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Determination of Activated Partial Thromboplastin Time (APTT) on STA® Analyzers

Purpose

This procedure provides instructions for the activated partial thromboplastin time (APTT) testing with STA-R® Max and/or STA Compact® Max.

Principle

The **STA® - PTT A 5** kit provides reagent 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) with STA-R® and/or STA Compact®.

The APTT involves the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) and a particulate activator (silica).

The activated partial thromboplastin time (APTT) is a general coagulation screening test that uses clotting time to assess the integrity of the intrinsic pathway coagulation factors VIII (8), IX (9), XI (11), XII (12), and the common pathway coagulation factors V (5, proaccelerin), X (10, Stuart factor), II (2, prothrombin) and I (1, fibrinogen).

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

APTT typically prolonged more than PT (prothrombin time):	Both APTT and PT typically prolonged:
Congenital causes: • Factor VIII deficiency (hemophilia A) • Factor IX deficiency (hemophilia B) • Factor XI deficiency • Factor XI deficiency Acquired causes: • Anticoagulation therapy (heparin) or treatment with thrombin inhibitors (e.g., hirudin, argatroban, dabigatran, etc.). • Proteinuria – loss of factors XI and XII • Circulating inhibitor o lupus anticoagulant o specific inhibitor (e.g., factor VIII inhibitor)	• Liver diseases – most coagulation factors are made in the liver • Consumptive coagulopathy (e.g., DIC, disseminated intravascular coagulation) • Fibrinolysis

Scope

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

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Specimen sources

Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Follow CLSI guideline documents GP41 (10) and H21-A5 (11). 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
- Cuvette roll – 1000 (REF 38669)
- Centrifuge
- Deionized Water

Pipettes & tips

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

1) **STA® - PTT A 5:** (REF 00595) reagent containing cephalin (2) prepared from rabbit cerebral tissues and a particulate activator (silica) in a buffered medium, lyophilized.

   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.

   Preparation: Reconstitute each vial with 5 ml of deionized water. Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Mix vigorously by turning the vial upside down, 5-10 times, to obtain a homogeneous solution and install the perforated plastic cap on the vial.

   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, the reagent is stable:
   - 24 hours on STA-R® Max and/or STA Compact® Max with the perforated cap in place
   - 7 days at 2-8 °C in its original capped vial.
   - **Do not freeze the reagent.**

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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.

2) **STA® - $CaCl_2$ 0.025 M (REF 00367):** 0.025 M $CaCl_2$ solution.

Solution Preparation: If the solution is refrigerated, allow it to stand at room temperature (18-25 °C) for 30 minutes, before use. Do not install either an STA® - Reducer or a perforated cap on the solution bottle if it is to be used on analyzers of the STA® line.

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

Solution Storage: The solution in intact bottles is stable until the expiration date indicated on the box label, when stored at 2-25 °C. After opening, it remains stable for:

- 24 hours at 37 °C
- 3 days on STA-R® Max and/or STA Compact® Max

In case of partial use, the remaining solution stored at 2-8 °C in its original bottle with the cap on top, is stable, when free of any contamination, until the expiration date indicated on the bottle.

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

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

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.

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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).

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

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. 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.

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4) **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® 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.

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

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 and STA Compact® Max (see the Reference Manual).

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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. 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.

5) **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.

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

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

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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.

Reagents
1-3): ALL
4): Med Cntr
5): 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 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): 15 – 200 seconds
Clinical reportable range: 15 – 200 seconds

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Calculations

No calibration of the system is necessary for performing an APTT if the results are reported in seconds.

Reference Interval

APTT test orders at KPSC:

	Reference Range	Critical Value
APTT *(no anticoagulation)*	25 – 37 seconds*	>68 seconds
HEPARIN THERAPY, APTT	68 – 105 seconds	> 149 seconds

*The APTT is statistically lengthened in newborn babies. By contrast, shortened times can be observed in older populations.

Reporting Results

The APTT 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.

Additional review of the sample is recommended for the following:

- When the APTT result is close to the lower limit of the analytical range (e.g., 20 seconds), 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 APTT 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 the "Approved KP SCAL Coagulation Autoverification Rules, 05-2019 version", for details on regional autoverification rule settings.

Limitations

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.

Non-Controlled

The following non-controlled documents support this procedure:

Determination of Activated Partial Thromboplastin Time (APTT) on STA® Analyzers, Continued

Documents / 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.

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. "Etude des différents paramètres intervenant dans les variables préanalytiques (revue de la littérature)". Sang Thromb. Vaiss., 10, 5-18, 1998.

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

11. 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, May 2008.

12. STA® – PTT A 5 (REF 00595): Determination of Activated Partial Thromboplastin Time (APTT) Package insert for use in APTT determinations May 2019.

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13. STA® - CaCl2 (REF00367): 0.025 M CaCl2 solution for coagulation tests. Package insert May 2018.

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

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

16. 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