|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Heparin Assays of Plasma** | | | | | | | | |
| **Purpose** | This procedure provides instructions for PERFORMING HEPARIN ASSAYS OF PLASMA,  Unfractionated Heparin (HEPU), Low Molecular Weight Heparin (HLMW). | | | | | | | |
| **Principle** | The INNOVANCE HEPARINassay is a one stage chromogenic assay. The reagent kit consists of  two components. One component (Reagent) contains Xa, the other (Substrate) a chromogenic  substrate specific for Xa. Upon mixing Reagent Xa and Substrate the chromogenic substrate is  converted into two products, one of them is paranitroaniline. The formation of paranitroaniline  can be quantified by the coagulation analyzer employing light absorption at a specific  wavelength (405 nm).  In the presence of a sample containing heparin the formation of paranitroaniline will be  reduced in a time dependent manner. This is due to inhibition of Xa by the heparin/AT  complex. This complex is formed in the patient's plasma and competes with the substrate  conversion by Xa. The concentration of the complex is not only dependent on the  concentration of heparin but also on the availability of the patient’s endogenous antithrombin.  By comparison to a reference curve the heparin activity of the sample can be quantified.  To reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran  sulfate is included in the reaction mixture. | | | | | | | |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist. | | | | | | | |
| **Materials** | **Equipment** | | | **Reagents** | | | **Supplies** | |
|  | * Behring Coagulation System (BCS-XP): analyzer, personal computer, printer and associated non-disposable parts. | | | * **INNOVANCE® Heparin**   REF OPOA05  Heparin Xa Reagent: 5 x 3.2 ml  Substrate Reagent: chromogenic  substrate 5 x 4 ml  Stability once opened 7 days if left on analyzer, 4 weeks in original capped vial at 2-8°C.   * **INNOVANCE® Heparin Calibrator**   REF OPOB05  Five levels, 5 x 1ml  Dilute each vial with 1ml water.  Allow to stand at least 15 minutes before use.  **Stability after reconstitution:**  **24 hours at 15-25°C.**  **48 hours at 2-8°C.**  **HEPU Controls:**   * **INNOVANCE UF Control 1**   REF OPOC05 5 x 1ml  Dilute with 1 ml water.  Allow to stand at least 15 minutes before use.  **Stability after reconstitution:**  **24 hours at 15-25°C.**  **48 hours at 2-8°C.**   * **INNOVANCE UF Control 2**   REF OPOD05 5 x 1ml  Dilute with 1 ml water.  Allow to stand at least 15 minutes before use.  **Stability after reconstitution:**  **24 hours at 15-25°C.**  **48 hours at 2-8°C.**  **HLMW Controls:**   * **INNOVANCE LMW Control 1**   REF OPOE05 5 x 1ml  Dilute with 1 ml water.  Allow to stand at least 15 minutes before use.  **Stability after reconstitution:**  **24 hours at 15-25°C.**  **48 hours at 2-8°C.**   * **INNOVANCE LMW Control 2**   REF OPOE05 5 x 1ml  Dilute with 1 ml water.  Allow to stand at least 15 minutes before use.  **Stability after reconstitution:**  **24 hours at 15-25°C.**  **48 hours at 2-8°C.** | | | * Disposable 4 mL sample cups, available from Dade Behring OVIS31. * Plastic transfer pipets, available from storeroom. * BCS-XP disposable cuvettes, available from Dade Behring OVIP11 * Owrens Veronal Buffer   REF B4234-25.  Open vial stability is 7 days.   * SCS Clean: Dade Behring, OQUB, 6 x 5-mL. An aqueous solution of sodium hydroxide (< 2.0 %) and a detergent.   Outdate on unopened  vial. Open vial stability  (capped vial) is 2 weeks.   * Type 1 distilled water (from Millipore) * Washing Solution for Behring Coagulation Analyzers: Dade Behring OWZC35 * Barbicide   disinfectant solution.  King Research chc# 31111. Prepare working solution by diluting one 125ml bottle of concentrate to 2.0 L with deionized water.  Working Barbicide solution is stable for 8 weeks.  **Do not use this product for cleaning surfaces, lanes or racks on the analyzer.** | |
| **Sample** | 1. Collect blood from a clean venipuncture; avoid foaming. 2. Mix nine parts of freshly collected blood with one part 3.2% (0.105 M) sodium citrate: 3. Add 1.8 mL whole blood to 0.2 mL 3.2% sodium citrate (blue-top vacutainer tube)   - or -   1. Add 2.7 mL whole blood to 0.3 mL 3.2% sodium citrate (blue-top vacutainer tube)   - or -   1. Special tubes must be prepared for patients whose hematocrit is > 55%. See procedure entitled *Citrate Concentration Adjustments.* 2. Invert to mix well; transport to lab at room temperature. 3. Check sample for clots with applicator sticks. 4. Centrifuge in Stat Spin for five minutes – or - 10 minutes at 3000 rpm at room temperature. 5. Sample for testing: Remove plasma from RBCs (Procedure Notes #4) and place in a 4 mL plastic cup; allow for 100 μl of dead space. 6. Specimen Stability: 7. Plasma two (2) weeks when stored -20°C. 8. Plasma six (6) months when stored -70°C (rapidly frozen). 9. Plasma must be frozen if testing cannot be completed within two (2) hours.   f. Samples should be centrifuged within one hour from the time of specimen collection.   1. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately. 2. If there is a delay in sample transport: 3. Notify supervisor or pathologist 4. If approval is given to run test, append one of the following to the result:  * “-DELA” (transport delayed)  1. Reject specimen if: 2. Clotted 3. Tubes insufficiently filled (tubes may vary by no more than -10%, see comparison tubes by centrifuge). 4. Incorrect ratio of anticoagulant to blood. 5. Grossly hemolyzed specimens, unless a new specimen cannot be drawn without causing the patient trauma or a non-hemolyzed sample is unobtainable (post-op heart, ECMO, etc.).   **If a hemolyzed sample is tested, add one of the following comments to the result depending on the amount of hemolysis:**   * “-HP” (hemolysis present may affect results)   - or –   * “-GRH” (gross hemolysis may interfere with testing)  1. Notify unit or physician of unacceptable specimens; enter appropriate comment in computer. 2. Lipemic samples: If the BCS-XP is unable to detect an endpoint because of lipemia, ultracentrifuge the sample and run using the normal wavelength. | | | | | | | |
| **Calibration** | Please Note: This is a Hybrid curve, one curve completes the calibration for both HEPU and HLMW.  1. Check the concentration of the calibrators, change if necessary (may be a new lot number). 2. Click on Definitions 3. Click on LotInfo, select Innovance HEPARIN CAL 1. 4. Highlight calibrator 5. Check concentration by highlighting appropriate lot number    * Change concentration by double clicking on the reference value line    * Enter value from package insert.    * Repeat this process for each calibrator (1-5).    * Load calibrator set in a 5ml rack with bar code facing left, place on BCS-XP (lane 6-14). 6. Load Factor Xa and Substrate in a cooler rack (lane 1 - 4) with bar code facing left. 7. Owrens Veronal Buffer should be loaded in a cooler rack where the sample arm has access   (lanes 3 or 4) with the bar code facing left.   1. Place SCS Clean in a 5 mL rack, any lane 6 through 14, with bar code facing left. 2. Click on the Calibration button. 3. Click on New. 4. Click the HEPU.IN assay from the selection box on the left side of the screen. 5. Select the correct lot number for all of the reagents.  * Click on the inverted triangle of the lot number selection box (right side of screen). * Highlight the correct lot number from the pop-up menu.  1. Click on Measure Curve. 2. Click on Close. 3. View the curve when completed:  * Click on the Calibration button. * Highlight the curve in the Curve Overview box. * Click on Show Curve. * Print the curve.  1. View individual points on the curve:  * Highlight the curve in the Curve Overview box. * Click on the Info button * Highlight the point in the Individual Results box. * Each measurement can be viewed in the Individual Measurement box.  1. If any point is flagged, the curve will be labeled invalid and the point must be rerun.  * Close the Info box being viewed. * Click on Show Curve * Point and click on the invalid point * Click on the Repeat button * NOTE: The request to repeat a point must be made within 30 minutes of obtaining the initial curve. After the point has been repeated, the curve will be updated. | | | | | | | |
|  | 1. To activate a specific curve when several curves of the same assay are present 2. Click on the specific calibration curve 3. Click on the Reactivate button on the bottom left side of the screen.  Auto Calibration  1. Load the new/old reagents into the appropriate racks (cooler and 15 mL racks); place on the BCS-XP. 2. Load appropriate calibrator set (as defined above) into a 5 mL rack; place on the BCS-XP. 3. Request control or patient samples tests first. 4. Once processing is complete, the BCS-XP will perform an AutoCalibration for a heparin assay. 5. When the calibration is complete, the patient and control results will be displayed. 6. Check curve and repeat appropriate points as discussed above (Manual Calibration). | | | | | | | |
| **Quality Control** | 1. Level 1 and Level 2 Heparin Controls are run: 2. At the beginning of each shift or once every eight (8) hours as needed 3. Each time a reagent is changed 4. Codes for controls are listed on the appropriate worklist 5. Place controls on the BCS-XP in their original vial using a 5 mL bottle rack 6. Order controls as if a patient:    * Highlight HEPU.IN or HLMW.IN on the Joblist    * Analysis will begin 7. Patient results cannot be reported unless control values are within expected tolerance limits. 8. If values do not fall within the expected range, test new controls then new reagents. 9. If QC is still out of range, notify the supervisor. 10. Control values are recorded daily. 11. All control values must be entered into Sunquest whether in or out of control range. Out of control values must have an appropriate modifier appended. 12. When QC data is entered, it is reviewed using Westgard rules. If a Westgard rule fails in Sunquest, the computer displays the result’s standard deviation from the mean. 13. To enter corrective action in Sunquest; after the standard deviation is displayed, the prompt ENTER QC MODIFIER is displayed, use the QC modifier that best describes the action taken from [Table A – Exclusion Comment Codes (QC Modifiers).](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/200705.pdf) | | | | | | | |
| **Procedure** | Follow the activities in the table below for PERFORMING HEPU and HLMW HEPARIN ASSAYS OF PLASMA. | | | | | | | |
|  | **Step** | **Action** | | | | | | |
|  | 1 | Load Heparin Xa reagent, and Substrate reagent in either cooler rack (lane 1 - 4) with bar codes facing left. Load Owrens Veronal Buffer in lanes 3 or 4, (sample arm must have access). | | | | | | |
|  | 2 | Place Cleaner SCS and controls in a 5-mL rack; load onto BCS-XP in any available lane (6 through 14). | | | | | | |
|  | 3 | To load patients:   1. Insert rack loaded with barcoded samples in any available lane (6 through 14). 2. The barcodes are read and the sample numbers are entered on the Job List. 3. Click on the Job List button; all patient sample numbers will appear on the job list with an analyzer symbol preceding the sample number and a red X in the appropriate test cell. 4. The run will begin. | | | | | | |
|  | 4 | Results appear on the job list when completed. Copy the results on the C1 worklist.   1. *If the instrument is online*, the results are transmitted to Sunquest and appear dark green on the Joblist. 2. *If the instrument is offline*, enter result in computer following directions listed for manual entry mode under Result Reporting section of this procedure. | | | | | | |
|  | 5 | Additional Notes:   1. Linearity: 0.01 – top point of curve, generally around 1.6. 2. Samples should **not** be collected from a heparinized line. 3. Samples should be proceeded by 3 rinses after a sample with Hepzyme® has been analyzed. 4. Samples should not be left on the RBCs, as heparin will be absorbed onto the cell surface or neutralized by the release of platelet factor 4. Dextran sulfate has been added to the INNOVANCE ® Heparin reagent to reduce the influence from heparin antagonists such as platelet factor 4. 5. If the values are above the calibrated measurement range, the sample must be diluted 1 + 1 with BEN. Testing will reflex to either HEPU.IN.x2 or HLMW.IN.x2. Load the BEN on a white rack in any lane 5-14 with the bar code facing to the right. The rack will be ejected, identify the BEN as BenDiluent and reinsert the rack. The BCSXP will dilute the sample and multiply the result x2 so it can be directly reported.   If samples are still above the measurement range they will be reported as greater than two times the upper limit of the measurement range.   1. If the values are <0.10 IU/mL and the patient is receiving heparin, call the unit to obtain a new sample. 2. The correct test must be selected to determine the heparin level. 3. Generally, in-house patients are treated with unfractionated heparin. 4. Generally, outpatients are treated with low molecular weight heparin. 5. Different lot numbers of heparin, especially unfractionated, can cause the curve to shift dramatically. If the patient’s results seem too elevated as compared to previous results or the control, call pharmacy to check on the type of heparin the patient is receiving. 6. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis. 7. Results with flags or markings are to be examined in more detail. 8. Repeat patient samples with an invalid or questionable result flag. 9. There has been evidence to suggest that dextran sulfate dissociates protamine/heparin complexes which may lead to inadequate management of heparin reversal. | | | | | | |
|  |  | 13. If a value of <0.00 is obtained, verify the result by performing testing on the other  analyzer before reporting. Abnormal results can be encountered with reagents and  samples that contain air bubbles at the surface; remove all bubbles in reagents and  samples. If results match a value of <0.01 should be reported.  [Table T - Comparative properties of Unfractionated vs Low Molecular Weight Heparin](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200687.pdf)  [Table U - Trade names of LMW heparin in the United States](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200688.pdf) | | | | | | |
| **Interpretation/**  **Results/Alert Values** | 1.Critical Value: All ages:  **1.00 IU/mL**   1. Call results to the patient’s caregiver within 10 minutes 2. Documentation:  * In Sunquest, append all of the following * - RP * -;first and last name of caregiver and time called.+   2. Various anticoagulants may affect results for heparin assays  [Effect of various anticoagulants on commonly used coagulation assays](https://starnet.childrenshc.org/References/labsop/coag/res/effect-of-various-anticoagulants-on-commonly-used-coagulation-assays.pdf)  3. A class of anticoagulants referred to as Direct Thrombin Inhibitors such as Hirudin (Refludan) and argatroban (Novastan®) may cause a falsely elevated heparin level. | | | | | | |
| **Reference Intervals** | See [Table V – Heparin Assay of Plasma – Reference Ranges](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200689.pdf) | | | | | | |
| **Result Reporting** | Sunquest:   1. On-line mode (OEM):   Function: OEM <CR>  Device: XP1 or XP3 (Mpls) or  XP2 or XP4 (St.Paul)  <CR>  Workload data for - <CR>  Last Cup Received = xxxx Last Cup Processed = xxxxx  Start at Cup Enter cup # if appropriate (same as sequence #)  WAITING (ENTER \* TO EXIT ‘OE’)  Accession numbers appear as results are transmitted. Check flagged results on the BCS, if all results are acceptable:  Accept (A), Modify (M), or Reject (R): A <CR>  If results are unacceptable:  Accept (A), Modify (M), or Reject (R): R <CR>   1. Manual entry mode (MEM):   Function: MEM <CR>  Worksheet: C1 <CR>  Test-1: <CR>  Test-2: <CR>  CAP Method: M <CR>  Lots of tests appear one at a time Enter XP1 or XP3 (Mpls) or XP2 or XP4  (St.Paul) <CR>  (A)ccept, (M)odify or (R)eject: A <CR>  Workload data for - <CR>  Acc. No.: Enter ##### <CR>  HEPU or HLMW: Enter results (xxx.x) <CR>  Accept (A), Modify (M), or Reject (R): A <CR> | | | | | | |
| **Maintenance** | 1. Night shift performs daily maintenance: 2. See procedure on the back side of the BCS-XP Maintenance form 3. Document on the BCS-XP Maintenance form. 4. Day Shift performs weekly, monthly, and “as needed” maintenance is performed by day shift 5. See procedures in the front of the BCS-XP Logbook 6. Document on the BCS-XP Maintenance form | | | | | | |
| **Troubleshooting** | 1. Reoccurring problems are documented in the BCS-XP Action Log. 2. Call Dade Behring Technical Services (TAC) 1-877-457-4BCS; enter account number 511404# and be prepared to give the following:  * Serial number * What was going on at time of instrument malfunction | | | | | | |
| **References** | 1. Andrew, M., et al. Clinical Problems in Anticoagulation Therapy. HEMATOLOGY – 1997. Education Program American Society of Hematology, pp. 8-28, 12/97. 2. Behring Coagulation System Instruction Manual, Dade Behring 1 000 074.0698, Dade Behring Marburg GMBH, Version 2.0, June 1998. 3. Behring Coagulation System Customer Training Guidebook, Document #CT26, Dade Behring, Newark, DE, 04/10/00. 4. Bick, R.L., Heparin Therapy and Monitoring: Guidelines and Practice Parameters for Clinical and Laboratory Approaches. Clin Appl Thrombosis HEMOSTASIS. 2(Suppl 1) 512-520, 1996. 5. Check, W. In Coagulation, a Cascade of Questions, CAP TODAY, 1/98, Vol 12, No 1. 6. Chromogenix AB. Heparin, Taljegardsgatan 3, S-421 53 Molndal, Sweden, version 1.1. 7. Cleaner SCS, Dade Behring product insert OQUB G19 C0530 (1785) W, Dade Behring Marburg GMBH, edition July 1998. 8. Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, 2nd edition, NCCLS Document H21-A2, Vol 11, No 23, December 1991. 9. Corriveau, D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107. 10. Enoxaparin Guidelines, Children’s Thrombophilia Network, 4/22/96, pp. 1-6. 11. Heparin, Elkins-Sinn Product Insert J-1432K. Elkins-Sinn, Inc. Cherry Hill, NJ. 12/92 edition. 12. Hirsh, J., et al; “Low molecular weight heparin”. Blood, 79.1, 1-17, 1992. 13. Kovacs, M.J., et al. A Comparison of Three Methods of Measuring Low Molecular Weight Heparin after Total Knee or Hip Arthroplasty, Laboratory Hematology, Vol 2, pp. 111-114. 14. Lovenox® Package Insert IN-1107M, Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, PA, Rev 3/97. 15. Massicotte, P., et al.: Low-molecular-weight-heparin in pediatric patients with thrombotic disease: A dose finding study. J Pediat. 128:313, 1996. 16. Rosborough, T.K., Comparison of Anti-Factor Xa Heparin Activity and Activated Partial Thromboplastin Time in 2773 Plasma Samples from Unfractionated Heparin-Treated Patients. AJCP 108:6, 662-668. 17. Simonneau, G., et al.: Subcutaneous Low-Molecular Weight Heparin Compared with Continuous Intravenous Unfractionated Heparin in the Treatment of Proximal Deep Vein Thrombosis. Archives of Int Med, 7/93. 18. Triplett, D.A.:Laboratory Monitoring of Heparin Therapy, Hemoliance Times, 7/97, Vol 7 No 7. 19. Heparin / INNOVANCE® Heparin Application Sheet (V.O1) 20. INNOVANCE® Heparin Calibrator IFU 10873530GU11 Rev. 01 – en 2017-01 21. INNOVANCE® Heparin QC IFU 10873534GU11 Rev. 01 – en 2017-01 22. INNOVANCE® Heparin Reagent IFU 10873535GU11 Rev. 01 – en 2017-01 23. Siemens INNOVANCE® White Paper A91LD-HHS-171634-P1-4A00 · Printed in USA · 10-2017 · © Siemens Healthcare Diagnostics Inc., 2017 24. Dextran Sulfate included in factor Xa assay reagent overestimates heparin activity in patients after heparin reversal with protamine. Mouton C., Calderon J., Janier G., Vergnes MC., PMID: 14693175 [PubMed – Indexed for MEDLINE] | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | **Effective Date:** | **Summary of Revisions** | |
| 1 | | Laura Rachford | | 11/2001 | Update for STP conversion to Sunquest | |
| 2 | | Laura Rachford | | 05/2001 | Added ISTH standard, deleted SHPL | |
| 3 | | Al Quigley | | 12/2007 | Changed QC TO Ci-Trol, added SHPL | |
| 4 | | Al Quigley | | 11/2008 | BCS-XP Application | |
| 5 | | Al Quigley | | 06/01/11 | Updated, renamed (formerly Coag.H.01), reformatted | |
|  | 6 | | Al Quigley | | 02/11/13 | Changed from Rotochrom to Liquid Xa Kits. | |
|  | 7 | | Al Quigley | | 05/05/17 | Added Barbicide as routine disinfectant solution. | |
|  | 8 | | Al Quigley | | 12/2/19 | Siemens Innovance application | |
|  | 9 | | Al Quigley | | 02/19/20 | Added additional note #5 for diluting samples that are above the measurement range. | |