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| **Heparin (Liquid) Anti-Xa**  **on the ACL TOP 500** | *Procedure #:* | ***HCO# 260*** |
| *Version #:* | ***1.0*** |

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| **Purpose** | This procedure provides instructions for the quantitative determination of unfractionated (UF) heparin and low molecular weight (LMW) heparin on the ACL TOP Family analyzers. | |
| **Principle/ Clinical Significance** | 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 LMW heparin, besides being as useful therapeutically as UF heparin, also has a longer half-life. The Liquid Anti-Xa kit is a one stage chromogenic assay based on a synthetic chromogenic substrate and on Factor Xa inactivation.  Heparin is analyzed as a complex with antithrombin present in the samples. 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 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.  **This assay is calibrated for use with only the following types of heparin:**   * **Unfractionated heparin (including Calciparin® and Leo®)** * **Fragmin® (dalteparin sodium)** * **Innohep® (tinzaparin sodium)** * **Lovenox® (enoxaparin sodium)** * **Fraxiparine**® **(nadroparin calcium)**   This assay is not calibrated for use with the following types of anticoagulants:  *Note: samples that contain some of these drugs may produce anti-Xa results, but these results should be considered false positives.*   * Warfarin * Synthetic heparins (Arixtra®/fondaparinux and Orgaran®/danaparoid) * Direct thrombin inhibitors * Factor X inhibitors | |
| **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** | |  |  | | --- | --- | | **Responsible Party** | **Responsibilities** | | Hematology Supervisor | * review this procedure biennially and make any necessary revisions in a timely manner | | Medical Director | * review all new or substantially revised procedures, before implementation | | Staff | * read this procedure in its entirety and ask any questions before implementation * govern yourself according to the contents of this procedure after implementation | | |
| **Safety Precautions** | Standard Precautions:   * Gloves * Fluid resistant laboratory coat | |
| **Sample Requirements** | SAMPLE COLLECTION:   1. Collect 9 parts fresh venous whole blood to 1 part **3.2% Sodium Citrate anticoagulant** at the time frames indicated below: 2. anytime for UNFRACTIONATED Heparin monitoring 3. **4 hours after dosing** for LOW MOLECULAR WEIGHT Heparin monitoring   SAMPLE PROCESSING:   1. **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.* 2. Alternately, to check for clot formation, you may remove the cap and insert two wooden sticks into the sample, then remove the sticks and observe for clots. *Note: removal and replacement of the cap may cause errors with a cap-piercing probe. If the sample cap has been removed, you may have to pour off the centrifuged plasma into a sample cup and run it in a rack designated for uncapped samples.* 3. Centrifuge coagulation samples in ONLY those centrifuges designated to have a speed and time which is known to produce **platelet poor plasma**. 4. When preparing a coagulation sample to be frozen, transfer the plasma from the initial centrifugation to a non-activating plastic tube, using a plastic pipette, then re-centrifuge the sample in a coagulation centrifuge a second time. Aliquot the second spin plasma to a secondary plastic tube, taking care to not include the residual platelets that may have collected at the bottom of the centrifuge tube. Frozen sample stability is indicated below.   SAMPLE STABILITY:   1. 3.2% Sodium Citrated plasma stored at ROOM TEMP (18-25°C) or REFRIGERATED (2-8°C) may be analyzed within **4 hours of collection.** 2. 3.2% Sodium Citrated plasma may be stored FROZEN (-20°C) for up to 2 weeks. 3. 3.2% Sodium Citrated plasma may be stored FROZEN (-70°C) for longer periods. 4. Frozen plasma specimens should be rapidly thawed at 37°C (using the Blood Bank water bath) for approximately 5 minutes. After thawing, each sample should be gently mixed and tested immediately. | |
| **Equipment/ Materials** | This test is intended for use on the ACL TOP 500. | |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | **Item** | **Part No.** | **Storage** | **Packaging/Use** | | Factor Xa Reagent | 0020302610 | 2-8º C | Open expiration = **7 days** loaded on the TOP analyzer | | Chromogenic substrate | 0020302620 | 2-8º C | Open expiration = **7 days** loaded on the TOP analyzer | | UF (Low) control | 0020300320 | 2-8º C | Open/prepared expiration =  **24 hours** on the TOP analyzer | | UF (High) control | 0020300310 | 2-8º C | Open/prepared expiration =  **24 hours** on the TOP analyzer | | LMW (Low) control | 0020300220 | 2-8º C | Open/prepared expiration =  **24 hours** on the TOP analyzer | | LMW (High) control | 0020300210 | 2-8º C | Open/prepared expiration =  **24 hours** on the TOP analyzer | | Heparin Calibrators | 0020300600 | 15-25 º C | Open expiration = **24 hours** loaded on the TOP analyzer | | Nerl Reagent Grade Water | IM#21895 | 4D-135 | Open expiration = **30 days** |   **PREPARING REAGENTS:**   1. Factor Xa reagent: invert gently to mix before use. 2. Chromogenic substrate: invert gently to mix before use. | |
| **Quality Control** | The chart below provides an outline of when Heparin QC is run under normal circumstances. In the event of analyzer downtime, all Heparin controls are analyzed at least once every 8 hour shift of patient testing.   |  |  |  | | --- | --- | --- | | **Control Material:** | **Performed on the**  **Heme ACL TOP:** | **Performed on**  **the Stat ACL TOP:** | | *UF LOW –*  *Unfractionated Heparin Control*  *(yellow cap)* | By DAY SHIFT  (0800-1600) | By EVENING SHIFT (1600-MIDNIGHT)  By NIGHT SHIFT (MIDNIGHT-0800) | | *UF HIGH –*  *Unfractionated Heparin Control*  *(green cap)* | | *LMW LOW –*  *Low Molecular Weight Heparin Control (yellow cap)* | | *LMW HIGH –*  *Low Molecular Weight Heparin Control (green cap)* |   **PREPARING CONTROLS:**   1. Reconstitute the contents of each vial with 1 mL of Nerl reagent grade water. 2. Replace the stopper and swirl gently. 3. Write on each vial the 24-hour on-board expiration date/time. 4. Make sure each vial is completely reconstituted. 5. Keep the control at 15-25º C for 30 minutes. 6. Invert gently to mix before use. DO NOT SHAKE. ***Note: bubbles on top of the liquids may interfere with the instrument’s liquid sensors.*** 7. Weekdays: DAYSHIFT will make the Heparin controls, run them on the Heme Lab analyzer and then transfer them to the Stat Lab analyzer prior to 4pm each day. Weekends: DAYSHIFT will make the Heparin controls and leave them on the Stat Lab analyzer for 24 hours. | |
| **QC Corrective Action** | |  |  |  | | --- | --- | --- | | **IF:** | **QC IS:** | **THEN:** | | First run | Acceptable | Proceed with patient testing | | First run | Unacceptable | *Repeat affected QC material* | | Second run | Acceptable | Proceed with patient testing | | Second run | Unacceptable | *Check all reagents and QC vials for expiration, degradation, discoloration, or other abnormal findings. If found, repeat QC using in-date/normal reagents/materials.* | | Third run | Acceptable | Proceed with patient testing | | Third run | Unacceptable | *Discontinue patient testing and notify supervisor.* |  * **Document all QC corrective action in the ACL TOP software by following these steps:**  1. Select the failed data point in the Levy-Jennings chart by clicking directly on the point. Selected points will appear with a circle around them. 2. Click on the COMMENT icon  at the top of the screen. 3. Enter an appropriate **corrective action comment** and **your initials** in the pop-up box then choose OK. | |
| **Calibration** | Calibration and storage of a valid Liquid Heparin calibration is required to obtain Heparin results.  **Heparin calibration is performed:**   * With every change of reagent lot numbers * At least every 6 months for calibration verification * As needed based on QC shifts/trends * After major parts replacement, as determined by vendor service   **PREPARING CALIBRATORS:**   1. Reconstitute one vial of each level of calibrator with 1.0 mL of Nerl Reagent grade water. (Level 1 = 0, Level 2 = 0.8, Level 3 = 2.0 IU/mL) 2. Replace the stopper and swirl gently. 3. Write on each vial the 24-hour on-board expiration date/time. 4. Keep the calibrators at 15-25º C for 30 minutes. 5. Make sure each vial is completely reconstituted. 6. Invert gently to mix before use. DO NOT SHAKE. ***Note: bubbles on top of the liquids may interfere with the instrument’s liquid sensors.***   **PERFORMING CALIBRATION:**   1. Choose **Setup, Materials List.** 2. Double-click on the appropriate Heparin Calibrator to open the **Materials Definition** screen. 3. Choose the **Lot Specific Information** tab and enter the calibrator lot # and expiration date. 4. Enable **Lot Management** from the Lot Specific Information tab. 5. Select the **Save** icon to store the lot #. 6. Choose the **Previous Screen** icon to exit. 7. Load the Factor Xa reagent, Substrate, Calibrators (3), and Diluted Clean B in appropriate racks on the analyzer. 8. Select **Calibration, Status List.** 9. Double-click on the Anti-Xa test code to open the **Calibration Details** screen. 10. Choose the **Run** icon. 11. Select **OK** at the “Do you confirm the operation?” prompt. 12. Choose the **Previous Screen** icon to exit. 13. Once the calibration is complete, **review the calibration results with the Hematology Supervisor**. *An acceptable r2 – value is ≥ 0.990.* 14. If there are no errors/failures and the calibration is acceptable, choose the **Validate** icon to validate the calibration curve. | |
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| PROCEDURE | **Step** | **Action** |
|  | 1 | * Place a properly labeled sample tube in a sample rack with the barcode facing outward:  1. Racks with BLUE handles are for capped tubes when using the cap-piercer on the ACL TOP 500. 2. Racks with plain BLACK handles are for open tubes when the cap-piercer is not available, or for SAMPLE CUPS, when needed. |
|  | 2 | * Select an available sample track and load the sample rack when the barcode reader is in position. * Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually from the test and programming window. |
|  | 3 | * Click on the RUN icon if the ACL TOP is not currently running. |
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| **Results Interpretation/ Expected Results** | | * Liquid Heparin results are reported in **IU/mL**. * To obtain an optimal effect with minimum risk of bleeding or thromboembolic complications the heparin activity should be in the range recommended by the heparin manufacturer. * Therapeutic ranges for the types of heparin that are approved for use with this assay are listed below:  |  |  | | --- | --- | | **Type of heparin:** | **Therapeutic Range (IU/mL):** | | UF – Unfractionated | 0.3 – 0.7 | | ***When measured 4 hours after dosing:*** | | | LMW – Enoxaparin (Lovenox) | ***Therapeutic ranges for LMW heparin to be deployed***  ***at a later date.*** | | LMW – Tinzaparin (Innohep) | | LMW – Dalteparin (Fragmin) | |
| **Critical Values** | | * + - **UF Heparin results > 1.0 IU/mL should be called to the provider or designee.**     - ***Critical results for LMW Heparin to be determined at a later date***     - Refer to *HAD 010 – Hematology Laboratory Critical/Panic Value Reporting* for more information. |
| **Results Reporting** | | * + - Heparin anti-Xa results are interfaced directly to the LIS (Vista):  1. Access the automated result entry routine in Vista (EA – enter/verify data, auto instrument). 2. Choose the BCOAGULATION worklist (BCO). 3. 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 interface, as they may be in need of further action.*** |
| **Method Performance Specifications** | | The linearity and reportable range for Anti-Xa results on the ACL TOP are indicated below:  **Linearity & Reportable range:**  **0.04 – 2.0 IU/mL** |
| **Method Limitations** | | 1. Heparin anti-Xa results are not affected by:  * Hemoglobin up to 300 mg/dL * Bilirubin up to 20 mg/dL * Triglycerides up to 800 mg/dL  1. This assay is not calibrated for use with the following types of anticoagulants:   *Note: samples that contain some of these drugs may produce anti-Xa results, but these results should be considered false positives.*   * Warfarin * Synthetic heparins (Arixtra®/fondaparinux and Orgaran®/danaparoid) * Direct thrombin inhibitors * Factor X inhibitors |
| **Technical Support/**  **Service Information** | | **Instrumentation Laboratories Technical Support Hotline (24/7)**  **1-800-678-0710**  ***Document all instrument troubleshooting/service and store all troubleshooting/ service records in the analyzer Maintenance Log binder.***  Important analyzer information:  **ACL TOP 500 – Hematology Laboratory**  Serial #: 09080763  **ACL TOP 500 – Stat Laboratory**  Serial #: 09080761 |
| **References & Attachments** | | * Liquid Anti-Xa, package insert. Instrumentation Laboratory. Bedford, MA 01730-2443. * Heparin Calibrators, package insert. Instrumentation Laboratory. Bedford, MA 01730-2443. * UF & LMW Heparin Controls, package inserts. Instrumentation Laboratory. Bedford, MA 01730-2443. * CLSI. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition.* CLSI Document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. |

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| **Prepared by:** | **Date Adopted:** | **Approved by:** |
| Heather Crum, MT(ASCP) |  |  |

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| **Review Date:** | **Revision Date:** | **Reviewed/Revised by:** |
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