# SCOPE:

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This document applies to UPMC – Hanover Laboratory.

# PURPOSE:

This procedure provides instructions for the analysis of Anti-Xa on the ACL TOP 500 hemostasis analyzer.

**PRINCIPLE:**

HemosIL Liquid Anti-Xa is an automated chromogenic assay for in vitro diagnostic use by laboratory professionals in clinical laboratories. The assay provides quantitative results on 3.2% citrated human plasma for the following analytes based on the calibrators used:

**When used with HemosIL Heparin Calibrators**: Quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity on the ACL TOP® Family, ACL TOP Family 50 Series, and ACL TOP Family 70 Series. This laboratory uses only HemosIL Heparin Calibrators.

Heparin is analyzed as a complex with antithrombin present in the sample. The concentration of this complex is dependent on the availability of the patient’s endogenous antithrombin. When the heparin-antithrombin complex is formed, two competing reactions take place:

1. Factor Xa is neutralized by the heparin-antithrombin complex.
2. Residual Factor Xa is quantified with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the heparin level in the sample.

In order to reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran sulfate is included in the reaction mixture.

The assay is not a stand-alone test and the results should be used in conjunction with other clinical and laboratory findings. For use in adult population. For prescription use only.1

**REAGENTS:**

* **Chromogenic substrate** –
  + Ready to use- Opened reagent is stable: 1 month at 2-8°C or 4 days at 15° -25°C on-board the ACL TOP in the original vial.1
* **Factor Xa reagent** –
  + Ready to use- Opened reagent is stable: 1 month at 2-8°C or 4 days at 15° -25°C on-board in the original vial.1

**QUALITY CONTROL:**

* **Low UF** control (0020300320) and **High UF** control (0020300310):
  + Pipette 1 mL of Deionized water into each vial using 1mL volumetric pipette.
  + Replace stopper.
  + Allow to stand at 15 – 25°C for 30 minutes.
  + Gently swirl and invert to mix before use. Do not shake, avoid foam formation.
  + STABILITY after reconstitution: 48 hours at 2-8°C in the original vial or 24 hours at 15 - 25°C on-board instrument.3
  + Label bottle with open date, time and expiration date.
* Assayed controls: **Low LMW** control (0020300220) and **High LMW** control (0020300210):
  + Pipette 1 mL of Deionized water into each vial using 1mL volumetric pipette.
  + Replace stopper.
  + Allow to stand at 15 – 25°C for 30 minutes.
  + Gently swirl and invert to mix before use. Do not shake, avoid foam formation.
  + STABILITY after reconstitution: 48 hours at 2-8°C in the original vial or 24 hours at 15 - 25°C on-board instrument.4
  + Label bottle with open date, time and expiration date.

**SUPPLIES**

* 4- 1mL volumetric pipettes for reconstitution of controls
* Cuvettes
* Diluent Rack/Reagent Rack
* Patient/Test tube Rack

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. **If the hematocrit is >55% follow the procedure for Adjusting Volume of Anticoagulant for >55% Hematocrits**, then append the result with: “Results corrected for a hematocrit of >55%.”
* Specimens for hemostatsis assays must be centrifuged in a centrifuge that has been verified to produce platelet-poor plasma [<10 x 109 /L]
* **Interference**: no interference up to:

Hemoglobin 300 mg/dL

Bilirubin 20 mg/dL

Triglycerides 800 mg/dL

**Stability of specimen**:

* **Anti-Xa for samples containing UFH: Must be centrifuged within 1 hour of collection**, and testing should be completed within 4 hours.2
* **Anti-Xa for samples containing LMWH:** Uncapped tubes (plasma or whole blood) are stable for 24 hours at room temperature.2
* If anti-Xa testing cannot be performed within the allotted time, platelet-poor plasma should be removed from the cells and stored frozen at -20°C for 1 month.
* Frozen plasma samples should be thawed at 37°C for up to 10 minutes, or until completely thawed, then thoroughly mixed and tested immediately.2
* Refer to the Hemostasis Testing Specimen Policy [COA 3101.03] for additional critical information on specimen collection, handling, testing suitability, stability, and rejection.

1 Stability based on in-house studies.

**PROCEDURE:**

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| **Analyzer Preparation** | | |
| **Step** | **Action** | |
| **1** | Ensure that all required instrument maintenance has been performed. | |
| **2** | **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 “Factor Xa” row. The pop-up box details the number of tests that can be performed with each of the test component requirements [Factor Xa and Chromogenic Substrate]. |
| **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/Control Loading** | |
| **Step** | **Action** |  |
| 1 | Remove the Diluent Rack from the the D1 position on the analyzer. |
| 2 | Load the Controls LMH and LMLO for low molecular weight heparin high and low or UFH and UFL for unfractionated heparin high and low, 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 R3 position from the analyzer. If there is no rack located in the R3 position, obtain an unused Reagent rack.   * **Note:** Reagent racks are labeled with a two-letter sequence beginning with an “R.” |
| 5 | Gently mix the HemosIL Liquid Anti-Xa and Substrate reagents by inversion. |
| 6 | Remove the caps from the HemosIL Liquid Anti-Xa and Substrate vials and place them in vacant, adjacent positions in the rack. Be sure that the reagent barcode label is visible through the slit in the rack. |
| 7 | Load additional reagents/assays on the rack and/or return the rack to the R4 position on the analyzer. |
| 8 | Run and evaluate Quality Control materials prior to patient testing with the newly-prepared reagent/s and every 8 hour shift afterwards. |
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| **Quality Control** | |
| Two levels of LMW and UF 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 “Factor Xa” 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 “Factor Xa”. Note that check marks also appear in the boxes adjacent to “LMH”and “LMLO” and “UFH” and “UFL”.  **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 run on the analyzer do not flow to Epic. The Laboratory will be using the ACL Top software for Quality Control statistics and documentation. |
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| **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 and a yellow “dot” on the handle. * Uncapped samples must be run in an “Uncapped Sample” rack, indicated by a black handle-end without the blue plastic insert or yellow “dot”.   **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 EPIC 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. |
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| **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:**

Reportable Range

* 0.04 – 2.00 IU/mL

Reference Range

* **Low Molecular Weight Heparin**: (Lovenox): Measure anti-Xa level 4 hrs post dose.

Target anti-Xa concentration varies depending upon indication and dosing regimen.

* + Treatment of venous thromboembolism:

1.5 mg/kg dosing 1-2 IU/mL

1 mg/kg dosing 0.6 – 1 IU/mL

* + Prophylaxis in Pregnancy
  1. – 0.2 IU/mL
  + Therapeutic range in Pregnancy

0.6 – 1.0 IU/mL

* **Unfractionated Heparin**:

0.3 – 0.7 IU/mL

Analyzer Measurement Range

* 0.04 IU/mL – 2.00 IU/mL.

**REFERENCES:**

1. HemosIL Liquid Anti-Xa Package insert, current revision. Werfen, Instrumentation Laboratory Company.
2. CLSI. *Collection*, *Transport, and Processing of Blood Specimens for Testing Plasma-Based Assays.* 6th.ed. CLSI guideline H21. Clinical and Laboratory Standards Institute; 2024
3. HemosIL UF Heparin Controls Package insert, current revision. Werfen, Instrumentation Laboratory Company.
4. HemosIL LMW Heparin Controls Package insert, current revision. Werfen, Instrumentation Laboratory Company.