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| **Title:** Activated Partial Thromboplastin Time (APTT) - IL ACL TOP 350 |

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| **Author:** | **Effective Date:**  *Note: The Effective Date is assigned after all approval signatures are obtained* | **Supersedes Procedure #** |
| Sue Baker |  | New |

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| **Approval Signature** | **Approval Date** |
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**REVISION HISTORY**

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| **Procedure #** | **Revision Date** | **Reason for Revision** |
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# TITLE: Activated Partial Thromboplastin Time (APTT) - ACL TOP 350

1. Purpose
   1. This procedure provides instructions for the analysis of Activated Partial Thromboplastin Time (APTT) using HemosIL™ SynthASil or APTT-SP on ACL™ TOP 350. The test is a basic screening test used for the evaluation of the intrinsic coagulation pathway, including congenital and acquired deficiencies of the intrinsic pathway (factors XII, XI, IX, and VIII), APTT substitution test and the monitoring of heparin therapy.
2. Principle
   1. A plasma sample is incubated with an optimal quantity of phospholipids and a negatively charged contact activator which initiates the activation of the intrinsic coagulation pathway. Calcium is added after incubating at 37°C for a specific period of time, and the time required for clot formation is measured.
3. SCope
   1. To provide UR Medicine laboratory personnel with a guide to accurately and precisely measure activated partial thromboplastin time (APTT) on the IL ACL TOP 350 instruments at the Strong West Laboratory, 156 West Ave., Brockport, NY 14420.
4. RESPONSIBILITIES

| **Group/Person** | **Responsibility** |
| --- | --- |
| Quality Assurance | * Supports the development of this document |
| Medical Director | * Ensures that the procedure is followed. * Review and approval of this document. |
| Supervisor/Manager | * Ensures that the procedure is followed. * Review and approval of this document. |
| Technical Staff | * Follows the procedure. |

1. ACRONYMS/DEFINITIONS

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| UR | University of Rochester |
| SW | Strong West |
| HH | Highland Hospital |
| BR | Bailey Road Laboratory |
| SMH | Strong Memorial Hospital |
| aPTT | Activated Partial Thromboplastin Time |
| PT | Prothrombin Time |
| TT | Thrombin Time |
| IL | Instrumentation Laboratory |
| RPM | Revolutions per minute |
| RCF | Relative centrifugal force |

1. SPECIMENS
   1. Nine parts of freshly drawn venous blood are collected into one part 3.2% trisodium citrate. Refer to the most recent Clinical and Laboratory Standards Institute (CLSI) Document H21-A5 for further instructions on specimen collection, handling and storage. No other anticoagulant is acceptable.
   2. Centrifuge specimens for 12 minutes at 4000 rpm (RCF=3756g) or 2 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge). PTT may be processed by centrifuging specimens for 5 minutes at 4000 rpm (if testing is performed on fresh not frozen plasma.)
   3. Plasma Storage
      1. Uncentrifuged at 4 hours at 18-24°C
      2. Centrifuged 4 hours at 2-8°C or 18-24°C yields acceptable results
      3. 2 weeks at -20 ± 5ºC
      4. 6 months at -70 ± 5ºC
   4. Sample volume is CRITICAL to obtain accurate coagulation results. A 90% draw is the minimum volume acceptable for accurate testing. Do not run or report unacceptable samples. Place “.ND” in the result field with the canned text comment, “Sample volume inadequate, unable to perform required testing”. In Order Entry add the SPROB test by following procedure. See Documentation of Patient/Specimen” SH.CP.loe.0180 in the comment field and call for redraw. Refer to the minimum volume indicator on the tube. See the BD Tube draw volume guide.
   5. Elevated hematocrit specimens (specimens determined to have a hematocrit >55%) may require the preparation of a special collection tube. SW.CP.GL.jad.0016 for specific instructions on handling these samples.
   6. Clotted samples: Each specimen is checked visually for the presence of clots prior to analysis. If a clot is suspected, the tube is uncapped and checked with a pair of applicator sticks. *ANY* clot present in the specimen makes it inadequate for ALL coagulation testing. Clotted specimens are rejected and a new specimen should be requested.
   7. Plasma Coloration Interference (hemolysis, lipemia, icterus):Refer to TOP 350 SOP # for instructions on how to handle specimens with plasma coloration interference
2. QUALITY CONTROL
   1. Normal and abnormal controls are recommended for a complete quality control program.
   2. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing.
   3. Controls should be analyzed at least once every 8 hour shift of patient testing and with each reagent change in accordance with good laboratory practice.
   4. Refer to the instrument’s On-Line Help for additional information.
   5. Refer to Westgard et al for identification and resolution of out-of-control situations.
   6. QC data is transmitted to HemoHub. Remedial action for out of range values is documented in HemoHub.
      1. When a value is out of range, the operator should:
         1. Repeat the control using the same control material. If acceptable, document in HemoHub and continue running patient samples.
         2. If still not acceptable, reconstitute a new control and rerun. If acceptable document in HemoHub and continue running patient samples.
         3. If still not acceptable, reconstitute new reagent for the out-of-range test and rerun. If acceptable, document in HemoHub and continue running patient samples.
         4. If still not acceptable notify Hematology Supervisory personnel to begin maintenance and/or troubleshooting procedures as necessary.
         5. If supervisory personnel are not immediately available, set up backup analyzer for STAT/routine testing until issue is resolved.
         6. **FOR SPECIMENS RUN PRIOR TO THE OUT OF RANGE VALUES**, perform a lookback on the specimens prior to the out-of-range result.
            1. Rerun the five most recent specimens on the backup analyzer.

If no clinically significant difference is noted then no further action is necessary.

If clinically significant differences are noted, then continue to perform result look back until comparison is within acceptable bias, usually 10%. Correct all results not within acceptable bias limit.

1. SPECIAL SAFETY PRECAUTIONS
   1. All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC (Center for Disease Control) recommendations and in compliance with the Federal OSHA (Occupational Safety and Health Administration) Blood-borne Pathogen Standard, 29 CFR (Code of Federal Regulations) part 1910.1030. All animal products should be treated as potentially infectious. Avoid contact with skin and eyes. Do not empty into drains. Wear suitable protective clothing. Follow specimen handling as outlined by Laboratory Safety Policy (SW. CP.GLU.adm.0005).
   2. Wherever there are moving parts use caution with correcting malfunctions and when operating system.
   3. The Calcium Chloride in both kits, APTT-SP and SynthASil contains sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures. Please refer to the Material Safety Data Sheet for this product for more detailed safety information.
   4. For in vitro diagnostic use only.
   5. Disposal of all waste material should be in accordance with local guidelines.
2. MATERIALS
   1. Equipment
      1. IL ACL TOP 350 LAS Coagulation Analyzer (hereafter referred to as “TOP 350”)
      2. Centrifuge
   2. Supplies
      1. Pipettes and pipette tips
      2. CLSI CLRW Type (or equivalent) water
   3. Reagents
      1. HemosIL SynthASil Kit (PN 0020006800) contains:
         1. APTT Reagent: 5 x 10mL vials of a buffered synthetic phospholipid reagent containing a colloidal silica activator, stabilizers and a preservative.
         2. Calcium Chloride: 5 x 10mL vials of aqueous solution of calcium chloride (0.020mol/L) with preservative.
      2. The following are not supplied with the kit and those required may be purchased separately:
         1. HemosIL Cleaning Agent (PN 9832700)
         2. HemosIL Cleaning Solution (PN 9831704)
         3. HemosIL ACL TOP Rinse Solution (PN 20302400)
         4. Factor Diluent (PN 9757600)
   4. Controls
      1. HemosIL Normal Control, Assayed (PN 20003110)
      2. HemosIL Abnormal Control 3, Assayed (PN 20014100)
3. PROCEDURE – (STEP/ACTION)

**Note:** Please refer to the IL ACL TOP 350 onboard help manual for additional information on the procedures below.

* 1. Reagent/Control Preparation and Stability:
     1. SynthASil
        1. Each vial of APTT reagent must be equilibrated at 15-25˚C for at least 15 minutes and mixed thoroughly before use.
        2. Opened reagents are stable 30 days at 2-8°C in the original vial or 10 days (SynthASil) at 15°C on the ACL TOP® Family.

**Note: Do not freeze**.

* + 1. Calcium Chloride
       1. Is ready for use (Packaged with either reagent).
       2. Opened reagent is stable 30 days at 2-8°C.
    2. Cleaning Agent (Clean B Diluted)
       1. Dilute Cleaning Agent 1:8 with CLSI CLRW Type water or equivalent (2 mL Cleaning Agent: 14mL Diluent).
       2. Diluted Clean B is stable onboard for 24 hours.
    3. HemosIL Normal, Assayed and Abnormal 3, Assayed Controls
       1. Dissolve the contents of each vial with 1 mL of CLSI CLR Type water or equivalent.
       2. Replace the stopper and swirl gently.

**Note:** Make sure of complete reconstitution of the product.

* + - 1. Keep the reagent at 15-25˚C for 30 minutes and invert to mix before use.

**Note:** Do not shake to avoid foam formation.

* + - 1. Unopened controls are stable until the expiration date shown on the vial when stored at 2-8°C.
      2. Stability after reconstitution:
         1. 24 hours at 2-8˚C in the original vial
         2. 24 hours at 15-25°C in the original vial or onboard the TOP 350.
  1. Calibration: Calibration is not necessary for performing an APTT testing.
  2. Procedure:
     1. Make sure all maintenance has been done and is up to date.
     2. Place the APTT reagents (SynthASil and Calcium Chloride materials) in a Reagent Rack and load onto the TOP 350.
     3. Verify that QC for APTT is up to date. The **QC Results List** screen displays the results obtained from the *most recent* QC measurement and contains the following information for tests that have QC enabled and defined
        1. Test Code
        2. Material Name
        3. Last QC results
        4. Unit
        5. Last QC Job Status

If it is necessary to run QC, proceed with steps 4-9. If QC status is “OK”, skip to step 10.

4. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the TOP 350 analyzer in a Diluent track. (If running QC from the sample rack, refer to **Quality Control, Performing a QC Test** in the On-line Help Manual)

5. Choose **QC** from the Main Menu and select **Test Status List.**

6. Double click on a test code to reveal the **Test Materials** definition tree, making sure that the tree is organized by **Material/Tests.**

7. Select the box in front of the APTT QC Control and choose the **Program QC** icon. This will run all QC levels for that test.

8. Verify that all QC is in before running patient samples by viewing the QC **Test Status** list and validating the QC values in HemoHub.

9. If QC is out of range, the instrument will alert the operator by posting a “FAILED” result and with an audible beeping sound and a flashing red exclamation point on the **Alarm Status** bar. Investigate and repeat QC according to the laboratory protocol (See section VII).

10. Place sample tubes in a sample rack with barcodes facing outwards.

11. Select an available sample track and load the sample rack when the barcode reader is in position.

12. Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually using the **Rack Details** screen (refer to **Samples Analysis, Managing Patient Samples, Programming Bar Coded Samples, and Programming No-Bar Coded Samples** in the ACL TOP online help manual).

13. Choose the ***Run*** icon if the TOP 350 analyzer is not currently running to start processing the sample.

14. If the test has completed successfully the mean result for each sample is displayed. If the test completed, but the results failed, the work “FAILED” is displayed. IF the test failed, the operator will need to investigate and rerun or load reagents, if necessary.

15. If the result is:

**Purple and Bold** – result is outside the Test Range

**Red and Bold** – results is outside of Linear Range but within Test Range

**Orange and Bold** – result is out of Therapeutic Range

Blue and not bold – result is out of Normal Range, but within Linear Range

Black and not bold – result is within Normal Range

16. The results will auto-validate (indicated by a green V) if they are within the test range for APTT and there are no error codes associated with the result.

17. If the results do not auto-validate, the operator will need to investigate and address whatever issues are preventing the result from validating. IF any flags or alarms are present, refer to Online Help for details including viewing individual clot curves. IF the sample reruns and is still out, the operator may need to validate the result on the TOP 350 as well as in HemoHub and/or the LIS.

18. When a patient result exceeds the upper limit of the reportable range for APTT at URMC labs, the TOP 350 will automatically rerun the sample in extended mode. IF not error flags occur, both results will be sent to HemoHub to be validated by the operator.

1. LIMITATIONS
   1. APTT results may be affected by many commonly administered drugs. Further studies should be made to determine the source of unexpected abnormal results.
   2. Studies have shown SynthASil to be sensitive to decreased concentration of intrinsic factors resulting in an abnormal APTT value when factors VIII, IX, XI and XII levels were in the 35-60% range for SynthASil.
   3. APTT results on the IL ACL TOP® Family are not affected by these substances up to:

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| **Hemoglobin** | **Triglycerides** | **Bilirubin** |
| 500 mg/dL | 1000 mg/dL | 26 mg/dL |

1. CALCULATIONS
   1. Heparin Therapy
      1. Due to many variables (i.e. different source of heparin) which may affect the clotting times, each laboratory should establish its own unfractionated heparin (UFH) therapeutic range.
      2. The therapeutic APTT range should be checked and changed, if necessary, whenever there is a:
         1. Change of lot number of APTT reagent
         2. Change of type of heparin
         3. Change of instrument
         4. When requested by vendor
         5. As required for regulatory purposes

**Note:** For further information, see Beckman Coulter Hemostasis Performance Verification manual, Section 9, Dec. 2009.

1. INTERPRETATION
   1. Prolonged clotting times may be observed in the following situations:
      1. Deficiency of Factor XII, XI, X, IX, VIII, V, II fibrinogen
      2. Liver diseases
      3. Vitamin K deficiency
      4. Presence of heparin
      5. Lupus anticoagulant or other inhibitor
2. RESULT REPORTING

A. Reportable range (Test Range): APTT = 16.0 – 400.0 seconds. Also known as Test Range

**NOTE**: For reporting purposes within the URMC laboratory network, all APTT results that are greater than 200 seconds will be reported out by the LIS as “>200 seconds”.

* 1. Normal Reference Range: APTT = 25.8 – 37.9 seconds

1. PROFICIENCY TESTING
   1. Proficiency testing is performed on:
      1. College of American Pathologists (CAP) (5 samples) 3 times per year
      2. Other proficiency testing as applicable
2. TRAINING
   1. Staff is initially trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

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| **Role** | **Training Needed** |
| Management | Read the procedure. |
| Technical Staff | Read the procedure. |

1. REFERENCES
   1. HemosIL SynthASil (PN 0020006800) package insert
   2. ACL TOP® Family On-Line Help Manual.
   3. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition, CLSI Document H21-A5; Vol. 28, No. 5.
   4. Clinical and Laboratory Standards Institute. One Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline – Second Edition, CLSI Document H47-A2; Vol. 28, No. 20.
   5. UR.CP.GL.Coa.0001, ACL TOP 350 General Operation Procedure.
   6. Beckman Coulter Hemostasis Performance Verification manual, PN 722276BA, Dec. 2009.
   7. Lab Safety Manual. (SW. CP.GL.adm.0005)

**Training or Read/Review Signature Log**

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