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| cid:image001.png@01D22602.9D744AC0 | TITLE:**TITLE**: **FONDAPARINUX (Arixtra)** **(CS 5100)**  | **DEPT OF LAB MEDICINE****CLINICAL HEMATOLOGY****Policy and Procedure Manual** |
| **DOCUMENT #**HEM 221  |
| **WRITTEN BY:**Parveen Bahel H(ASCP) | **EFECTIVE DATE:****07-14-2020** | **REVIEW/REVISION:****H-1(New)** | **Pages 1 of 8** |

1. **INTENDED USE:**

In-vitro diagnostic automated chromogenic assay for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity in human plasma collected from venous blood samples in 3.2 % sodium citrate tubes on the SYSMEX CS-5100 System in the clinical laboratory. For use with plasma from patients undergoing heparin anticoagulant therapy with either UFH or LMWH.

The performance of this device has not been established in neonate and pediatric patient populations.

1. **PRINCIPLE:**

The Innovance® Heparin assay 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 of Reagent and Substrate Xa converts the chromogenic substrate 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 wave length (405 nm). In the presence of a heparin containing sample 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.

After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of paranitroaniline (pNA) that is released is inversely proportional to the concentration of heparin present in the plasma. Arixtra is used as a prophylaxis against thromboembolic events following hip and knee replacement surgery.

1. **SPECIMEN:**

The only acceptable sample for routine coagulation is plasma, from whole blood drawn into blue-stoppered tubes containing 3.2% sodium citrate (nine parts of freshly collected blood with one part of 3.2% sodium citrate anticoagulant).

4.5 ml-draw tube must have a MINIMUM ACCEPTABLE VOLUME of 4.0 ml total (3.5 ml blood +0.5 ml anticoagulant).

2.7 ml-draw tube must have a MINIMUM of 2.5 ml (2.2 ml blood + 0.3 ml anticoagulant).

 A “micro method” sample (newborn PT/PTT/FIB only) must be a full 1.0 ml specimen (0.9 ml of whole blood drawn with a syringe added to 0.1 ml 3.2% sodium citrate anticoagulant.)

 Invert the tube gently three or four times immediately after venipuncture to ensure proper mixing of blood and anticoagulant.

A syringe or evacuated tubes (blue top) may be used for collection. If multiple specimens are collected; the coagulation sample should be the second or third tube collected. If only coagulation testing is to be performed, a red-top tube, which has no additives, should be drawn first and discarded prior to drawing the blue-top coagulation tube.

The patient cannot be on anti-coagulants when the test specimen is collected. Sufficient time after discontinuance of heparin should be allowed for heparin to be cleared from the patient’s blood, usually 6 hours.

If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 mL saline and the first 5 mL of blood or six dead space volumes of the catheter discarded or used for other laboratory tests.

The citrate concentration must be adjusted in patients who have hematocrit values above 55%.

Specimens that are clotted, collected in the wrong tube or serum, or have less than the 90% expected fill should be rejected, the floor must be notified, a comment entered into the computer.

**Handling Condition and Stability**

The whole blood specimen is checked for clot formation by gentle inversion and observation. Centrifuge the capped blood specimen to produce platelet-poor plasma (platelet count <10x10 9/L for 10 minutes at 4000 g or 3 minutes at 8000 rpm (STAT centrifuge) for STAT samples and 7 minutes at 5200 RPM (DAS centrifuges) as soon as possible after collection. If immediate testing is to be done, the plasma may remain on the packed cells or separated.

Patient plasma should be tested within **4 hours**. If immediate testing is to be done, the plasma may remain on the packed cells. For special coagulation testing, spin samples **20 minutes at 4000 g**, separate plasma into plastic tubes, label and freeze all aliquots at –70C located in the Special coag area until ready to use. Always check for clots after aliquoting with applicator sticks. Always tracks aliquots in BEAKER under YH Coag Hold before freezing them. A frost-free freezer should not be used. Frozen plasma samples must be rapidly thawed at 37°C while gently mixing and tested immediately after thawing. If testing is delayed, the sample may be held for 2 hours at 4°C until tested immediately after thawing. If testing is delayed, the sample may be held for 2 hours at 4°C until tested.

 **Specimen stability** at ambient temperature: 4 hours; Frozen at -70° C 6 months.

**Specimen Labelling** Specimen should be properly labeled with at least 2 unique patient identifiers. Patient's full name and medical record number (MRN) and should have the date and time of collection. The patient's birth date may substitute for the MRN if the MRN is not available.

1. **ENVIRONMENTAL OPERATING CONDITIONS:**

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The room temperature and humidity percent are monitored and documented on the Routine Coagulation checklist

1. **SUPPLIES AND REAGENTS:**
	1. **Supplies**
		* Nerl Water: pH 7.0
		* Gauze
		* Citrated blue top tubes
		* Frosted tubes for aliquots
		* Cuvettes
		* SLD Mini - cups
		* CS5100 sample cups – 4 mL
		* CA Clean I – 50 mL (On-board stability – 5 days)
		* CA Clean II – 5 L (On-board stability – 2 months)
		* OVB (Buffer) (On-board stability – 24 hours)
		* Trash bags

**Reagents:**

**NOTE: After opening any vial to place onto the instrument, label that vial with the open and expiration date, referring to the stability information provided here. Discard reagent when expired.**

Reagents (Reagent and Substrate) are liquid and ready to use. Before use unscrew and remove caps. Place the reagents on the analyzer and start measurement. All kit components are lot-specific. The combination with components from other lots may lead to incorrect results.

1. **Arixtra Calibrators**:

Obtain a syringe of Arixtra (fondaparinux) from the pharmacy. If the concentration in the syringe is 2.5 mg/ 0.5 mL it is equivalent to 5000 mg/L. If the concentration in the syringe is other than 2.5mg/0.5 mL, calculate the mg/L and adjust these dilutions accordingly.

 Prepare dilutions for calibration as follows:

0.5 ml of 5000 mg/L + 4.95 ml OVB = 50 mg/L

 0.1 ml of 50 mg/L + 0.4 ml OVB = 10 mg/L

 (ARIX 1) 0.1 ml of 10 mg/L + 0.9 ml PNP= **1.0 mg/L**

 (ARIX 2) 0.5 ml of 1 mg/L + 0.5 ml PNP= 0.50 **mg/L**

 (ARIX 3) 0.5 ml of 0.50 mg/L + 0.5 ml PNP = **0.25 mg/L**

 (ARIX 4) 0.5 ml of 0.25 mg/L + 0.5 ml PNP = **0.125 mg/L**

 (ARIX 5) 0.5 ml PNP= **0 mg/L**

1. **Controls**:

 Two plasmas containing different levels of anti-Xa activity should be used:

A 0.50 mg/L and the 0.25 mg/L should be run as control material to validate the calibration curve.

To prepare controls, use reconstituted IHep LMW controls (as for Lovenox and Fragmin anti-Xa assays), but dilute each 1:2 with GK PNP. IHep LMW1 control should yield a result of approx. 0.25 mg/L; the IHep LMW 2 control should yield approx. 0.50 mg/L. Reconstituted stability on the CS5100 is 4 hours.

1. **Reagent 1: Ready to use: chromogenic substrate**

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| **Reagents** | **Description** | **Preparation** | **On board stability** | **Refrigerated stability** |
| Innovanace Heparin reagent | Aqueous solution containing:– Bovine Coagulation Factor Xa(appr. 0.7 IU/mL)- Tris(hydroxymethyl)aminomethane (TRIS)– Sodium Chloride (NaCl)– Ethylenediaminetetraacetic Acid (EDTA) Disodium Salt– Bovine Serum Albumine (BSA)– Dextran Sulfate– Preservativeb pH 8 | Ready to use | 72 hours | 8 weeks |
| Innovanace Heparin Substrate | Aqueous solution containing:– Suc-Ile-Glu(piperidin-1-yl)-Gly-Arg-pNA.HCl(chromogenic substrate; 1.25 mg/mL)– Sodium Acetate– PreservativebpH 5 | Ready to use | 72 hours | 8 weeks |

1. **CALIBRATION:**

**Instrument Set-Up and Calibration** (only if necessary): Calibration or recalibration frequency is based on Policy HEM 179 (Calibration and Analytical measurement Policy)

 **Arixtra calibrators**: Manually enter the calibrator information as stated in the Test Setup. Place each calibrator in the reagent after the information is entered.

1. All standard dilutions are automatically prepared by diluting the calibrators with OVB according to the parameters entered in the Test Set-up.
2. **To order calibration**:
3. Select **Order.**
4. Select **Switch Order.**
5. Select **Holder Calib Curve Order.**
6. Select the **Arixtra** to be calibrated.
7. Select **Change** and select the correct lot number.
8. Select **OK.**
9. Select the **correct calibrator lot number** from list. Assay value automatically displays.
10. For manual entry of assay value: Place the cursor in **the Assay Sheet Value field** and enter value using keypad.
11. Select **OK.**
12. Select **Start.**
13. To view the calibration status and progress, press **Joblist.**

**Note:** Calibrator plasma should be transferred into a SLD mini cup and placed in the vial. Place vial in a C-rack.

1. **Calibration Validation**
2. View new calibration curve
3. Select **Calib. Curve.**
4. Select **Change**
5. Select Arix Assay
6. Select the lot number.
7. Compare new/current calibration curve
8. Select **Detailed Display**
9. Select **Select Compared Calib. Curve**
10. Select a calibration curve to compare and press Load.
11. Compare calibration curves
12. Select **Close.**
13. Validate new calibration curve
14. Select **Validate** to validate the calibration curve.
15. Select **OK.**
16. Select **Print.**

**Notes:**

* Validate calibration by performing QC.
* If Calibration fails, then recalibrate instrument following steps from the Calibration Procedure Sections. Refer to Calibration or recalibration frequency is based on Policy HEM 179 (Calibration and Analytical measurement Policy)
* If repeat Calibration fails again, Call Siemens Technical Service Assistance, If Instrument is unacceptable for operation, use back-up analyzer.

**How to Order QC**

1. Load QC (Both levels of .25 and .50 arixtra) onto C-rack.
Refer to the Loading Reagents tab to load QC material in a C-rack using a SLD mini cup.
2. Select **Order.**
3. Select **Switch Order.**
4. Select **Holder QC Order.**
5. Select **Order Entry**.
6. Select control material (IHep .25 Arix) from the list.
7. Place cursor in the Lot No. boxand **select the lot number** from the list.
8. Select the Arixtra test
9. Press the down arrow to order the next control (0.50 Arix).
10. Press OK
11. Press Start button, once controls have been ordered.

Controls run as patient samples should be evaluated by the operator, and should closely approximate the expected values.

 Corrective action when tolerance limits are exceeded:

1. Recalibration may be necessary if control values are consistently outside the target range.
2. Verify reagent performance.
3. Check instrument performance.
4. Document actions taken to identify and correct the problem before reporting any patient data.

**Note: All Routine Coag Assays controls for the CS 5100 are not formatted in the BEAKER QC program but set up and reviewed in the instrument QC Software.**

**Note: Instrument for Arixtra should be set up on CS 5100-3 instrument only on M, W and F days unless approved by Lab resident.**

1. **PROCEDURE:**

 **Loading patient samples on the cs 5100**

* 1. **LIS order processing**
1. Load bar-coded samples into any sample rack (above #6) with the bar-code label visible through the window of the rack position and Place rack on the sampler.
2. After barcode reading, confirm sample order status and progress on the Joblist screen.
	1. **Micro mode**
3. For barcoded samples,
4. Press in **Mc** column on Order screen.
5. Load uncapped tube on to system.
6. Select **Start.**

**Refer to Sysmex 5100 Operational procedure for auto verification guidelines, reporting normal and flagged results.**

1. **CALCULATIONS:**

Results are reported in mg/L.Result should always be interpreted with in conjunction with the patient’s medical history, clinical presentation, and other findings.

1. **ANALYTICAL MEASUREMENT RANGE:** 0.10 – 1.0 mg/L
2. **REFERENCE RANGE**
	1. The therapeutic range for Arixtra (fondaparinux):
	2. Therapeutic levels needed may change due to clinical considerations specific to the patient’s condition.
	3. Detection threshold for the assay is 0.10 mg/L
3. **DILUTIONS:** no dilutions
4. **REPORTING RESULTS:**

For a result below the detection threshold, report “<0.10” U/mL.

**Any result <0.10 IU/mL (First time), always check for bubble and repeat the test.**

If instrument gives the results above the highest linearity point, report > 1.0 mg/L

**All Results reflex to MD Interpretations.**

1. **CRITICAL RESULTS:** No critical result for the procedure.
2. **INTERFERENCE:**

Following concentration of listed endogenous substances were found to cause no interference up to the indicated concentrations.



1. **LIMITATIONS OF THE PROCEDURE**
2. **The lab must know which heparin is being administered.**

The draw time should also be noted, as therapeutic levels are based upon a sample drawn approximately 3 hours post dose.

1. Any release of platelet factor 4 (PF4), which is a potent heparin inhibitor, will lead to an under estimation of the heparin level in the plasma being tested. Careful and adequate centrifugation is essential: the higher the level of residual platelets, the greater the risk of PF4 release.
2. Lipemic plasma may be ultracentrifuged before testing, if results are in question. Hemolyzed plasma may be run, but reported with a comment.
3. **REFERENCES:**
	1. Sysmex CS 5100 Operator manual
	2. Sysmex CS 5100 Instruction for Use.
	3. Sysmex CS 5100 Reference Guide
	4. Sysmex CS 5100 System Evaluation and Algorithm
4. **HISTORY:**

H-1 This procedure was written by P Bahel on 07/1/2020