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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 Sysmex CS-5100 is a fully automated coagulation analyzer. The CS-5100 can analyze samples using clotting, chromogenic and immunoassay methods. | | | | | | | |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist. | | | | | | | |
| **Materials** | **Equipment** | | | **Reagents** | | | **Supplies** | |
|  | * Sysmex CS-5100 System: analyzer, personal computer, printer and associated non-disposable parts. * Reaction Tubes Sysmex CS   PN 10488059  • Plastic transfer  pipettes  • 4ml sample cups  PN 10446526  • SLD Mini Cups  PN 10709524 | | | * INNOVANCE®Heparin Kit**,** PN 10873535   Consisting of;  Heparin Xa Reagent: 5 x 3.2 ml  Substrate Reagent:  5 x 4 ml  Stability: 72 hours on board analyzer, 8 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. | | | * TypeI deionized water, available in canisters used to collect Type I water from the Millipore system. Stable seven (7) days * Owrens Veronal Buffer (OVB) PN10445724, (10 x mL )   Stability: 4 days on board analyzer, 8 weeks 2-8°C   * CA System Buffer PN 10873440 ( 8 x 250 mL)   Stability: 4 days on board analyzer, 8 weeks 2-8°C   * CA Clean I PN 10445689,   (50 mL)  Stability: 5 days on board  analyzer, 1 month 2-8°C. | |
| **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. 1 A calibration **must** be done every time a new lot of reagents is opened. Dilute and prepare reagents according to directions. 2. Enter reagent and calibrator lot information in the Reagent Lot Master. The heparin calibrators come as a set of five vials at different concentrations. 3. Load reagents. Slowly dispense the entire volume of the calibrator(s) into a SLD Mini cup. 4. Insert the vial into a C-Rack and place back into the reagent Table. 5. Close the cover and press O.K. to read the barcode. 6. On the Reagent screen, highlight the vial just loaded and press Change to update the date and time.   Refer to the Supply and Reagent Management section of the System Training  Workbook pages 14-22 for more details on steps 2-6.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)   1. Order the calibration curve.   **Press Order / Switch Order / Holder Calib Curve Order / Select the desired assay to be calibrated / Press Change / Press O.K. / Select Calibrator / Press O.K. / Press** **Start / to view calibration status press job list.**   1. When calibration is complete view the new calibration curve.   **Press Calib. Curve / Press Change / Select correct assay / Select lot number.**   1. To compare new versus current calibration curve.   **Press Calib. Curve / Press Detailed Display on the Operation Panel / Press selct Compared Calib. Curve / Select a curve to compare, press Load / Compare curves / Press Close.**   1. Validate or Delete the new Calibration Curve.   **Display the desired calibration curve / Press Validate to validate the curve or Delete to delete the curve / Press O.K. / Press Print**  Note: Validate the new calibration curve by performing QC.   1. Restoring old Calibration Curves.   **Display the calibration curve / Press restore on the Operation Panel, if Restore is not displayed, press More / Select the desired curve to restore / Press O.K. / Press Validate.**  Refer to the Calibration section of the System Training  Workbook pages 42-46 for more details on steps 7-11.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  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:   1. At the beginning of each shift or once every eight (8) hours as needed. 2. Each time a reagent is changed. 3. Patient results cannot be reported unless control values are within expected tolerance limits. 4. If values do not fall within the expected range, test new controls then new reagents. 5. If QC is still out of range, notify the supervisor. 6. Control values are recorded daily. 7. 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. 8. 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.   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 P - Exclusion Codes](https://starnet.childrenshc.org/References/labsop/heme/res/table-p-exclusion-codes.pdf) | | | | | | | |
| **Procedure** | Follow the activities in the table below for FONDA MEASURING ARIXTRA (FONDAPARINUX) IN PLASMA. | | | | | | | |
|  | **Step** | **Action** | | | | | | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load the Heparin Xa reagent and the Substrate reagent in any reagent rack.  Load FONDA controls into a C-Rack using SLD Mini cups.  Load the Owren’s Veronal Buffer (OVB) or CA System Buffer on the Buffer Table.  Training Workbook Pages  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) | | | | | |  |
|  | 2 | To load patients, follow the procedural steps below that match the situation: | | | | | |  |
|  | 3 | **To load patients using Manual Order Processin**g:   1. Place rack with sample tubes on the sampler. 2. Press **Order**. 3. Enter the Rack number. 4. Select a tube position to input an order. 5. Press **Order Entry** on the Operation Panel. 6. Select **Ordinary Sample**. 7. Place the cursor in Sample No. and input the sample ID if the sample does not have a barcode. If the sample has a barcode, the 2D barcode reader can be used to input the sample ID. 8. Select the assays to be analyzed. 9. Use the down arrow to order the next sample. 10. Press **O.K**. 11. Press **Start**. 12. Confirm the sample order status on the Joblist screen.   Training Workbook, *page* 27.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) | | | | | |  |
|  |  | **LIS Order Processing (Sample with barcode):**   1. Place rack with barcoded sample tube on sampler. 2. Check the host connection status. The host connection status icon must be green or orange. 3. **Press Start**. 4. After the barcodes have been read, confirm the sample order status and progress on the Joblist screen.   Training Workbook, page 26. [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) | | | | | |  |
|  |  | **Micro Mode Sampling:**   1. Follow the Manual Ordering Processing steps. 2. Select the **Mc** column on the Order screen. 3. Load the un-capped tube onto the system. 4. Press **Start**.   Note: Reflex testing is not available in the Micro Mode. | | | | | |  |
|  | 4 | Job analysis progress will be displayed on the Joblist; | | | | | |  |
| **Result Reporting** | 5 | Result Reporting;  Sunquest:   1. On-line mode (OEM):   Function: OEM <CR>  Device: CS5M1 or CS5M2 (Mpls) <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 CS-5100, 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 CS5M1 or CS5M2 (Mpls)  (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> | | | | | |  |
| **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.High values should be reported as “greater than” the highest measurable point on the calibration curve. **Do not dilute samples.**  The antithrombin activity in samples with pathological low antithrombin levels will be compensated by diluting with products such as Standard Human Plasma, which may lead to an overestimation of the anticoagulation effect in these patients.  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." | | | | | | | |
| **Maintenance** | 1. Night Shift performs daily maintenance:   [MAI 2.2 Performing CS-5100 Daily Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.2%20Performing%20CS-5100%20Daily%20Maintenance.docx)   1. Day Shift performs weekly, monthly, and “as needed” maintenance:   [MAI 2.3 Performing CS-5100 Weekly Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.3%20Performing%20CS-5100%20Weekly%20Maintenance.docx)    [MAI 2.4 Performing CS-5100 Monthly - As Needed Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.4%20Performing%20CS-5100%20Monthly%20-%20As%20Needed%20Maintenance.docx) | | | | | | | |
| **Troubleshooting** | 1. Call Dade Behring Technical Services (TAC) 1-800-242-3233, be prepared to give the following:  * Serial number * Functional location number * What was happening 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     1. Heparin / INNOVANCE® Heparin Application Sheet (V.O1) 2. Sysmex CS-5100 Training Workbook, Effective Date: 14-Jan-2021 JobAid HOOD05162003158941 [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) | | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | **Effective Date:** | **Summary of Revisions** | | |
| 1 | | Al Quigley | | 9/19/22 | Initial Version, CS-5100 application | | |