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 solvent 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 kinogen. The aPTT is also used in assays which quantitate these factors and in monitoring heparin therapy. An activated partial thromboplastin time reagent was described by Proctor and Rapaport in 1961 that improved upon the partial thromboplastin time\(^1\) 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 (lift-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.

For Sales, Technical and Order Information and Service Assistance, call 800-231-5683 toll free.

Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement or repair at our option of any amount not to exceed the purchase price attributable to the goods as to which such claim is made. These alternatives shall be buyer’s exclusive remedies.

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In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised of the possibility of such damage.

The foregoing warranties are in lieu of all warranties expressed or implied including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.
**Specimen Preparation:** Centrifugate 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 within 2 hours. Do not allow 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:** Error 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.

**PROCEDURE**

**Materials Provided:**

- Helena aPTT-SA Reagent
- 5 x 10 mL 0.025 M Calcium Chloride Reagent
- 5 x 5 mL 0.025 M Calcium Chloride Reagent
- Helena aPTT-SA Reagent
- 5 x 5 mL 0.025 M Calcium Chloride Reagent
- aPTT-SA Reagent (10 x 10 mL)
- 0.025 M Calcium Chloride Reagent
- (10 x 10 mL)

**Materials and Equipment Required but not Provided:**

- (10 x 10 mL)
- Pipettes to deliver 0.1 mL
- Control Plasmas:
  - Helena Norm-Trol 1
  - Ab-Trol 2
  - Ab-Trol 3
  - Centrifuge

**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. Centrifugate 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 at 37°C to 39°C (requires approximately 5 minutes).
6. Pipette 0.1 mL of patient plasma or control plasma into a reaction tube.
7. Gently mix the aPTT Reagent by inversion to resuspend any sediment.
8. Incubate the 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 5 MINUTES.
10. Add 0.1 mL of prewarmed 0.025 M Calcium Chloride while simultaneously starting a timer.
11. If using the manual-tit method, gently rock the reac-
tion 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. **Automatic 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 if the test is performing within the 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 X ± 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 thrombocytopenia (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**
- **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.

**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.$^7$ The following precautions should be considered when monitoring heparin therapy:

1. Time of collection is important, since heparin has an in-vivo half-life of only 1.5 hours.
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 2.6% 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:

- **mean aPTT (seconds)**
  - Instrument
  - Reagent
  - ± 2 S.D.
  - C.V.
  - Cascade 480 aPTT-SA 29 25 ± 1.5 8.7%
  - Cascade 480 Comparison 29 28 ± 4.9 8.7%

**FACTORS 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 = 0.9967 Factor XI: r = 0.9959
**PROCEDURE**

**Materials Provided:**
- **Item**
  - Helena aPTT-SA Reagent
  - 5 x 10 mL 0.025 M Calcium Chloride Reagent
  - 5 x 10 mL aPTT-SA Reagent
  - Helena Norm-Trol 1
  - Cascade M-4
  - Cascade M

**Materials and Equipment Required but not Provided:**
- **Cat. No.**
  - 5389
  - 5388
  - 5387
  - 5396
  - 1710
  - 1711
  - 5186
  - 5187
  - 5183

**Laboratory Setup:**
- **Item**
  - Coagulation Instrument
  - Cascade M
  - Cascade M-4
  - Pipettes to deliver 0.1 mL
  - Control Plasmas: Helena Norm-Trol 1
  - Ab-Trol 2
  - Ab-Trol 3
  - Centrifuge

**STEP-BY-STEP METHOD**

**Preparation:**
- Collect blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING SECTION.
- Prepare 50 mL of blood for aPTT testing.

**Procedure:**
1. Time of collection is important, since heparin has an in-vivo half-life of only 1.5 hours.
2. Place red blood cells and 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 (aPTT) within 1 hour of collection. Do not allow 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.

**Quality Control:**
- Each laboratory should establish a quality control procedure that includes normal and abnormal controls to evaluate instrument, reagent and technologist performance. The control should be performed daily prior to performing tests on patient plasmas and with 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.

**Technical Assays:**
- The aPTT test should be reported to the nearest 1/10 of a second. The mean and C.V. values for the aPTT test are shown below:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Reagent</th>
<th>N</th>
<th>± 2 S.D.</th>
<th>C.V.</th>
<th>N (pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cascade 480</td>
<td>aPTT-SA</td>
<td>29.43</td>
<td>0.29</td>
<td>0.99</td>
<td>20</td>
</tr>
<tr>
<td>Ab-Trol 2</td>
<td></td>
<td>51.64</td>
<td>0.60</td>
<td>1.16</td>
<td>20</td>
</tr>
<tr>
<td>Ab-Trol 3</td>
<td></td>
<td>68.21</td>
<td>1.31</td>
<td>1.92</td>
<td>20</td>
</tr>
</tbody>
</table>

**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
- **X ± 5 minutes**

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.

**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.

**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. This variation among laboratories must be standardized and carefully controlled. Unexpected results should be confirmed by additional tests. This variation among laboratories must be established its own expected values for patients and well defined performance standards for the control.

**PERFORMANCE CHARACTERISTICS**

**I. PRECISION STUDIES**

**Results are shown below:**

**A. Precision studies were performed in replicate using three levels of control materials.**

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.</th>
<th>N (pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm-Trol 1</td>
<td>29.43</td>
<td>0.29</td>
<td>0.99</td>
<td>20</td>
</tr>
<tr>
<td>Ab-Trol 2</td>
<td>51.64</td>
<td>0.60</td>
<td>1.16</td>
<td>20</td>
</tr>
<tr>
<td>Ab-Trol 3</td>
<td>68.21</td>
<td>1.31</td>
<td>1.92</td>
<td>20</td>
</tr>
</tbody>
</table>

**Between Run (aPTT in seconds)**

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

<table>
<thead>
<tr>
<th>Control</th>
<th>C.V.</th>
<th>N (pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm-Trol 1</td>
<td>2.7%</td>
<td>60</td>
</tr>
<tr>
<td>Ab-Trol 2</td>
<td>2.8%</td>
<td>60</td>
</tr>
<tr>
<td>Ab-Trol 3</td>
<td>4.9%</td>
<td>60</td>
</tr>
</tbody>
</table>

**B. COMPARISON STUDIES**

**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**
aPTT REAGENTS

<table>
<thead>
<tr>
<th>Items</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helena aPTT-SA Reagent</td>
<td>5387</td>
</tr>
<tr>
<td>5 x 10 mL aPTT-SA Reagent</td>
<td>5387</td>
</tr>
<tr>
<td>5 x 10 mL 0.025 M Calcium Reagent</td>
<td>5386</td>
</tr>
<tr>
<td>Helena aPTT-SA Reagent</td>
<td>5388</td>
</tr>
<tr>
<td>5 x 5 mL aPTT-SA Reagent</td>
<td>5387</td>
</tr>
<tr>
<td>5 x 5 mL 0.025 M Calcium Reagent</td>
<td>5387</td>
</tr>
<tr>
<td>aPTT-SA Reagent (10 x 10 mL)</td>
<td>5387</td>
</tr>
<tr>
<td>Calcium Chloride 0.025 M (10 x 10 mL)</td>
<td>5387</td>
</tr>
</tbody>
</table>

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) 5283
- Factor VIII Deficient Substrate (10 x 1.0 mL) 5193
- Factor IX Deficient Substrate (10 x 1.0 mL) 5194
- Factor XII Deficient Substrate (10 x 1.0 mL) 5197
- Owens Veronal Buffer (10 x 25 mL) 5375

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 sole activating substrate (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, prekalikoerin, and high molecular weight kinogen. The aPTT is also used in assays which quantitate these factors and in monitoring heparin therapy. An activated partial thromboplastin time reagent was described by Proctor and Rapaport in 1961 that improved upon the partial thromboplastin time 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 (lift-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. The specimens must be handled in accordance with all applicable government regulations. Because of the potential for exposure to infectious agents, all equipment should be clean and dry, and should be exchanged between patients.

**Specimen:** Blood collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Raw specimens are not accepted. Using a high quality coagulation instrument, perform the activated partial thromboplastin time (APTT) test within 1 hour of collection.

**Preservation:** Whole blood collected with heparin anticoagulant, regardless of the anticoagulant, should be separated within 1 hour of collection.

**Storage:**Store specimens at 2 to 8°C if not analyzed immediately or prior to separation of serum or plasma.

Signs of Deterioration: The specimen should be discarded if not processed within 2 hours of collection or if separation is delayed.

**Ordering:** Specimen: aPTT

**Test:** aPTT

**Result:** The APTT result is the time in seconds from the initiation of the test.

**Reference Range:** The reference range is determined by the laboratory and is included in the final test report.