aPTT-SA (Soluble Activator)

Cat. No. 5387, 5388, 5389

INTENDED USE

For use in the determination of activated partial thromboplastin times and related coagulation procedures using dehydrated rabbit brain extract with ellagic acid as a soluble activator (SA). The test system can be used on manual, semi-automated and automated methods.

SUMMARY

The activated partial thromboplastin time (aPTT) is used to detect disorders in the intrinsic coagulation system, which involves coagulation factors VIII, IX, XI, XII, prekallikrein, and high molecular weight kininogen. The aPTT is also used in assays which quantitate these factors and in monitoring heparin therapy.[1,2] An activated partial thromboplastin time reagent was described by Proctor and Rapaport in 1961[3] that improved upon the partial thromboplastin time[4] by adding an activator (kaolin) to phospholipid, offering greater reproducibility and sensitivity. The aPTT is now routinely used for presurgical screening and monitoring of heparin therapy. Commercially available reagents typically use one of three activators: kaolin, silica, or ellagic acid. Ellagic acid has gained increasing popularity as an aPTT activator because it is generally soluble and thus easier to keep in homogeneous suspension on automated instruments. Ellagic acid based reagents, such as Helena's, typically require a shorter activation time (approximately 3 minutes vs. 5 minutes).

PRINCIPLES

In the basic screening test, the activated partial thromboplastin time indirectly measures the formation of thrombin by its action on fibrinogen forming the fibrin clot. In the test, citrated test plasma is mixed with aPTT reagent for a specified period of time (typically 3 minutes) at 37°C followed by the addition of pre-warmed (37°C) calcium chloride (0.025 M). Timing is begun from the time of addition of calcium chloride. The time required for clot formation is the activated partial thromboplastin time (aPTT). Clot detection can be by mechanical, manual (tilt-tube), or photo-optical (e.g., Cascade M-4) measurement.

REAGENTS

1. aPTT-SA Reagent

Ingredients: The reagent contains 0.1 mM Ellagic acid with a suspension of phospholipids extracted from dehydrated rabbit brain. Buffers, stabilizers, and preservatives have been added, including 0.2%phenol.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY

Preparation for Use: The reagent is ready for use as packaged. Gently invert the vial several times until a homogeneous suspension is obtained.

Storage and Stability: Helena aPTT-SA Reagent should be stored at 2 to 8°C when not in use and is stable until the date indicated on the vial. Avoid contamination of reagent by following appropriate laboratory cleanliness procedures. DO NOT FREEZE.

Signs of Deterioration: The reagent is normally a homogeneous pale green solution. A green wispy sediment may form upon standing that should dissipate easily upon mixing by inversion. Appearance other than described, or especially failure of normal plasma or controls to fall within established laboratory quality control ranges, may be indicative of product deterioration.

2. Calcium Chloride Reagent 0.025 M

Ingredients: The reagent is a 0.025 M solution of calcium chloride.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY

Preparation for Use: The reagent is ready for use as packaged.

Storage and Stability: The reagent should be stored at 2 to 8°C when not in use and is stable until the date indicated on the vial.

Signs of Deterioration: A turbid solution may be indicative of product deterioration.

INSTRUMENT

Any high quality electro-mechanical or photo-optical coagulation instrument designed for performing activated partial thromboplastin times may be used. The Helena Cascade® M or the Cascade® M-4 is recommended. The procedure may also be performed manually.

SPECIMEN COLLECTION AND HANDLING

Throughout the procedure for determination of activated partial thromboplastin times (aPTT) all test tubes, syringes and pipettes must be plastic or siliconized glass.

Specimen: Plasma obtained from whole blood collected with sodium citrate as an anticoagulant is the specimen of choice. The concentration of the sodium citrate should be 3.8% (0.129 M) or 3.2% (0.109 M).

Specimen Collection:

Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. According to NCCLS guidelines, blood specimens with hematocrits (HCT) of < 55% should be obtained by adding 9 parts of freshly collected whole blood to one part anticoagulant.9 For blood specimens with hematocrits > 55%, adjust the amount of whole blood added to the anticoagulant according to the following formula:[5]

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1-.\text{HCT})} \times 9$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

Specimen Preparation: Centrifuge the whole blood specimen at an appropriate rcf and length of time to obtain a platelet poor plasma. Specimens may be spun twice, if time permits. Immediately separate the plasma from the red blood cells and place it in a plastic test tube with cap.

Storage and Stability: Perform the activated partial thromboplastin time assay within 2 hours. Do not allow specimen to stand at 37°C for more than 5 minutes. The plasma sample should be stored in capped plastic test tubes at 2 to 8°C. If testing is delayed for more than 2 hours, plasma may be stored at -20°C or colder for up to one month. Thaw quickly at 37°C prior to testing but do not allow to stand at 37°C for more than 5 minutes.

Interfering Substances: Erroneous results may be caused by contamination with tissue fluids or stasis. Avoid agitation, air bubbles or foaming. For the effects of commonly administered drugs, refer to Young, et al.[6]

PROCEDURE

Materials Provided:

Item	Cat. No.
Helena aPTT-SA Reagent 5 x 10 mL aPTT-SA Reagent 5 x 10 mL 0.025 M Calcium Chloride Reagent	5389
Helena aPTT-SA Reagent 5 x 5 mL aPTT-SA Reagent 5 x 5 mL 0.025 M Calcium Chloride Reagent	5388
aPTT-SA Reagent (10 x 10 mL)	5387
0.025 M Calcium Chloride Reagent (10 x 10 mL)	5386

Materials and Equipment Required but not Provided:

	Cat. No.
Coagulation Instrument:	
Cascade M	1710
or	
Cascade M-4	1711
Pipettes to deliver 0.1 mL	
Control Plasmas:	
Helena Norm-Trol 1	5186
Ab-Trol 2	5187
Ab-Trol 3	5183
Centrifuge	
Materials for specimen collection (see above)	

STEP-BY-STEP METHOD

Throughout the procedure all test tubes, syringes and pipettes must be plastic or siliconized glass.

I. Manual and Electromechanical Method

1. Collect blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING SECTION.
2. Centrifuge the anticoagulated whole blood specimen at 1600-2000 x g for 10 minutes or equivalent force-time.
3. While the blood specimen is in the centrifuge, reconstitute the control plasma (i.e., Helena Norm-Trol 1, Ab-Trol 2 or Ab-Trol 3) according to the package insert included with the control.
4. Immediately after centrifugation, separate the plasma from the red blood cells and place in a plastic tube with cap at 2 to 8°C until assayed. The maximum storage time at 2 to 8°C is 2 hours.
5. Place an aliquot of 0.025 M Calcium Chloride Reagent into a test tube and prewarm to 37°C (requires approximately 5 minutes).
6. Pipette 0.1 mL of the patient plasma or control plasma into a reaction tube.
7. Gently mix the aPTT Reagent by inversion to resuspend any sediment.
8. Pipette 0.1 mL of aPTT Reagent into the reaction tube containing patient plasma or control plasma.
9. Incubate the aPTT Reagent and patient plasma at 37°C for EXACTLY 3 MINUTES.
10. Add 0.1 mL of prewarmed 0.025 M Calcium Chloride while simultaneously starting a timer.
11. If using the manual-tilt method, gently rock the reaction tube back and forth in the 37°C waterbath and continually look for clot formation. Immediately upon formation of a fibrin clot, stop the timer and record the test results.
12. Perform all tests in duplicate. If the difference in results of duplicate tests is greater than the allowable variance established in your laboratory (typically 5%), repeat the assay.

II. Automated Methods

If using the Cascade M-4 or other instrument to perform this test, refer to the appropriate Operator's Manual for instructions.

Quality Control: Each laboratory should establish a quality control program that includes normal and abnormal controls to evaluate instrument, reagent and technologist performance. The quality controls should be performed daily prior to performing tests on patient plasmas and with each change of personnel. Monthly quality control charts provided by Helena's Quality Assurance Review (QAR) program are recommended to determine the mean and standard deviation of each control. The Helena controls Norm-Trol 1 (Cat. No. 5186), Ab-Trol 2 (Cat. No. 5187) and Ab-Trol 3 (Cat. No. 5183) are recommended. If the controls do not perform as expected, patient results should be considered invalid.

RESULTS

The results of the aPTT test should be reported to the nearest 1/10 of a second. The normal range (usually $\bar{X} \pm 2$ Standard Deviations) for each individual laboratory should be established. Results greater than the upper limits of the normal range should be considered abnormal and follow-up testing should be performed. aPTT values less than the lower limits of the normal range should be repeated on a new blood sample. Short aPTT values may be seen in association with in-vivo thrombosis (i.e., deep vein thrombosis and disseminated intravascular coagulation).

REFERENCE VALUES

A reference range study was conducted for Helena Laboratories using frozen plasma samples from 27 normal adult donors. Results for Helena aPTT-SA run on the Cascade 480 with samples from blood collected into evacuated collection tubes containing 3.8% sodium citrate were as follows:

Range + 2 S.D. = 20.7 - 32.7 seconds

$\bar{X}$ = 26.7 seconds

These values should serve only as guidelines. Because

differences may exist among instruments, laboratories and local populations, it is recommended that each laboratory establish its own range of expected values.[10]

Heparin Monitoring

When monitoring heparin therapy, it is important to construct an in-vitro reference curve which reflects the average heparin response, since individual patients respond differently to heparin. In general, one can consider the therapeutic range for heparin to be 0.2 to 0.5 units/mL.[2,7] The following precautions should be considered when monitoring heparin therapy.

1. Time of collection is important, since heparin has an in-vivo halflife of only 1.5 hours.[8]
2. Base line data on the aPTT of each patient should be established before therapy in order to determine the respective patient aPTT as it relates to the normal range established in the testing laboratory.
3. Heparin response curves should be constructed using the same heparin employed in therapy to eliminate variables connected with heparins from different sources (e.g., porcine mucosa or bovine lung).
4. Heparin response curves should be reestablished when lot numbers of reagent change and at periodic intervals with the same lot number.

LIMITATIONS

Expected values for the aPTT test will vary from one laboratory to another, depending on the technique used. The method of clot detection, temperature, pH, collection technique, type of anticoagulant and time and method of specimen storage are all very important. Plasma sample collection and storage conditions should be standardized and carefully controlled. Unexpected results should be confirmed by additional tests. Thus, laboratories should establish their own expected values for patients and well defined performance standards for the control.

PERFORMANCE CHARACTERISTICS

I. PRECISION STUDIES

Results are shown below:

Within Run (aPTT in seconds) Precision studies were performed in replicate using three levels of control materials.

Control	Mean	S.D.	C.V.	N (pairs)
Norm-Trol 1	29.43	0.29	0.99%	20
Ab-Trol 2	51.64	0.60	1.16%	20
Ab-Trol 3	68.21	1.31	1.92%	20

Between Run (aPTT in seconds)

Precision studies were performed in replicate using three levels of control materials run on three different Cascade 480 instruments to evaluate between run variation.

Control	C.V.	N (pairs)
Norm-Trol 1	2.7%	60
Ab-Trol 2	2.8%	60
Ab-Trol 3	4.9%	60

II. COMPARISON STUDIES

A. Comparison of Helena aPTT-SA Reagent and a commercially available reagent was performed using blood samples from individuals with normal clotting activity. The following results were obtained:

Instrument	Reagent	N	mean aPTT (seconds) ± 2 S.D.	C.V.
Cascade 480	aPTT-SA	29	29.1 ± 5.1	8.7%
Cascade 480	Comparison	29	28.0 ± 4.9	8.7%

B. PATIENT TESTING COMPARISON

Comparison of Helena aPTT-SA Reagent and a commercially available reagent was performed for Helena Laboratories using blood samples with normal clotting activity and those with abnormal activities due to heparin therapy, factor deficiencies, or both. A total of 68 samples, measured in duplicate, were examined. Parameters of the linear regression equation (Y = Helena) were:

slope = 0.992
intercept = 1.92
correlation coeff. (r) = 0.978

C. FACTOR ASSAY LINEARITY

Factor assay standard curves (using the 4 point Helena method) were performed on the Cascade 480. Excellent linearity was obtained as indicated by the following log-log linear correlation coefficients(r):

Factor VIII: r = 0.9913 Factor IX: r = 0.9987
Factor XI: r = 0.9959 Factor XII: r = 0.9958.

BIBLIOGRAPHY

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4. Langdell, R.D. et al., Effect of Antihemophilic Factor on One-Stage Clotting Tests, J Lab Clin Med 41:637-647, 1953.
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8. Estes, J. and Poulin, P., Pharmacokinetics of Heparin, Throm Diath Haemorr 33:26-35, 1974.
9. NCCLS, Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, 2nd ed. - H21-A2, 1991.
10. NCCLS, How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory Proposed Guideline - C28P, 1992..

aPTT REAGENTS

Items	Cat. No.
Helena aPTT-SA Reagent	5389
5 x 10 mL aPTT-SA Reagent	
5 x 10 mL 0.025 M Calcium Chloride	
Helena aPTT-SA Reagent	5388
5 x 5 mL aPTT-SA Reagent	
5 x 5 mL 0.025 M Calcium Chloride Reagent	
aPTT-SA Reagent (10 x 10 mL)	5387
Calcium Chloride 0.025 M (10 x 10 mL)	5386
Equipment and Supplies	
Cascade M	1710
Cascade M-4	1711
Norm-Trol 1 Coagulation Control (10 x 1.0 mL)	5186
Ab-Trol 2 Coagulation Control (10 x 1.0 mL)	5187
Ab-Trol 3 Coagulation Control (10 x 1.0 mL)	5183
Factor VIII Deficient Substrate (10 x 1.0 mL)	5193
Factor IX Deficient Substrate (10 x 1.0 mL)	5194
Factor XI Deficient Substrate (10 x 1.0 mL)	5196
Factor XII Deficient Substrate (10 x 1.0 mL)	5197
Owrens Veronal Buffer (10 x 25 mL)	5375

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