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| **Fondaparinux ( Arixtra ) Assay** | | | | | | | | |
| **Purpose** | This procedure provides instructions for MEASURING FONDAPARINUX ( ARIXTRA ) IN PLASMA. | | | | | | | |
| **Principle** | Fondaparinux (Arixtra) is a synthetic anticoagulant with selective inhibition of activated factor X (factor Xa). Fondaparinux induces a conformational change in antithrombin and increases its affinity for factor Xa. Inhibition of factor Xa leads to decreased thrombin generation and thrombus development. Fondaparinux has a half life of approximately 17 hours which allows for once daily dosing. It is almost completely excreted by the kidneys. Fondaparinux is approved for the prophylaxis and treatment of venous thromboembolic events.  Laboratory monitoring of Fondaparinux is possible by utilizing factor Xa inhibitory activity of the drug.  This is the same principle used in the assay of low molecular weight and unfractionated heparins. Because Fondaparinux inactivates Xa at a different rate than either unfractionated or low molecular weight heparin the assay must be calibrated using Fondaparinux.  The amount of Xa remaining is determined by a reaction with a chromogenic substrate. The change in absorbance at 405 nm will relate directly to the concentration of Xa and indirectly to the concentration of Fondaparinux.  The Behring Coagulation System (BCS-XP) is a fully automated photometric instrument used to perform a wide range of coagulation assays rapidly and efficiently. It can be used to determine clotting, chromogenic, immunologic and agglutination-based assays. | | | | | | | |
| **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 * **Disposable 4 mL sample cups**, available from Allegiance OVIS31 * **Plastic transfer pipets** * **BCS-XP disposable cuvettes**, available from Allegiance OVIP11 | | | * **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.  **Arixtra Calibrator – ANIARA Biophen chc# 26157.**  Four level calibration plasma (0.0, 0.5, 1.00, 1.50 mcg/ml)  Dilute each level with 1ml water. Let sit 30 minutes before use.  **Stability 7 days 2-8**°C**hrs. at room temperature. Do not freeze.**  Arixtra Control – ANIARA Biophen chc# 26156.  Two levels; Level 1 approx. 0.4 IU/ml, Level 2 approx. 1.20 mcg/ml.  Dilute each with 1ml water. Let sit 30 minutes before use.  **Stability 7 days 2-8**°C**hrs. at room temperature. Do not freeze.** | | | * **Type I deionized water** * **Washing Solution for Behring Coagulation Analyzers:** Siemens OWZC35. Contains hydrochloric acid and detergent * 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.**  **●** Owrens Veronal Buffer  REF B4234-25.  Open vial stability is 7 days. | |
| **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.      1. Sample for testing: Remove plasma from RBCs and place in a 4 mL plastic   cup; allow for 100 l of dead space.   1. Specimen Stability: 2. Plasma must be frozen if testing cannot be completed within four (4) hours. 3. Plasma two (2) weeks when stored -20°C. 4. Plasma six (6) months when stored -70°C (rapidly frozen). 5. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately. 6. If there is a delay in sample transport: 7. Notify supervisor or pathologist. 8. 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 should be rejected 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 the computer. | | | | | | | |
| **Calibration**  **Quality Control** | Please Note: FONDA uses FONDA (ARIXTRA) Calibrator Set:  1. Check the concentration of the calibrators, change if necessary (may be a new lot number).  a. Click on Definitions  b. Click on Lot Info, select Biophen Fondaparinux Cal Set  c. Highlight calibrator  d. Check concentration by highlighting appropriate lot number  Change concentration by double clicking on the reference value line  Enter value from package insert.  Load calibrator set in a 2.5ml rack with bar code facing left, place on BCS-XP (lane 6-14).  2. Load Factor Xa and Substrate in a cooler rack (lane 1 - 4) with bar code facing left.  3. 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.  4. Place SCS Clean in a 5 mL rack, any lane 6 through 14, with bar code facing left.  5. Click on the Calibration button.  6. Click on New.  7. Click the IN.FONDA CAL 1 assay from the selection box on the left side of the screen.  8. 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.  9. Click on Measure Curve.  10. Click on Close.  11. 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.  12. 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.  13. 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.  14. To activate a specific curve when several curves of the same assay are present  Click on the specific calibration curve  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 BCSXP.  2. Load appropriate calibrator set (as defined above) into a 2.5 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 Fondaparinux 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).  1. Assayed Control Plasmas (FONDA Low FONDL, and Fonda High FONDH) should have their ranges verified when there is a change in lot number of reagent or control material.  2. Assayed Control Plasmas (FONDA Low, and Fonda High) are run:  At the beginning of each shift or once every eight (8) hours  Each time a reagent is changed  Codes for controls are listed on the appropriate worklist  Place controls on the BCS in their original vial using a 2.5 mL (small) bottle rack  Order controls by:   * Click CONTROL JOURNAL button * Highlight FONDA on both controls * Click New * Analysis will begin   3. Patient results cannot be reported unless control values are within expected tolerance limits.   1. If values do not fall within the expected range, test new controls then new reagents. 2. If QC is still out of range, notify the supervisor.   4. Control values are recorded daily.  5. 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.  6. 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.   7. 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](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/200705.pdf) | | | | | | | |
| **Procedure** | Follow the activities in the table below for FONDA MEASURING ARIXTRA (FONDAPARINUX) IN PLASMA. | | | | | | | |
|  | **Step** | **Action** | | | | | | **Related Document** |
|  | 1 | Load Factor Xa and Substrate 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 | Place controls in a 2.5-mL rack, load onto BCS-XP in any available lane (5-14). | | | | | |  |
|  | 4 | To load patients:  a. Insert rack loaded with barcoded samples in any available lane (6 through 14).  b. The barcodes are read and the sample numbers are entered on the Job List.  c. 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.  d. The run will begin. | | | | | |  |
|  | 5 | Results appear on the job list when completed. Copy the results on the C1 worklist.   1. If the analyzer is online, the results are transmitted to Sunquest and appear dark green on the Joblist. 2. If the analyzer is offline, enter result in computer following   directions listed for manual entry mode under Result Reporting  Section of this procedure. | | | | | |  |
| **Procedure Notes** |  | Additional Notes:  1. Linearity:  0.0 mcg/ml – 1.50 mcg/ml (top point on curve). Do not report values of 0.0, these should be reported as <0.01.  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. Unlike samples containing unfractionated heparin, fondaparinux is relatively stable (although there are no published studies) because the interaction of platelet factor 4 is weaker and therefore so is its neutralizing potential.  5. If the values are above the calibrated measurement range, the sample must be diluted 1 + 1 with BEN. Load BEN on the analyzer as BEN Diluent and the analyzer will make a 1 + 1 dilution. The results obtained are then corrected for the dilution factor by the analyzer. If samples are still above the measurement range they will be reported as greater than two times the upper limit of the measurement range.  6. This assay is not specific for fondaparinux, specimens containing unfractionated or low molecular weight heparin will yield results by this assay that will not be an accurate estimation of concentration for fondaparinux. It should also be recognized that fondaparinux will yield an erroneous result in the assay for unfractionated or low molecular weight heparin. For these reasons it is important that the provider orders the assay correctly.  7. Similar to unfractionated heparin, fondaparinux is dependent upon antithrombin for its anticoagulant activity. Low levels of antithrombin will influence the effect of fondaparinux.  The anticoagulant effect can be restored by either increasing fondaparinux concentration or adding antithrombin. These observations are relevant for fondaparinux therapy in patients with hereditary antithrombin deficiency or in intensive care patients with low antithrombin levels.  8. Although laboratory monitoring is thought to be unnecessary in most patients receiving prophylactic and therapeutic dose fondaparinux it may be important in patients with antithrombin deficiency as well as in patients receiving antithrombin concentrates in combination with fondaparinux or in patients with impaired renal function. Determination of antithrombin levels during treatment with fondaparinux may identify patients with an expected lower response who might benefit from antithrombin supplementation or dose adjustment of fondaparinux. | | | | | |  |
| **Interpretation/**  **Results/Alert Values** | The therapeutic anti-Xa range for fondaparinux has not been established. In patients treated with 2.5 mg fondaparinux daily the peak steady-state and plasma concentration is on average 0.39-0.50 mcg/ml approximately 3 hours post dose and the minimum steady-state concentration is 0.14-0.19 mcg/ml.  In patients treated with 5.0 mg (body weight <50 kg), 7.5 mg (body weight 50-100 kg), and 10.0 mg (body weight >100 kg) fondaparinux once daily, the mean peak steady-state plasma concentration is approximately 1.20-1.26 mcg/ml and the mean minimum steady-state plasma concentration is approximately 0.46-0.62 mcg/ml.  (Arixtra Prescribing Information version 9/2013, GlaxoSmithKline, Research Triangle Park NC and Garcia et al. Chest 2012, 141:e24s-e43s).  All results will be appended with the coded comment “ASR” in Sunquest translated as the following:  "This test was developed and its performance characteristics determined by Children's Hospitals and Clinics. It has not been cleared or approved by the U.S. Food and Drug Administration. Analyte Specific Reagents(ASR's) are used in many laboratory tests necessary for standard medical care and  generally do not require FDA approval." | | | | | | | |
| **Result Reporting** | Sunquest:   1. On-line mode (OEM): MPLS- See procedure “Autoverification of Coagulation”   Function: OEM <CR>  Device: XP1 or XP3<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-XP, 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  (A)ccept, (M)odify or (R)eject: A <CR>  Workload data for - <CR>  Acc. No.: Enter ##### <CR>  FONDA 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: 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 Siemens Technical Services (TAC) 1-877-457-4BCS, be prepared to give the following:  * Serial number * What was going on at time of instrument malfunction | | | | | | | |
| **References** | 1. BCS®XP System Instruction Manual 1 000 767.0506 Manual Version 1.0, Siemens Diagnostics Inc., Marburg Germany, Copyright 2006. 2. Behring Coagulation System Customer Training Guidebook, Document #CT26, Siemens Diagnostics Inc., Newark, DE, 04/10/00. 3. Biophen Arixtra Calibrator Ref. A222501-RUO. Product Insert D.750.02/BI/2501/RUO   ANIARA 6560 Grove Court – Mason, OH 45040.   1. Biophen Arixtra Control Plasma Ref.A224001-RUO. Product Insert D.750.02/BI/4001/RUO   ANIARA 6560 Grove Court – Mason, OH 45040.   1. 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. 2. Automated assay for Fondaparinux (Arixtra) on the Dade Behring BCSXP   <http://ajcp.ascpjournals.org/content/132/4/608.full>   1. Glaxo Smith Kline Product Monograph ARIXTRA®   GlaxoSmithKline Inc 7333 Mississauga Road Mississauga, Ontario L5N 6L4  <http://www.gsk.ca/english/docs-pdf/product-monographs/Arixtra.pdf>   1. The Reduced Anticoagulant Effect of Fondaparinux at Low Antithrombin Levels   Copyright 2009 International Anesthesia Research Society  DOI: 10.1213/ane.0b013e3181ae94b0   1. Coagulation assays and anticoagulant monitoring   ASH Education Program Book  asheducationbook.hematologylibrary.org  doi:10.1182/asheducation-2012.1.460  ASH Education Book December 8, 2012 vol.2012 no. 1 460-465  10. Heparin / INNOVANCE® Heparin Application Sheet (V.O1) | | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | **Effective Date:** | **Summary of Revisions** | | |
| 1 | | Al Quigley | | 05/08/17 | Initial Version | | |
|  | 2 | | Al Quigley | | 05/18/20 | Siemens Innovance Application | | |