# SCOPE:

This document applies to UPMC– Hanover Laboratory.

# PURPOSE:

This procedure provides instructions for the analysis of Prothrombin Time (PT) on the ACL TOP 500 hemostasis analyzer.

**PRINCIPLE:**

The prothrombin time (PT) is a global screening procedure with three major applications:

* a rapid screening test to detect single or combined deficiencies of the extrinsic coagulation pathway indicative of hereditary and acquired coagulation disorders, liver disease or vitamin K deficiency.
* a sensitive monitoring test for oral anticoagulant therapy utilizing the International Normalized Ratio [INR].
* an assay for specific extrinsic coagulation factors.

Oral anticoagulants depress the production of factors II, VII, IX and X in the liver by inhibiting the action of vitamin K. Since the PT is sensitive to the levels of factor II, VII and X, it is widely used to monitor patient therapy with oral anticoagulants.

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.

**REAGENTS:**

• **HemosIL ReadiPlasTin [cat # 0020301400]**

* Each box contains 5 – 1 mL vials of a solution of recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer

And 5 – 19 mL vials of an aqueaous solution of calcium chloride, polybrene and a preservative.

* Instructions for reagent preparation are located in the reagent package insert and in the Reagent Preparation subsection of the procedure, below.
* Reconstituted ReadiPlasTin reagent is stable for 10 days at 2-8°C or 10 days on the analyzer.

• **HemosIL** **Cleaning Agent [cat# 0009832700] as “diluted Clean B”**

* Supplied in an 80 mL bottle stored at ambient temperature.
* Cleaning Agent is used in the Prothrombin Time assay in a diluted form referred to as “diluted Clean B.”
* “Diluted Clean B” is prepared and loaded as a component of Daily Maintenance on the analyzer or replaced as needed. Refer to the procedure COA 1501 ACL Top 500 Daily Maintenance and Setup for detailed instructions on preparation and loading of diluted Clean B.
* Diluted Clean B is stable for 24 hours on the analyzer.

Instructions for reagent/control loading are given in the Reagent/Control Loading subsection of the procedure below. Instructions for the preparation and loading of diluted Clean B are located in the procedure COA 1501 ACL Top 500 - Analyzer Daily Maintenance and Setup.

**QUALITY CONTROL:**

* **HemosIL Assayed Normal Control.**
* **HemosIL Assayed High Abnormal Control**

Each box contains 10 – 1mL vials of the specific level. Instructions for preparation of control materials are located in the control package insert and in the Control Preparation subsection of the procedure, below.

Reconstituted QC is stable for 24 hours on the analyzer.

Instructions for reagent/control loading are given in the Reagent/Control Loading subsection of the procedure below.

**SPECIMEN:**

Venous blood collected in 3.2%, 0.109 Molar Sodium Citrate [light-blue cap].

* The proper 9:1 ratio of whole blood to anticoagulant is critical to obtaining accurate results. Reject any tube that is under- or over-filled.
* Specimens for hemostatsis assays must be centrifuged in a centrifuge that has been verified to produce platelet-poor plasma [<10 x 109 /L]
* Specimens for PT/INR testing are stable up to 24 hours at room temperature. If unable to test within this time frame, platelet-poor plasma from the sample may be frozen at [-]200C for up to two weeks. Refrigeration is not desirable due to the potential cold activation of Factor VII.
* Refer to the Hemostasis Testing Specimen Policy [COA 3101] for additional critical information on specimen collection, handling, testing suitability, stability, and rejection.

**PROCEDURE:**

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| **Analyzer Preparation** | | |
| **Step** | **Action** | |
| **1** | Ensure that all required instrument maintenance has been performed. | |
| **2**  **2(cont)** | **Step** | **Action** |
| **a** | Access the Reagent Area screen by clicking on the Reagent Area icon on the Tool Bar. Loaded Diluent and Reagent racks are represented on the display. Individual colored circles indicated each of the currently loaded reagents, diluents, cleaning solutions, and controls.   * GREEN indicates a supply that is within on-board stability and volume guidelines. * YELLOW indicates a supply that is within the “warning” range for stability [near expiration] or of low volume. * RED indicates a supply that is expired or depleted.   Double-click on any circle in a rack to access the “Rack Details” screen. This screen displays the complete contents of the rack, including name, lot, volume, and on-board stability. |
| **b** | Click on the “Test Feasibility List” icon on the Tool Bar..  **Note:** Hover the mouse pointer over an icon to display the name of that icon. |
| **c** | The pop-up “Feasibility List” consists of a grid with test names listed down the left column. The third column from the left, indicated by the “Patient Feasibility” icon, displays the number of patient [and control] tests that can be run with currently loaded reagents, diluents, and Clean B. If a required reagent, diluent, or Clean B is not loaded or expired, the box will contain a red “X”, instead of a number.  Double-click on the number [or “X”] in the “PT-RP” row. The pop-up box details the number of tests that can be performed with each of the test component requirements [Clean B diluted and PT RecombiPlasTin]. |
| **3** | With the information gathered in 2, above, determine which reagents, controls, diluent, and cleaning solutions need to be prepared and loaded and proceed to the appropriate step, below. | |

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| **Reagent Preparation** | |
| **Step** | **Action** |
| **1** | Pour the entire contents of the HemosIL ReadiPlasTin Diluent vial into the HemosIL ReadiPlasTin Reagent vial. |
| **2** | Invert to gently mix prior to loading on the analyzer. |
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| **Control Preparation** | |
| **Step** | **Action** |
| **1** | For each of the 2 control levels, dissolve the contents of the vial with **1 mL** of reagent grade [NERL] water. Replace the stopper and swirl the vial gently to completely reconstitute the lyophilized material |
| **2** | Allow the control to equilibrate at room temperature for 30 minutes. |
| **3** | Invert gently to mix before use. Avoid foam formation. |
| **Reagent/Control Loading** | |
| **Step** | **Action** |
| 1 | Remove the Diluent Rack from the the D1 position on the analyzer. |
| 2 | Load the Routine Controls 1 and 3 into small wells on the Diluent Rack. Make sure that the vial barcode is visible through the slot in the rack. |
| 3 | Load the Diluent Rack into the D1 position on the analyzer. |
| 4 | Remove the rack in the R4 position from the analyzer. If there is no rack located in the R4 position, obtain an unused Reagent rack.   * **Note:** Reagent racks are labeled with a two-letter sequence beginning with an “R.” |
| 5 | If not already loaded, load prepared diluted Clean B in the first position [closest to the handle] of the rack   * **Note:** Refer to the Procedure COA 1501 ACL Top 500 Daily Maintenance and Setup for detailed instructions on preparation and loading of diluted Clean B. |
| 6 | Gently mix the prepared ReadiPlasTin by inversion. |
| 7 | Remove the cap from the ReadiPlasTin vial and place it in a vacant position in the same rack as the diluted Clean B. Be sure that the reagent barcode label is visible through the slit in the rack. |
| 8 | Load additional reagents/assays on the rack and/or return the rack to the R4 position on the analyzer. |
| 9 | Run and evaluate Quality Control materials prior to patient testing with the newly-prepared reagent/s and every 8 hour shift afterwards. |
| **Quality Control** | |
| Two levels of controls must be run for each 8-hour shift of patient testing and each time new reagent is loaded. | |
| **Step** | **Action** |
| 1 | Click on “QC” on the display Menu Bar. |
| 2 | Click “Test Status List” from the drop-down box. |
| 3 | The displayed screen lists tests configured on the analyzer in the left-most column and the status [Pass,Fail] of the last QC run in the next column. The column on the extreme right displays the time and date of the last QC run. |
| 4 | Double-click on the test “PT-Read” to bring up the “QC Statistics” screen. This screen displays the QC mean, SD, and Levy Jennings chart for the selected level of control. |
| 5 | Note on the left side of the screen the “QC Tree.” Click on the check boxes next to the listing “PT-Read”. Note that check marks also appear in the boxes adjacent to “Normal Control” and “Abnormal High Control”.  **Note:** Click to deselect other QC test options if not being run concurrently or rerunning a single level. |
| 6 | Click on the “Program QC” icon on the Tool Bar to initiate the QC run. |
| 7 | When the QC run is completed, click on “QC” and “Test Status List”. Verify that the “Last Completed QC” column reflects the date and time of the current QC run. Verify that the “Active QC Lot Status” column indicates that the performed QC “PASSED”. Repeat analysis for any QC level that is noted as “FAILED” and verify that acceptable results are obtained prior to patient testing.  **Note:** QC runs from the analyzer do not flow to Meditech. The Laboratory will be using the ACL Top software for Quality Control statistics and documentation. |
| **Patient Testing** | |
| Patient testing can be performed on capped or uncapped tubes:   * Capped samples [ie have not been uncapped or re-capped] must be run in a “Capped Sample” rack, indicated by a blue plastic insert at the handle-end of the rack. * Uncapped samples must be run in an “Uncapped Sample” rack, indicated by a black handle-end without the blue plastic insert. * **Warning:** **Running a capped sample in an “uncapped” rack or an uncapped sample in a “capped” rack will damage the cap-piercer system.** | |
| **Step** | **Action** |
| 1 | Place capped tubes in a “capped tube” rack and uncapped tubes in an “uncapped tube” rack with barcodes facing and visible through the slot in the racks.. |
| 2 | Load the rack on the analyzer. The analyzer will query the LIS to determine patient demographics and ordered tests. |
| 3 | If patient testing does not commence automatically, click on the “Run Tests” icon on the Tool Bar. |
| **Manual Sample Programming** | |
| During computer downtime or as required or appropriate, samples can be manually ordered in the analyzer computer system. | |
| **Step** | **Action** |
| 1 | Click on the Sample Area icon. |
| 2 | On the left hand side of the display is a depiction of an empty rack. Double click anywhere on the rack to access the “Rack Details” screen. |
| 3 | In the “Rack Details” screen, click on the position on the left side of the screen where the sample will be place. [Note: The bottom of the screen is he handle-end of the rack]. Type a patient identifier in the “Sample ID” field. The corresponding position on the rack depiction will turn blue. |
| 4 | Double click any box in the grid to the right of the “Sample ID” field. A pop-up box will appear listing available test assays. Select tests by clicking on the desired items. Click “Close” when done. |
| 5 | Place the sample in the corresponding position of a sample rack.   * **Capped specimens in a “capped” rack, uncapped specimens in an “uncapped” rack.** |
| 6 | Click on the “Insert Rack” icon on the Tool Bar. At the prompt “Insert a new rack”, select an available sample position, S1 – S8, and load the rack onto the analyzer. |
| 7 | If testing does not commence automatically, click on the “Run Tests” icon on the Tool Bar. |

**INTERPRETATION OF RESULTS:**

Normal Range

* 9.4 – 12.5 seconds

Therapeutic Range [Oral anticoagulant therapy]

Critical Range Low Dose INR 1.5 – 2.5

* INR > 5.0. High Dose INR 2.5 – 3.5

Reportable Range Analyzer Measurement Range

8.0 – 300.0 seconds 8.0 – 320.0 seconds

INR 0.68 – 22.9 seconds

**REFERENCES:**

* Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition, H21 – A5, Vol. 28 No. 5. Clinical and Laboratory Standards Institute.
* HemosIL ReadiPlasTin package insert. 000305656 R00 11/2022.

**RELATED DOCUMENTS:**

* COA 3101 Coagulation Specimen Policy
* COA 1501T ACL Top 500 Daily / Weekly Maintenance Chart
* COA 1502 ACL Top 500 – Changing Bulk Rinse and Clean Solutions and Adding Cuvettes.

**Document History**

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See document control system for approvals and periodic review.