Deficiencies and Abnormal Conditions
    ◊ Liver diseases
    ◊ Consumptive coagulopathy
    ◊ Circulating anticoagulants (antiprothrombinase or circulating anticoagulant against a factor)
    ◊ During heparin or oral anticoagulant therapy
    ◊ Treatment 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 particulate activator (silica).
The APTT explores the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II and I) except the platelets.

4/ KIT REAGENT

PTT Automate: reagent containing cephalin (2) prepared from rabbit cerebral tissues and a particulate ativator (silica) in a buffered medium, lyophilized.

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. These reagents are to be used only by certified medical laboratory personnel authorized by the laboratory.
Exercice 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.

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.). Use sample collection tubes made of plastic or siliconized glass. (In the USA follow CLSI guideline H3-A6).
  When monitoring heparin therapy, use preferably CTAD tubes specially designed sample collection tube to prevent heparin inactivation (6).
- Centrifugation: 15 minutes at 2,500 g. Collect the plasmas in plastic tubes.
- Plasma storage: 4 hours at 20 ± 5 °C (8).
  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**
  Reconstitute each vial of:
  – PTT Automate ⑤ (REF 00480) with 5 ml of distilled water
  – PTT Automate ⑩ (REF 00482) with 10 ml of distilled water.

  Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Then, swirl the reagent vial gently to obtain a homogeneous suspension.
- **Storage**
  The reagent in intact vials is stable until the expiration date indicated on the box label, when stored at 2-8 °C.
  Once reconstituted, it remains stable for:
  24 hours at 20 ± 5 °C
  7 days at 2-8 °C.
  Do not freeze.

8/ 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.
- Common clinical laboratory equipment and materials.

9/ PROCEDURE

The APTT of the plasma being studied is compared with several normal plasmas tested alone or as a pool (see "11/ Limitations" section). Follow the instrument manufacturer's instructions for APTT determination.
For instance:

In a glass test tube at 37 °C:	
• Undiluted plasma (reference, patient's or control)	0.1 ml
• PTT Automate well resuspended	0.1 ml
• Mix, incubate at 37 °C for exactly	3 min.
• Starting a stopwatch, add 0.025 M $CaCl_2$ prewarmed at 37 °C	0.1 ml
Mix. Note the clotting time (seconds).	

10/ RESULTS

Note the clotting time 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.

11/ LIMITATIONS

- The incubation time should be 3 minutes. In special cases, this time may be prolonged (maximum 10 minutes), as long as the control and the patient's plasma are treated in the same way.
- For reference use normal human plasmas. A normal plasma is defined as one collected from a healthy individual, either male or female, aged between 18 and 55, not taking any medication and giving blood voluntarily.
- 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.

  Whatever the type of heparin (unfractionated or low molecular weight heparin) and whatever the dose may be, it is recommended that frequent platelet counts be performed before and during treatment in order to detect any thrombocytopenia that may eventually be induced by the heparin (9). These heparin-induced thrombocytopenias (HIT) may be detected with the **Asserachrom® HPIA** kit (REF 00615) which allows the detection of the anti-heparin-platelet factor 4 antibodies present in the great majority of HIT.
  Depending on the clinical context, an antithrombin determination may be useful.

12/ REFERENCE INTERVAL

Normal values may vary depending on local conditions (type 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, 30 normal human plasmas have been tested with the PTT Automate on the KC 10 instrument. The observed mean time was 32.7 seconds with a standard deviation of 2.5 seconds.

The APTT is statistically lengthened in young subjects. By contrast, shortened times are obtained in older populations (4).

13/ PERFORMANCE CHARACTERISTICS

Different samples were used for the intra-assay and inter-assay reproducibility studies on the ST art®. Results obtained with PTT Automate are shown below:

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
n	24	24	10	10
$\bar{X}$ (s)	32.9	54.2	36.3	51.3
SD (s)	0.26	1.14	0.68	1.50
CV (%)	0.8	2.1	1.9	2.9

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Significant changes are indicated by dotted lines in the margin.

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