Liquid Anti-Xa Level on the ACL TOP

I. PRINCIPLE

Heparin is the most frequently used antithrombotic drug. The biological activity of this sulphated glycosaminoglycan resides in its ability to accelerate (up to 2000-fold) the inhibitory effect of antithrombin on coagulation proteases. In recent years, it has been shown that LMWH, besides being as useful therapeutically as UFH, also has a longer half-life.\(^1\)

Heparin and heparin-like anticoagulants reduce the formation of fibrin clots. The major potentiator of anticoagulation used in hospitals, unfractionated heparin must be provided either intravenously or subcutaneously. Heparin is a naturally occurring sulfated polysaccharide polymer with a molecular weight of about 15,000. Commercially prepared heparin is extracted from bovine or porcine tissue.

This procedure provides instructions for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) in human citrated plasma using HemosIL® Liquid Heparin on the ACL TOP.\(^1\)

In this assay, 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.\(^1\)

In order to reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran sulfate is included in the reaction mixture.\(^1\)
II. **SPECIMEN**

*For patients on low molecular weight heparin, specimen should be drawn 3 to 4 hours after administration of heparin.*

Nine parts of venous blood is collected into one part of 3.2% sodium citrate, avoiding stasis and contamination of the specimen with tissue fluids. It is not recommended that blood for coagulation testing be drawn through arterial lines, since heparin can contaminate the specimen. After checking for clots, the specimen is centrifuged at approximately 3,000 rpm for 20 minutes at 4°C to obtain platelet poor plasma (PPP). Transfer PPP to polypropylene tubes and perform a platelet count to verify that it is less than 10,000/μL. If not, the plasma must be re-spun until the desired count is obtained. Immediately place the PPP on ice. Aliquot into one mL cryotubes, place in ice block, and freeze at −70°C within four hours of centrifugation. Specimens may remain at −70°C for up to six months. When ready to use, the specimen is thawed in a 37°C waterbath for 5 minutes. Mixing of the sample is critical before testing, as precipitation of certain proteins may occur with freezing.

The allowable time between specimen collection and the centrifugation and removal of the plasma is 4 hours.

Technologists must wear gloves throughout the procedure.

III. **REAGENTS**

HemosIL Liquid Anti-Xa Kit (pn 20302600) contains chromogenic substrate (S-2832) and Factor Xa reagents. Please refer to the package insert for more detail.1

HemosIL Heparin Calibrators (pn 20300600)

HemosIL LMW Heparin Controls (pn 20300200)

HemosIL UF Heparin Controls (pn 20300300)

CLSI Type CLRW water or equivalent

**A. Reagent Preparation**

**Anti Chromogenic substrate:** Invert to mix before use.

**Anti Factor Xa reagent:** Invert to mix before use.

**Calibrators:** If required, reconstitute one vial of each concentration (0, 0.8, 2.0 IU/mL) with 1.0 mL of CLSI Type CLRW water or equivalent. Replace the stopper and keep controls at 15-25°C for 30 minutes. Ensure the complete reconstitution of the calibrators. Gently swirl and invert to mix before use. Do not shake. Avoid foam formation.4,5

**Controls:** Reconstitute each required vial with 1.0 mL of CLSI Type CLRW water or equivalent. Replace the stopper and keep controls at 15-25°C for 30 minutes. Ensure the complete
reconstitution of the controls. Gently swirl and invert to mix before use. Do not shake. Avoid foam formation.\textsuperscript{5,6,7}

**Cleaning Agent:** dilute 1:8 (1 part plus 7 parts) with CLSI type CLRW water (or equivalent).\textsuperscript{5}

### B. Reagent Storage and Stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.\textsuperscript{1,4,6,7}

**Anti Chromogenic substrate** – Opened reagent is stable: 1 month at 2-8°C or 7 days at 15° - 25°C on-board the ACL TOP Family Systems in the original vial.\textsuperscript{1}

**Anti Factor Xa reagent** - Opened reagent is stable: 1 month at 2-8°C or 7 days at 15° - 25°C on-board the ACL TOP Family Systems in the original vial.\textsuperscript{1}

**Heparin Calibrators** – Reconstituted calibrators are stable: 24 hours at 15-25°C on-board the ACL TOP Family Systems, or 48 hours at 2-8°C in the original vial.\textsuperscript{4}

**LMW Heparin Controls** – Reconstituted calibrators are stable: 24 hours at 15-25°C on-board the ACL TOP Family Systems, or 48 hours at 2-8°C in the original vial.\textsuperscript{6} In-house studies show that controls are also stable for two months at -80°C.

**UF Heparin Controls** – Reconstituted calibrators are stable: 24 hours at 15-25°C on-board the ACL TOP Family Systems, or 48 hours at 2-8°C in the original vial.\textsuperscript{7} In-house studies show that controls are also stable for two months at -80°C.

For optimal stability remove vials from the system and store them at 2-8°C in the original vial.\textsuperscript{1,4,6,7}

### IV. EQUIPMENT

ACL TOP CTS
Sample cups
Transfer pipettes
Pipette and tips, 75 – 200 µL
37° Waterbath

### V. PROCEDURE

1. **Calibration**

Calibration of the Liquid Anti-Xa test and storage of the calibration curve is required to obtain Heparin results. Samples cannot be analyzed on the same run as the calibration curve. Calibration is performed:
- With a change of reagent lot numbers
- When troubleshooting doesn’t resolve control recovery issues
• After major parts replacement, as specified by Instrumentation Laboratories
• At laboratory discretion

Note: Based on QC data, it has not been necessary to routinely recalibrate every six months.

Method for Calibration (if necessary):
1. Define Materials if necessary (Setup, Materials, Material List and Material Definition from the On-Line Help Manual)
3. Define Results Units and Rerun Rules in the Liquid Anti-Xa Test Definition if necessary (Setup, Tests, Anti-Xa, Result Units and Rerun Rules from the On-Line Help Manual).
5. Double-click on the appropriate calibrator to open the Materials Definition screen.
6. Choose Lot Management from the General Definition tab.
7. Choose the Lot Specific Information tab and enter the HemosIL Heparin Calibrators lot number and expiration date, if they have changed. The codes for these calibrators are HEP CAL 1 (0.0), HEP CAL 2 (0.8), and HEP CAL 3 (2.0).
8. Select the Save icon to store the lot numbers. Once the lot numbers are saved, the Assign Values icon becomes available.
9. Select the Assign Values icon.
10. Verify the calibration values. They should never change from 0.0, 0.8, and 2.0.
11. Choose the Previous Screen icon to exit.
12. Double-click on the appropriate reagent (Anti Factor Xa and Anti Chromogenic substrate) to open the Materials Definition screen.
13. Choose Lot Management from the General Definition tab.
14. Choose the Lot Specific Information tab and enter the lot number and expiration date, if they have changed. Choose the Save icon.
15. Choose the Previous Screen icon to exit. Place the three calibrators in a ‘D’ rack in decreasing concentration order: 2.0 (vial #3), 0.8 (vial #2) and 0.0 (vial #1) IU/mL, respectively, where the highest concentration vial (#3) would be closest to the back of the instrument.
16. Place Anti Factor Xa reagent, Anti Chromogenic Substrate and Clean B diluted (1 part Clean B plus 7 parts water) in appropriate racks on the TOP. Use the Test Feasibility icon located on the Sample, Reagent, and Diluent screens to determine the appropriate positions.
17. Select **Calibration, Status List**.

18. Double-click on the **Anti-Xa** test to open the **Calibration Details** screen.

19. Choose the **Run** icon.

20. Select **OK** at the “Do you confirm the operation?” prompt.

21. Choose the **Previous Screen** icon to exit.

22. Verify the Job Status for the **Anti-Xa** calibration says **Active**.

23. Once the calibration is complete, review calibration results. An acceptable $r^2$ value is $\geq 0.990$. If there are no errors/failures and the calibration is acceptable, choose the **Validate** icon to validate the calibration curve. If the calibration is not acceptable, it must be repeated.

24. Choose **Actions** and then **Print** to print out a copy of the calibration. Place printout in “Standard Curve” book.

2. **Procedure**

1. Load the Anti Factor Xa reagent and Anti Chromogenic substrate onto the ACL TOP (**Preparing the System, Managing Materials, Loading Racks** from the On-Line Help Manual or see Calibration Details section above). Use the **Test Feasibility** icon found on the Sample, Reagent, and Diluent screens to determine accurate placement.

2. Calibrate, if necessary (Calibration Section of this procedure).

3. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the TOP in the D1 or D2 tracks. Use the UF Heparin controls when running patients on unfractionated heparin and use the LMW Heparin controls when running patients on LMW heparin.

4. Choose **QC** from the Main Menu and select **Results List**.

5. Double-click anywhere on the screen to reveal the Test/Materials Definition tree.

6. Select the appropriate Heparin Control and choose the **Program QC (Run)** icon.

7. Place patient samples in sample cups and load onto a non-cap piercing sample rack. Go to the Sample area screen and double-click on the off-line rack to access the Rack Details screen. The Sample Type field should be left on “Patient”. Click on the Sample ID field corresponding to the position of each patient sample in the rack and enter the accession number in alpha-numeric format.

8. To program the test, select the **Add/Remove Tests** icon, choose the “Heparin” profile, and then choose “Anti-Xa” for each sample. You may also choose “Apply to all samples” before choosing the test and the test will be ordered on all samples in that rack without having to add it to each sample individually.
9. Select the Insert Rack icon and insert the rack into any available sample track.

10. Choose the Run icon if the TOP is not currently running.

11. For more instructions on loading samples without barcodes or LIS, please refer to Samples Analysis, Managing Patient Samples, Programming Bar Coded Samples and Programming Non-Bar Coded Samples.

12. For those samples that are not autovalidated, each individual test must be validated. To do so, place the focus on the desired sample ID, select the Sample Details icon or double click on the sample to display the Test Information screen. Select the left-most column for the test you want to validate and place a check mark in that column. Select the Test Details icon or double-click on the test. The Validate icon is enabled and can be selected. Validate the test after checking the curve and verifying the absence of errors. See “Interpretation of Results” section for more information. Clot curves should always be checked in the presence of the following flags: CE, RE, CW, RW.

Note: Autovalidation is always enabled on the TOP, so only rare results will have to be validated manually.

13. Sample results can be uploaded from the Sample List screen or from the Test Details screen by clicking on the Upload icon. The LIS must be enabled before uploading can occur and only validated results can be uploaded.

14. When testing is complete, place a check mark next to each sample, go to Actions and print out a Sample Results Report.

VI. QUALITY CONTROL

Two levels of control are run each time the assay is performed. LMW and UF Heparin Controls are designed for this assay. The mean and standard deviation of these controls is verified by our laboratory for each new lot and this data can be found in the “Control Reassay and Verification” book. Daily control values are logged into the “Standard Curve” book and are monitored using the database on the ACL TOP.

When QC values are outside of two standard deviations, they must be repeated. If they continue to be out, repeat with new control and/or reagents. If they are still out, the assay should be recalibrated.

VII. RESULTS

Interpretation of Results

Results are autovalidated on the ACL TOP but, rarely, they may need to be validated manually. Before results are validated, they must be reviewed using the clot curves and the display on the Sample List screen. The following displays provide the information necessary to assess the validity of each result:

- If the result starts with an * -- this test has triggered a rerun.
• If the result starts with an &-- the result is a diluted rerun result.
• If the result is underlined – more than one result exists for this test and the result displayed is the most recent.
• If the result is italicized – there is at least one warning against it.
• If the result is purple and bold – the result is out of Test Range.
• If the result is red and bold – the result is out of Linear Range but within Test Range
• If the result is blue and unbolded – the result is out of Normal Range but within Linear Range
• If the result is black and unbolded – the result is within Normal Range.
• An exclamation mark (!) is displayed when a result fails.

**Linear Range**

0 – 2.0 IU/mL

• When a result exceeds the linear range of the assay, dilute the patient sample with Factor Diluent and multiply by the appropriate dilution factor.

**Reporting of Results**

• Heparin results are reported in U/mL. A general assumption is made that anti-Xa heparin activity kits give equivalent results and that the heparin therapeutic range using anti-Xa activity is 0.3 to 0.7 U/mL. It has also been reported that the therapeutic range for enoxaparin is estimated at 0.6 to 1.0 U/mL for twice-daily dosing (levels drawn 3-4 hours after administration), or >1.0 U/mL for once-daily dosing (levels drawn 4 hours after administration). Results between different manufacturer’s kits are not equivalent.

• All validated results, whether they have been autovalidated or validated by the user, are uploaded to the LIS. The results are then released in OEM. The device is TOP2, unless the backup instrument (TOP1) is being used. The test code is “XA”.

• The interpretation will automatically come up at the “XAIN” prompt.

The interpretation when testing for unfractionated heparin will come up as “XA5” and will read:

“Typical therapeutic range for unfractionated heparin in venous thrombosis is 0.3 – 0.7 U/mL anti-Xa activity.”

The interpretation for low molecular weight heparin will come up as “XA6” and will read:

“Typical therapeutic ranges for enoxaparin are between 0.6 – 1.0 U/mL, for twice daily dosing, or >1.0 U/mL for once daily dosing. Samples are typically drawn 3 – 4 hours after SQ administration.”

• The LIS code for the type of heparin used is “ATC3” and is requested in the REI function.

The codes for each heparin are as follows:
“UFH” for unfractionated heparin
“LOV” for Lovenox
“FRAG” for Fragmin

- When all results on the worksheet have been entered into the LIS, print out a completed worksheet and verify each result for accuracy. Attach this worksheet to the Sample Results Report and save for review.

**To Enter Results in the LIS using MEM:**

- The test name for this test in the LIS is the “AXA”.
- Always enter results in the worksheet mode.
- When entering results, enter the U/mL at the “XA” prompt and report to two decimal places.

**VIII. LIMITATIONS**

Heparin results on the ACL TOP Family are not affected by:
Hemoglobin up to 300 mg/dL,
Bilirubin up to 20 mg/dL,
Triglycerides up to 800 mg/dL.¹

**IX. PATHOLOGY AND BIOCHEMISTRY**

Heparin catalyzes the activation of antithrombin (AT), a factor normally present in its active form in blood. AT combines with and inactivates thrombin (activated factor II), and also activated factors IX, X, XI, and XII, components of the intrinsic system of the coagulation cascade. The action of heparin is normally monitored by measuring the activated partial thromboplastin time (aPTT). Increased bleeding is the most common adverse effect, and its anticoagulant effects are additive when used with other anticoagulants. The drug causes moderate transient thrombocytopenia in many patients and severe thrombocytopenia in a small percentage. Prolonged use is associated with osteoporosis.

The anti-Xa assay may be used to monitor unfractionated heparin in patients with baseline prolonged aPTT, for example due to lupus anticoagulant. The anti-Xa assay can also be used to dose unfractionated heparin in patients who require unusually large doses of heparin to attain a therapeutic aPTT, for example because of elevated Factor VIII or fibrinogen levels.⁹

The anti-Xa assay may be used to monitor low molecular weight heparin in renal insufficiency, obese or pregnant patients, or small pediatric patients. Routine monitoring in other groups is not necessary.⁹

**X. REFERENCES**
9. Hirsh, Eighth ACCP Guidelines, “Parenteral Anticoagulants”, Chest 2008;133;141S-159S.

Revisions:
April 2013: Name of test kit changed to Liquid anti-Xa from Liquid Heparin. Name of test on TOP changed to “anti-Xa” from “Liq Heparin”. Change in names only, performance characteristics of test remain the same.