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| **Document Title:** Prothrombin Time (PT/INR) - 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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| Xiaolan Ou MD, Medical Director | | | |  | | |
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| Mary Johnson, Chief Supervisor | | | |  | | |
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# REVISION HISTORY

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# TITLE: Prothrombin Time (PT/INR) – ACL TOP 350

1. Purpose
   1. This procedure provides instructions for the analysis of Prothrombin Time (PT) using a thromboplastin reagent on the ACL TOP® Family. The test is used for the evaluation of the extrinsic coagulation pathway and the monitoring of oral anticoagulant therapy (OAT).
2. PRINCIPLE
   1. The addition of tissue thromboplastin and calcium ions (PT reagent) to the patient plasma initiates the activation of the extrinsic pathway. This results in the conversion of fibrinogen to fibrin, with formation of a solid gel. The time required for clot formation is measured.
   2. The prothrombin time is used to monitor warfarin therapy because of its sensitivity to variations in the concentration of the Vitamin-K dependent factors II, VII and X. Because of the variations in the prothrombin time results with different thromboplastins and instruments, it is recommended that the prothrombin time results be converted to an INR (International Normalized Ratio). The INR corresponds to the value of the ratio of the patient’s PT and the geometric mean PT of the normal reference population raised to the ISI (International Sensitivity Index) power:



* 1. The ISI value of a given thromboplastin is determined by performing PT’s on normal plasmas and Coumadin-treated patient plasmas with the given thromboplastin and the WHO reference thromboplastin. The slope of this regression curve of the matched pairs is the ISI for the thromboplastin.

1. SCOPE
   1. To provide UR laboratory personnel with a guide to accurately and precisely measure prothrombin time and INR levels on the ACL TOP 350 (TOP 350) instruments at the Strong West Laboratory, 156 West Ave., Brockport, NY 14420.
2. 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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| --- | --- |
| URMC | University of Rochester Medical Center |
| SW | Strong West Laboratory |
| BR | Bailey Road Laboratory |
| SMH | Strong Memorial Hospital |
| CLSI | Clinical and Laboratory Standards Institute |
| PTT | Partial Prothrombin Time |
| TT | Thrombin Time |
| PT | Prothrombin Time |
| INR | International Normalized Ratio |
| ISI | International Sensitivity Index |
| QC | Quality Control |
| LIS | Laboratory Information System |

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. Frozen samples: Thaw frozen specimens rapidly at 37°C and centrifuge plasma before testing. After thawing the assay must be performed within 2 hours.
   3. Samples for PT/INR and PTT testing may be processed by centrifuging specimens for 12 minutes at 4000 rpm (RCF = 3756g), or 3 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge.)
   4. Plasma Storage
      1. 24 hour 18-24 °C (room temp)
      2. 2 weeks at -20 ± 5º C
      3. 6 months at -70 ± 5º C
   5. 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 these 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 Problem test in the Laboratory Information System SH.CP.SM.loe.0180. Refer to the minimum volume indicator on the tube, see the BD Tube draw volume guide.
   6. Elevated hematocrit specimens (specimens determined to have a hematocrit >55%) may require the preparation of a special collection tube. See SW.CP.GL.jad.0016 for specific instructions on handling these samples.

G. 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.

H. Plasma Coloration Interference (hemolysis, lipemia, and icterus): Refer to TOP 350 SOP # section\_\_\_\_\_ for instructions on how to handle specimens with plasma coloration interference.

1. **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 both levels of control. 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. Refer to QC procedures SW.CP.GL.adm.0002
         7. **FOR SPECIMENS RUN PRIOR TO THE OUT OF RANGE VALUE:**
            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.GL.adm.0005).
   2. Wherever there are moving parts use caution with correcting malfunctions and when operating system.
   3. RecombiPlasTin2G contain sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures.
   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. 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. RecombiPlasTin 2G contains:
         1. RecombiPlasTin 2G: 5 x 20mL vials of solution of a recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer.
         2. RecombiPlastin Diluent: 5 x 20mL vials of an aqueous solution of calcium chloride, polybrene and a preservative.
      2. The following are not supplied with the kit and those required may be purchased separately:
         1. HemosIL Calibration Plasma (PN 20003700)
         2. HemosIL Factor Diluent (PN 9757600)
         3. HemosIL Cleaning Agent (PN 9832700)
         4. HemosIL Cleaning Solution (PN 9831704)
         5. HemosIL ACL TOP Rinse Solution (PN 20302400)
   4. Controls
      1. HemosIL Normal Control, Assayed (PN 2003110)
      2. HemosIL Abnormal Control 3, Assayed (PN 20014100)
3. PROCEDURE – (STEP/ACTION)

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

**Note**: Do not freeze.

**Note**: Make sure all maintenance has been done and is up to date.

* 1. Reagent/Control Preparation and Stability:
     1. RecombiPlastin 2G
        1. Allow each vial of and RecombiPlastin 2G Diluent to equilibrate at 15-25⁰C for at least 15 minutes before reconstituting with the diluent.
        2. Pipette exactly 20mL of the diluent into the vial of lyophilized reagent.

Note: Do not pour the contents of the diluent vial into the vial of RecombiPlastin 2G

* + - 1. Following reconstitution, replace the stopper and swirl gently to mix. Keep the reagent at 15-25°C for 15-20 minutes and invert to mix before use.
      2. Load reagent onto instrument.
    1. Unopened reagent is stable until the expiration date shown on the vial when stored at 2-8°C.
    2. Stability after reconstitution
       1. 10 days at 2-8˚C in the closed original vial.
       2. 5 days at 15-25°C in the original vial.
       3. 10 days at 15°C on the ACL TOP® Family in the original vial with no stirring.
    3. Cleaning Agent (Clean B Diluted): Dilute Cleaning Agent 1:8 with CLSI CLRW Type water or equivalent (2 mL Cleaning Agent: 14mL Diluent). Reconstituted Clean B is stable onboard for 24 hours.
    4. HemosIL Normal Control, Assayed and HemosIL Abnormal 3, Assayed Control
       1. Dissolve the contents of each vial with 1 mL of CLSI CLR Type water or equivalent.
       2. Replace the stopper and swirl gently. Make sure of complete reconstitution of the product.
       3. Keep the reagent at 15-25˚C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.
    5. Unopened controls are stable until the expiration date shown on the vial when stored at 2-8°C. Stability after reconstitution: 24 hours at 2-8˚C in the original vial, 24 hours at 15-25°C in the original vial or onboard the TOP 350.
    6. For optimal stability remove reagent from the system and store it at 2-8°C in the original vial.
  1. Calibration
     1. Calibration is not required for units of seconds or for INR.
     2. However, INR calculation must be set up for each lot number. If the INR calculation is not properly set up, erroneous patient results may be reported (see Section XI, Calculations). This process must be repeated with every new lot of PT reagent. For more details on setting up a new lot of PT reagent see section XV, Lot Conversion/INR Calculation.

C. Procedure

1. Make sure all maintenance has been done and is up to date.

2. Load RecombiPlasTin2G reagent and Clean B Diluted onto the TOP 350 analyzer using a reagent rack ® (Clean B Diluted should be loaded on the same rack as any thrombin-containing reagents)

3. Verify that QC for PT 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:

a. Test Code

b. Material Name

c. Last QC results

d. Unit

e. Last QC Job status

**NOTE:** this field displays the status of the last QC test.

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. (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 the test code to reveal the **Test Materials** Definition tree, making sure that the tree is organized by **Material/Tests**.

7. Select the box in from of the Prothrombin 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 as well as verifying the results in HemoHub.

9. If QC is out of range, the analyzer will alert the operator by posting a “FAILED” result and with an audible beeping sound and flashing red exclamation point in the **Alarm Status** bar. Investigate and repeat QC according to 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 Non-Barcoded Samples** in the SCL TOP 350 on-line help manual)

13. Choose the **Run** icon in 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 result 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 PT 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 on the LIS.

18. When a patient results exceeds the upper limit of the reportable range for URM labs (>100.0 sec), the TOP 350 will automatically rerun the sample. If no error flags occur, both results will be sent to the LIS to be validated by the operator.

19. Similarly, when a patient results exceeds the critical INR limit for URMC labs (>5.0), the TOP 350 will automatically rerun the sample. Any specimen that has an INR critical value of ≥5.0 should be physically checked for a clot prior to the reporting and calling of results. IF no error flags occur on the rerun, both results will be sent to the LIS to be validated by the operator.

20. It will be necessary for the operator to release the critical result from the LIS and document the follow-up for the critical value accordingly.

1. LIMITATIONS
   1. PT results may be affected by many commonly administered drugs. Further studies should be made to determine the source of unexpected abnormal results.
   2. Prothrombin Time (PT) results on the ACL TOP® Family are not affected by these substances up to:

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| --- | --- | --- | --- |
| **Heparin** | **Hemoglobin** | **Triglycerides** | **Bilirubin** |
| 1 U/mL | 500 mg/dL | 1000 mg/dL | 30 mg/dL |

1. CALCULATIONS
   1. An internal INR calculation check is performed for each PT result in the LIS. The PT test is a profile consisting of the PT in seconds and the INR calculated and sent from the instrument as well as 2 other tests – INRC and INRD. The LIS uses a calculation hook to determine the INR based on the PT sent from the instrument and the ISI and normal range and this result becomes the INRC. A second calculation is then performed comparing the INR sent from the instrument to the INR calculated based on the information in the LIS. A % Delta result becomes the INRD. If there is a > 0.2 difference between these two results it is noted on the next printed EXCEPTION REPORT. It can also be seen when bridging to the worksheet in the interface. It is strongly suggested that this be done several times each shift in order that more timely corrective action may be performed if necessary.
2. INTERPRETATION
   1. These products are particularly suited to the monitoring of oral anticoagulant therapy and factors of the extrinsic pathway.
3. RESULT REPORTING
   1. Reportable range: PT = 8.0 – 320.0 sec (also known as Test Range)

**NOTE**: For reporting purposes within the URMC laboratory network, all PT results that are greater than 100 seconds will be reported out by the LIS as “>100 seconds”. Similarly, if the INR exceeds the maximum URMC reported upper limit (see current lot-dependent upper limit), the INR result will be reported out as “>” whatever the current upper limit is.

* 1. Normal Reference Range: PT = 10.0 – 12.9 sec
  2. Normal Reference Range: INR = 0.9-1.1
  3. Therapeutic Range: INR = 2.0 – 3.0
  4. Critical Results: INR ≥ 5.0
  5. Prothrombin Time results may be reported in seconds, % activity, ratio and/or INR. If any flags or alarms are present, refer to the ACL TOP® Family On-Line Help Manual.In the case of a critically high INR (>5.0 ), the TOP 350 will automatically rerun the sample and send it to HemoHub, but it will be necessary for the operator to release it from HemoHub, the LIS and document the follow up for the critical value accordingly.
  6. **Any specimen that has an INR critical value of ≥ 5.0 should be physically checked for a clot prior to the reporting and calling of results.**

1. PROFICIENCY TESTING
   1. Proficiency testing is performed on:
      1. College of American Pathologists (CAP) (5 specimens) 3 times per year
      2. Other proficiency testing as applicable
2. LOT CONVERSION/INR CALCULATION:
   1. INR calculation procedure is performed under the following conditions:
      1. At the installation of a new analyzer
      2. With a change in the thromboplastin (RecombiPlasTin) lot number
      3. At the request of an IL Customer representative
      4. To follow the requirements of the appropriate regulatory agency
   2. Each new lot of RecombiPlasTin will have a unique ISI value and the laboratory must establish the lot specific mean of the normal reference interval (mean normal PT), which must be used as indicated below:
      1. INR = (PT Patient/Mean Normal PT)ISI
   3. Manual setup to obtain INR results for each new lot of PT reagent includes:
      1. Enable ISI value:
         1. Remove the PT reagent if it is currently on the analyzer.
         2. Choose **Setup, Material List.** Double –click on the name of the PT reagent in use to open the Materials Definition screen.
         3. Select **Enable Lot Management**, then the **Lot Specific Information** tab.
         4. Enter the lot number and expiration date of the RecombiPlasTin reagent in use.
         5. Select the **ISI value** box and enter the ISI value from the appropriate PT reagent (RecombiPlasTin) package insert.
         6. Select the **Save** icon to store the ISI value and the other changes that have been made.
      2. Verify/update (geometric) mean normal range:
         1. Choose **Setup, Material List**. Double-click on the name of the PT test in use to open the Test Definitions screen.
         2. Select **Normal Pool Plasma** from the navigational tree.
         3. Select **User Input Value** from the NPP mode dropdown list to activate the NPP field, if it is not already activated.
         4. Enter the geometric mean (in seconds) of the normal reference interval established for the new lot of RecombiPlasTin (thromboplastin) in use.
         5. Select the **Save** icon to store the changes made to the PT Test Definition screen.
3. 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 RecombiPlasTin (PN 0020003050) 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. ACL TOP 350 General Operations SOP, UR.CP.GL.Coa.0001
   6. Beckman Coulter Hemostasis Performance Verification manual, PN 722276BA, Dec. 2009.
   7. Laboratory Safety Policy (SW.CP.GL.adm.0005)

**Training or Read/Review Signature Log**

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| Brief Description: (i.e. Revised) |
| Trainer(s):  (if applicable, or NA) |

***Your signature below indicates that you have read/been trained and understood the information.***

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