Document Title: Prothrombin Time (PT/INR) on the ACL TOP 500

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Procedure #	Revision Date	Reason for Revision
Coa.0040.0002	1/27/17	Update Reference ranges; change name of controls; add centrifuge speed; update quality control section; add “FAILED” results to Limitations; remove NYS and update Proficiency Testing section

TITLE: Prothrombin Time (PT/INR) on the ACL TOP 500

I. PURPOSE

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).

II. PRINCIPLE

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.

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:

$$\text{INR} = \left(\frac{Patient's\ PT}{Geometric\ Mean\ PT} \right)^{\text{ISI}}$$

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.

III. SCOPE

To provide Hematology-Chemistry Laboratory personnel with a guide to accurately and precisely measure activated partial thromboplastin time (APTT) on the ACL TOP 500 instruments at UR Medicine Labs at Strong Memorial Hospital, 601 Elmwood Avenue, Rochester, NY 14642.

IV. 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	• Ensures that the procedure is followed. • Review and approval of this document.
End User/Staff	• Follows the procedure.

V. SPECIMENS

A. Nine parts of freshly drawn venous blood are collected into one part 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.

B. Unacceptable samples: Grossly hemolyzed samples, clotted samples, short draw samples

C. Frozen samples: Thaw frozen specimens rapidly at 37°C and centrifuge plasma before testing. After thawing the assay must be performed within 2 hours.

D. Centrifugation: Centrifuge specimens for 12 minutes at 4000 rpm (RCF = 3756g) or 2 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge. PT samples may be processed by centrifuging specimens for 7 minutes at 4000 r.p.m., if no specialized coagulation testing is needed.

E. Plasma Storage: 24 hours at 20 ± 5°C
24 hours at 2-8°C yields acceptable results

F. 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 performed required testing" In Order Entry add the SPROB test by following procedure. See Documentation of Patient/Specimen SH.CP.SM.loe.0180.0005.in the comment field and call for redraw. Refer to the Minimum volume indicator on the tube, see the BD Tube draw volume guide.

G. Elevated hematocrit specimens (specimens determined to have a hematocrit >55%) may require the preparation of a special collection tube: Refer to

General Operating Procedure for the ACL TOP 500, Section IV (SH.CP.AU.coa.0036 or see SH.CP.AU.jad.0136).

H. 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 coag testing. Clotted specimens are rejected and a new specimen should be requested.

I. Hemolyzed samples: Grossly hemolyzed samples should be rejected. If sample has been centrifuged and hemolysis is present, check for a clot. If a clot is present, follow procedure for reporting clotted samples.

VI. QUALITY CONTROL

Normal and abnormal controls are recommended for a complete quality control program. HemosIL Controls for Prothrombin Time are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift and with reagent change in accordance with good laboratory practice. Refer to Westgard *et al* for identification and resolution of out-of-control situations.

QC data is transmitted to the LIS. Remedial action for out-of range values is documented in the LIS and on the QC log for that instrument. When a value is out of range, the operator should:

A. Repeat the control using the same control material – if acceptable document in LIS.
B. If still not acceptable reconstitute a new control and rerun – if acceptable document in LIS.
C. If still not acceptable, run the Enhanced Probe Clean procedure and rerun the same well mixed control and well mixed reagent. If acceptable, document and continue processing samples.
D. If still not acceptable, reconstitute/use new reagent and rerun. If acceptable, a lookback of the last five samples analyzed (or an appropriate number of samples) for that assay on that analyzer should be done. These samples should be from the time when the last reagent vial was in use and the last QC point was in range, in accordance with the Quality Control Lookback Policy SH.CP.AU.gen.0002. If the samples do not agree, continue to look back at samples analyzed on that analyzer since the last successful QC run. Notify supervisor or technical specialist.
E. In the case of any if >4.0 SD QC failure (R1:XS in Soft QC), rerun the QC and evaluate according to Quality Control Policy SH.CP.AU.gen.0001. Perform a lookback as necessary according to the aforementioned policy if any patient samples were analyzed.
F. If still not acceptable, document and notify Hematology Supervisor or a Tech Specialist. Use backup analyzer for STAT/routine testing. Call service for further guidance and troubleshooting help.

VII. SPECIAL SAFETY PRECAUTIONS

A. 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, (SH.CP.AU.gen.0005).

B. Wherever there are moving parts use caution with correcting malfunctions and when operating system.

C. HemosIL RecombiPlasTin 2G and HemosIL PT-Fibrinogen HS PLUS buffer contain sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures

D. For in vitro diagnostic use only.

E. Disposal of all waste material should be in accordance with local guidelines.

VIII. MATERIALS

A. Equipment
1. ACL TOP 500 CTS Coagulation Analyzer (hereafter referred to as "TOP 500")
2. Centrifuge

B. Supplies
1. Pipettes and pipette tips
2. CLSI CLRW Type (or equivalent) water

C. Reagents

1. HemosIL RecombiPlasTin 2G (PN 0020002950 (8 mL) or 0020003050 (20 mL)) contains:
RecombiPlasTin 2G (RTF): 5 x 8 or 20 mL vials of lyophilized recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer.
RecombiPlasTin 2G Diluent (RTF Diluent): 5 x 8 or 20 mL 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:

HemosIL Calibration Plasma	PN 20003700
HemosIL Factor Diluent	PN 9757600
HemosIL Cleaning Agent	PN 9832700
HemosIL Cleaning Solution	PN 9831704
HemosIL ACL TOP Rinse Solution	PN 20302400

D. Controls

1. HemosIL Normal Control Assayed PN 20003110
2. HemosIL High Abnormal Control, Assayed PN 20003310

IX. PROCEDURE – (STEP/ACTION)

Note: Please refer to the ACL TOP 500 onboard help manual for additional information on the procedures below.

A. Reagent/Control Preparation and Stability:

1. RecombiPlasTin 2G: Allow each vial of reagent and diluent to equilibrate at 15-25˚C for at least 15 minutes before reconstitution. Pipette the exact amount required (either 8 mL or 20 mL) of diluent into the vial of reagent. **DO NOT POUR** the contents of the diluent vial into the vial of RecombiPlasTin 2G. Replace the stopper and swirl gently. Let sit for 15 to 20 minutes at 15-25˚C and invert to mix before use.

   Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C. Stability after reconstitution: 10 days at 2-8˚C, 10 days at 15°C on the ACL TOP® Family, 5 days at 15-25°C in the original vial. No stir bar is needed. For optimal stability remove reagent from the system and store it at 2-8°C in the original vial.

2. Cleaning Agent (Clean B Diluted): Dilute Cleaning Agent 1:8 with CLSI CLRW Type water or equivalent. Reconstituted Clean B is stable onboard for 24 hours.

3. HemosIL Normal Control, Assayed and HemosIL High Abnormal Control, Assayed: Dissolve the contents of each vial with 1 mL of CLSI CLR Type water or equivalent. Replace the stopper and swirl gently. Make sure of complete reconstitution of the product. Keep the reagent at 15-25˚C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.

   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 500.

For optimal stability remove reagent from the system and store it at 2-8°C in the original vial.

B. Calibration: Calibration is not required for seconds or INR. However, INR calculation must be set up for each lot number. If the INR calculation is not properly set up, then 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 RecombiPlasTin 2G reagent and Clean B Diluted onto the TOP 500 instrument using a reagent rack (R) (Clean B Diluted should be loaded on the same rack as any thrombin-containing reagents such as QFA thrombin or Recombiplastin).
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 500 instrument in a Diluent track. (If running the 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 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 and validating the QC values on the Instrument Menu in the LIS (SOFT GUI).
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 laboratory protocol (see section VI).
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-Bar Coded Samples** in the ACL TOP on-line help manual).
13. Choose the ***Run*** icon if the TOP 500 instrument is not currently running to start processing the sample, if necessary.
14. If the test has completed successfully, the mean result for each sample is displayed. If the test completed, but the results failed, the word "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 – result is outside of Linear Range but within Test Range
    Orange and bold – result is out of Therapeutic Range
    Blue and unbolded – result is out of Normal Range but within Linear Range
    Black and unbolded – result is within Normal Range
16. The results will autovalidate (indicated by a green V) if they are within the test range for PT (8.0 - 320 sec) and there are no error codes associated with the result.
17. If the results do not autovalidate, 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 500 as well as on the LIS.
18. When a patient result exceeds the upper limit of the reportable range for URMC labs (> 100.0 sec), the TOP 500 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 result exceeds the critical INR limit for URMC labs (≥ 5.0), the TOP 500 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.

X. LIMITATIONS

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

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

NOTE: See jad.0138 ACL TOP Exceptions and Limitations for processing samples with plasma interference issues

XI. CALCULATIONS

NOTE: 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 as INRCK test. It is strongly suggested that this be done several times each shift in order that more timely corrective action may be performed if necessary.

XII. INTERPRETATION

These products are particularly suited to the monitoring of oral anticoagulant therapy and factors of the extrinsic pathway.

XIII. RESULT REPORTING

A. 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.

B. Normal Reference Range: PT = 10.0-12.9 sec
INR = 0.9 - 1.1

C. Therapeutic Range: INR = 2.0 – 3.0

D. Critical Results: INR ≥ 5.0

E. 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 500 will automatically rerun the sample and send it to the LIS, but it will be necessary for the operator to release it from the LIS and document the follow up for the critical value accordingly.

F. **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.**

G. **FAILED Results:** Refer to SH.CP.AU.jad.0137, ACL TOP 500 Clot Curve Interpretation

XIV. PROFICIENCY TESTING

Proficiency testing is performed a minimum of three times per year.
Refer to SH.CP.AU.gen.0010, Proficiency Testing.

XV. LOT CONVERSION/INR CALCULATION:

NOTE: See SH.CP.AU.coa.0057 Reagent Lot Conversion for more details.
INR calculation procedure is performed under the following conditions:

- At the installation of a new analyzer
- With a change in the thromboplastin (RecombiPlasTin) lot number
- At the request of an IL Customer representative
- To follow the requirements of the appropriate regulatory agency

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:

$$INR = (PT\ Patient/Mean\ Normal\ PT)^{ISI}$$

Setup to obtain INR results for each new lot of PT reagent includes:

A. 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.

B. 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.

XVI. TRAINING

Staff are trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

XVII. REFERENCES

A. HemosIL RecombiPlasTin 2G (PN 0020002950/0020003050) package insert.

B. ACL TOP® Family On-Line Help Manual.

C. 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.

D. 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.

E. SH.CP.AU.coa.0036 – General Operation of the ACL TOP 500 CTS

F. Beckman Coulter Hemostasis Performance Verification manual, PN 722276BA, Dec. 2009.

Prothrombin Time (PT/INR) on the ACL TOP 500

Knowledge Check

In the event of a question answered incorrectly: Single-line through the incorrect answer, initial & date, then select the correct answer.

ALWAYS HAVE CHANGES INITIALED BY YOUR TRAINER.

Circle True or False for each of the following statements.

1. True or False — PT samples may be processed by centrifuging specimens for 7 minutes at 4000 r.p.m., if no specialized coagulation testing is needed.
2. True or False — When reconstituting Recombiplastin 2G, the contents of the diluent vial should be poured into the vial of RecombiPlasTin 2G.
3. True or False — Patient plasma is stable for PT for 48 hours at 2-8°C.
4. True or False — The current reference range for PT is 10.0-12.9 seconds.
5. True or False — In most cases, the first step an operator should take when QC fails is to repeat the control using new PT reagent.

Any incorrect answers I may have initially written have been discussed and corrected. I now understand the answers I may have gotten wrong.

PASSING GRADE IS 75% OR GREATER

_____
Employee name (print)

_____ _____
Employee signature **(Date)**

_____
Supervisor/Manager name (print)

_____ _____
Supervisor/Manager signature **(Date)**