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| **Prothrombin Time (PT) on ACLTOP 350 Analyzer** | *Procedure #:* | ***HCO# 210*** |
| *Version #:* | ***1.0*** |

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| **Purpose** | This procedure provides instructions for the analysis of Prothrombin Time (PT) using a thromboplastin reagent on the ACL TOP® Family.  |
| **Principle/ Clinical Significance** | 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 through turbidimetric. Turbidimetric clot detection is based on the principle that light passing through a medium in which fibrinogen is converted to fibrin will be absorbed by the fibrin strands.The test is used for the evaluation of the extrinsic coagulation pathway and the monitoring of oral anticoagulant therapy (OAT) in human plasma.  |
| **Scope** | This standard operating procedure applies to all laboratory technicians, technologists and supervisory personnel of the Baltimore VA Medical Center Pathology & Laboratory Medicine Service |
| **Responsibilities** |

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| **Responsible Party** | **Responsibilities** |
| Hematology Supervisor | * review this procedure annually and make any necessary revisions in a timely manner
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| Medical Director | * review all new or substantially revised procedures, before implementation
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| Staff | * read this procedure in its entirety and ask any questions before implementation
* govern yourself according to the contents of this procedure after implementation
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| **Safety Precautions** | Standard Precautions:* Gloves
* Fluid resistant laboratory coat.
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| **Specimen Requirements** | **Specimen Collection*** Collect 9 parts fresh venous whole blood to 1 part 3.2% **sodium citrate** anticoagulant. Citrated blood containing 0.5 ml of 3.2% **sodium citrate**.
* Centrifuge sample ONLY in a designated Coagulation centrifuge at the indicated speed and time which is known to produce platelet poor plasma.
* Blue top (3.2% citrate) tubes must be completely filled. Tubes under filled by more than 5% (<4m1 of whole blood) are unacceptable for analysis.

**Specimen Stability*** Specimens are stable for 24 hours after collection, provided the cap has not been removed.
* Open tubes are stored at ROOM TEMP (18-25°C) or REFRIGERATED (2-8°C) for up to 24 hours.
* Frozen specimens are stored FROZEN (-70°C) and good for 6 hours.
* Thaw frozen plasma specimens at 37°C (using the Blood Bank water bath) for approximately 15 minutes then re-centrifuge.
* After thawing, each sample should be gently mixed and tested within 2 hours.

**Specimen Processing*** Before centrifugation, check the whole blood sample for gross clot formation by gentle inversion and observation. *This is the preferred method for detecting clots when using analyzers with cap piercers*
* Centrifuge sample in a STAT SPIN EXPRESS at 3000 RPM for 180 seconds or on the Jouan CR3i centrifuge at 3900 RPM for 15 minutes during the dayshift.
* During the evening and night shifts, specimens run in the STAT LAB are spun in the EBA 21 centrifuge for 8 minutes at 5000 RPM.
* **Clotted** or **grossly** hemolyzed samples are unacceptable for analysis.
* Mildly lipemic samples may be initially run. If analyzer is unable to detect clot ("optics I" or "optics 2" error message) the remaining plasma should be airfuged (see procedure in Troubleshooting" section) and retested.
* Grossly lipemic (milky or white) plasma must be airfuged prior to testing.

**Plasma separation:*** During collection and centrifugation of the sample, hemolysis must be avoided. The breakage of red cells, whose phospholipid surfaces have thromboplastin activity, causes a change in coagulation times. Therefore, the samples should be centrifuged as soon as possible at a minimum of 2-500 g or a period long enough to obtain plasma with a platelet count less than 10,000/µL.
* If a sample is to be frozen, the plasma should be double-spun (10 g) in eppendorf tubes for 60 seconds to ensure it is platelet poor (<1000/4)
* Lipemic specimens should be spun in Beckman Airfuge to clear (see "Troubleshooting" section of individual tests for procedure).
* Hemolysis should be avoided. Hemolysis is generally associated with traumatic venipuncture which causes falsely decreased clotting times.
* If plasmas are to be stored, the recommended times and temperatures from the time of draw are:

22-24°C (room temperature) = 4 hours (aPTT),or 2-4°C (refrigerated) 24 hours (PT/FIB)-20°C (frozen plasma) 2 weeks-70°C (rapidly frozen plasma) = 6 months |
| **Reagents** | **RecombiPlasTin 2G** reagent consists of * **RecombiPlasTin 2G (RTF):** Lyophilized recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer.
* **RecombiPlasTin 2 G Diluent (RTF diluent)**: An aqueous solution of calcium chloride, polybrene and a preservative.

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| **Item**  | **Part No.** | **Storage** | **Packaging/Use** |
| 5x 20 ml RecombiPlasTin 2G (RTF) | 0020003050 | 2 – 8o C | Open expiration= 10 days on TOP analyzer |
| Factor Diluent 100 ml | 0009757600 | 15 – 25o C | **Replace/refresh analyzer aliquot every 7 days**: open bottle good until expiration on bottle |

**Preparation** * **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 (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. **Do not shake**.

**Stability*** **RecombiPlasTin 2G:** Stability after reconstitution: 10 days at 2-8°C, 5 days at 15-25°C in the original vial or 10 days at 15°C on the ACL TOP . No stir bar required.
* Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.
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| **Quality Control** | **HemosIL Normal and Low Abnormal Controls (Run once per shift).**

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| **Control Material** | **Part No.** | **Storage** | **Stability** |
| Normal Control (HemosIL 10 x 1 mL) | 0020013900 | 2 – 8o C | 24 hours on-board analyzer |
| Abnormal Control (HemosIL 10 x 1 mL) | 0020014000 | 2 – 8o C | 24 hours on-board analyzer |

**Preparation & Stability:** * Reconstitute each vial (normal and abnormal) with 1.0 ml of reagent grade water.
* Replace stopper and allow vial to stand for 30 minutes at room temperature. Swirl gently before use.
* Reconstituted product is stable for 24 hours when kept at 2-8°C (on the instrument).
* Reconstituted product stable for 24 hours when kept at 15-25°C in the original vial
* Reconstituted product stable for 24 hours when kept at 15-25°C in the original vial on-board the ACL TOP
* *For optimal stability remove control from the system and store it at 2-8°C in the original vial*
* **Running Controls:**
* Place QC in Diluent Rack.
* Load rack in either Reagent Area or Diluent Area.
* Go to Menu bar and choose "QC" then" Results List" from dropdown menu. Click on "QC Statistics".
* On Navigation Tree, select the appropriate QC to be run (only tests with check mark next to them will be run).
* Click on the "Run" tile.
* **Validation of Controls:**
* After controls have been run, click on the "Previous Page" tile.
* Select "QC" then "Results List" from the dropdown menu.
* Verify all QC is within acceptable range before processing patients.
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| **Procedure**  | **IMPORTANT!!** **IMPORTANT!! The instrument uses specific racks for closed and open mode processing. Do not place closed tubes in open mode rack or sampling probe may be damaged.*** **Closed Mode:**
* Place samples in correct Closed Mode Sample Rack (blue color on handle of rack) with barcodes facing out.
* From toolbar, click on the "Sample Area" tile.
* On instrument's "Track Control Panel" choose an empty or green track position (from S1 to S8). Load rack.
* If necessary, Add/Remove tests.
* Click on "Run" tile.
* **Open Mode:**
* Choose Open Mode Sample Rack (black color on handle of rack).
* From toolbar, click on the "Sample Area" tile.
* Click on the "Offline Rack" area.
* Enter Sample ID under the appropriate area then double-click on white box to the right of the Sample ID box. A "Tests and Profiles" window will open — click on desired tests then place sample in corresponding position of the Sample Rack. Repeat for allsamples.
* Click on "Insert Rack" tile.
* On instrument's "Track Control Panel" choose an empty or green track position (from S1 to S8). Load rack.
* If necessary, Add/Remove tests.
* Click on "Run" tile**.**
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| **Reference/Analyzer range**  | * **Reference range**

 Prothrombin time: 9.5 – 12.6 seconds* **Analyzer range**

7 - 320 seconds |
| **Report Reporting/ Critical Result**  | **Result Reporting*** Results are interfaced directly to the US (Vista):
* Access the automated result entry routine in Vista (EA — enter/verify data, auto instrument).
* Choose the BCOAGULATION worklist (BCO).
* Enter the accession # for the sample you wish to verify.
* **Note: certain analyzer flags will prevent results from crossing the interface. Investigate all results that do not cross the interface, as they may be in need of further action.**

**Critical Result*** "Critical" INR : = > 4.0
* Any INR result > 4.0 must be called to the provider or designee. Once the provider has been notified, the comments PV ("alert value. Called to ") and PV1 ("Results read back") must be attached to the patient's result in VistA.
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| **Interpretation**  | * The test measures Factors I (fibrinogen), II (prothrombin), V (labile factor), VII and X. All are made in the liver. Factors II, VII and X are dependent upon Vitamin K for their production. Therefore, the prothrombin time is prolonged in liver disease or Vitamin K deficiency, biliary obstruction and malabsorption syndromes. It is also prolonged in defibrination syndromes or congenital defects of any of the coagulation factors mentioned above. Circulatory anticoagulants against these factors also prolong the test.
* If the patient has received a bolus of intravenous heparin within the last four hours, the prothrombin time may be elevated due to heparin levels, but ever so little, unless the patient has a pre-existing prothrombin elevation.
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| **Troubleshooting** | **Handling Lipemic/Grossly Lipemic Plasma*** The Beckman Airfuge® ultracentrifuge is located near the Microbiology hood.
* Fit an empty 2.4 ml plastic liner inside the metal rotor.
* Draw lipemic plasma into a plastic Beckman transfer pipette (part # 343779). Insert the tip of the loaded pipette through the hole on top of the liner and into the outer chamber of the liner.
* Fill the outer chamber until the plasma just overflows into the inner chamber.
* Screw the metal rotor lid on tightly.
* Fill the inner chamber until the plasma just touches the inside of the top of the dome.
* Place the fully assembled rotor on the stator pad, then close the instrument door.
* Set the TIME dial by turning past the 30 minute point, then back to the desired time of 10 minutes.
* Secure the instrument door by turning the pressure regulator knob (located on top of the instrument door) clockwise, pushing down until the air pressure indicated on the PRESSURE gauge brings the rotor up to 90 kPa.
* After the rotor has stopped, turn the pressure regulator knob counterclockwise until the PRESSURE gauge reading is zero.
* Pipette the chylous material from the inner chamber while the rotor lid is in place, then unscrew the rotor lid.
* Using a new pipette, extract the clarified plasma from the outer chamber and into 0.5 ml sample cup.
* BE CAREFUL NOT TO MIX ANY FATTY MATERIAL LEFT ON THE WALL OF THE INNER CHAMBER WITH THE CLARIFIED PLASMA.
* Place 0.5 ml sample cup in sample rack and continue from Step 4 of "Procedure" section above.
* Note: RecombiPlasTin 2G results on the ACL TOP® Family are not affected by

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|  | **PT** |
| Heparin, up to: | 1.0 U/mL |
| Hemoglobin, up to: | 500 mg/dL |
| Bilirubin, up to: | 30 mg/dL |
| Triglycerides, up to: | 1000 mg/dL |

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| **Results outside of analyzer range** | **Results outside of analyzer range*** When a PT result is outside of the analyzer range, a numeric error code as well as HL or LL will be generated and result will show as FAILED.
* Review the curve by following the steps below:
1. Select "Sample List" from toolbar.
2. The Sample List screen is divided into 2 sections. Select the patient Sample ID desired from the top section — information about that sample will show on bottom half of screen.
3. Double click on the test for which curve is desired (page will open containing all test info as well as clot curve) NOTE: If extended curve is desired, make sure the extended result is selected — an "E" will be present under the "JOB TYPE" column of the extended result.
4. Print copy of clot curve by selecting the Printer icon on the top of the page.
* If curve is unacceptable, call floor and investigate whether specimen may have been drawn from a line. Do not report an estimated PT — answer test as "Possible IV contamination, redraw suggested". Refer to the "Think Quality" book for examples of acceptable and unacceptable graphs.
* If acceptable curve is present and clot is formed after 320 seconds, report the results in the computer. Note: **INR results that print in italics are outside of the instrument's linearity and should be reported as: PT = >320 seconds or**

**INR = >\_\_ (*INR calculation corresponding to 320 second PT for the reagent lot*).****Handling Samples with HCT> 55%** * A patient with HCT >55% will cause spurious coagulation results including falsely prolonged PT and APTT results and erroneous results for other calcium-dependent clotting tests due to excess anticoagulant in the plasma.
* The citrate anticoagulant is distributed only in the plasma but not into the blood cells.
* The amount of sodium citrate must be adjusted before re-drawing blood sample from patients with HCT>55%.
* When notified by the DxH800 tech about a patient with a HCT > 55%:
* Review patient Coagulation history by using the Interim Report for Select tests. Be sure to check from T (today) to T-365.
* If the patient has a PT/APTT requested, hold the current tube in reserve with the comment: "HCT > 55% requires redraw. Please call lab at x5499 for further instructions."
* The formula to calculate the appropriate amount of sodium citrate volume is:

**Formula:** (100 – HCT) / (595 – HCT) x Total Volume* Prepare the Adjusted Coagulation tube using the following table:

 **Amount of Anticoagulant Solution (0.5 ml) Adjustment at Different HCT for 3.2% Sodium Citrate, 2.7ML Draw**

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| **HCT % Range** | **Volume of Sodium Citrate to Remove** |
| 55 – 59 | 0.2 |
| 60 – 64 | 0.19 |
| 65 – 69 | 0.17 |
| 70 – 74 | 0.15 |
| 75 – 79  | 0.12 |

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**Reference** 1**.** ACL TOP Series Operation Manual

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

 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. HemosIL PT-Fibrinogen HS PLUS (PN 008469810) package insert.

 5. 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;

 6. Clinical and Laboratory Standards Institute. Preparation and Testing of Reagent Water

 in the Clinical Laboratory; Approved Guideline. Fourth, Edition, CLSI Document C3-

 A4;Vol.26 No.22

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| **Prothrombin Time (PT) on ACLTOP 350 Analyzer** | *Procedure #:* | ***HCO# 210*** |
| *Version #:* | ***1.0*** |

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| **Prepared by:** | **Date Adopted:** | **Approved by:** |
| Daniel Samaila, H(ASCP), MS |  |  |

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| **Date Rescinded** | **Procedure Rescinded** |
|  | HE 19b |

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| **Review Date:** | **Revision Date:** | **Reviewed/Revised by:** |
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| **Prothrombin Time (PT) on ACLTOP 350 Analyzer** | *Procedure #:* | ***HCO# 210*** |
| *Version #:* | ***1.0*** |

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| **“I, the undersigned, do hereby certify that I have read this new/revised procedure. I understand the instructions contained within and have the opportunity for any/all of my questions to be answered by the Hematology Supervisor and/or the Medical Director. I agree to govern myself accordingly.** |
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