1. **PRINCIPLE:**

The STA - Liquid anti-Xa Assay kit Cat.# 00311US is intended for the quantitative determination of the plasma levels of low molecular weight heparin (LMWH) by the measurement of their anti-Xa activity in a competitive system using a synthetic chromogenic substrate. This assay can also be used to measure the anti-Xa activity of Fondaparinux sodium (Arixtra), a synthetic and specific inhibitor of activated Factor X (Xa). **Only Fondaparinux can be used to calibrate this anti-Xa assay.** The compound can reduce thrombin generation *in vivo* via inhibition of factor Xa and does not inactivate thrombin (IIa). The normal function of a molecule of factor Xa, as soon as it appears in plasma, is to cleave its natural substrate, prothrombin, to generate thrombin, the enzyme responsible for the fibrin clot formation. In the presence of heparin, competition occurs between this mechanism and the inhibitory mechanism exerted by the heparin-antithrombin III complex, this inhibition being largely responsible for the action of heparin. The proposed method is a one-step reaction based on a similar principle: as soon as factor Xa is added to the plasma-substrate mixture, two reactions take place simultaneously:

* hydrolysis of the substrate by factor Xa
* inhibition of factor Xa by the heparin-antithrombin III complex

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:**
2. Citrated blood 9:1 (blood to anticoagulant) 3.2% or 3.8% sodium citrate. Follow NCCLS guidelines H21-A2 and H3-A3. No other anticoagulant is acceptable.
3. Centrifugation: 20 minutes at 3500 g - room temperature
4. Plasma storage: 2 hours for 20 C

6 months at -70 C. Frozen plasma should be thawed only once at 37 C for 5 minutes.

1. Unacceptable specimens: Samples that are QNS or clotted; hemolyzed samples may be run, but hemolysis should be noted and commented that results may be affected.
2. **REAGENTS AND EQUIPMENT:**
3. Centrifuge
4. Pipette tips
5. Cuvette roll (contains1,000cuvettes)
6. STA/STA Compact
7. TYPE –I Reagent Grade Water
8. STA - Owren-Koller Buffer
9. Arixtra Calibrators
10. STA - Quality HBPM/LMWH
11. **REAGENTS:**
12. **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 saline = 50 mg/L

 0.1 ml of 50 mg/L + 0.4 ml saline = 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 LMWH controls (as for Lovenox and Fragmin anti-Xa assays), but dilute each 1:2 with GK PNP. The LMWH 8 control should yield a result of approx 0.25 mg/L; the LMWH 14 control should yield approx 0.50 mg/L.

1. **Reagent 1: Ready to use: chromogenic substrate** CBS 02.44, approximately 4.5 µmoles (Cat. No. 00311US 4 ml) per vial. Allow the reagent to sit 30 minutes at room temperature before use. Swirl vial gently to ensure each vial is completely mixed; then install an STA - Reducer in the vial and replace the perforated plastic cap on top. Request the reagent drawer to open through the MAIN MENU under LOADING and bar code the reagent. Place the reagent into the reagent drawer. Reconstituted stability: 7 days on analyzer in its original vial with the STA 00322US)
2. **Reagent 2: Ready to use: bovine factor Xa,** approximately 1.0 IU (Cat. # 00311US 4 ml) per vial. Allow the reagent to sit at room temperature (18-25 °C) for 30 minutes. Swirl vial gently to ensure the vial is completely mixed; then install an STA - Reducer in the vial and replace the perforated plastic cap on top. Request the reagent drawer to open through the MAIN MENU under LOADING and bar code the reagent. Place the reagent into the reagent drawer. Reconstituted stability:7 days on analyzer in its original vial with the STA 00322US)
3. **STA - Owren-Koller Buffer: Ready to use. (**Cat. # 00360) Ready to use buffer. Used by the STA - Compact to perform dilutions of controls and patients’ plasmas. Request the sample drawer to open through the MAIN MENU under LOADING, scan the barcode on the reagent bottle. Press F1 for diluent. Press Enter  , and then the buffer into the sample drawer. Stability is 72 hours.
4. **CALIBRATION:**
5. **Arixtra calibrators**:

Request the product drawer to open. Manually enter the calibrator information as stated in the Test Setup. Place each calibrator in the product drawer after the information is entered.

B. All standard dilutions are automatically prepared by the STA/STA Compact by diluting the calibrators with Owren-Koller Buffer according to the parameters entered in the Test Set-up.

 C. **To order calibration**:

 Through the MAIN MENU select CAL/CONTROL.

 Select CALIBRATION and enter. Place the cursor on “ARIXROTA” and select by pressing **F1** and then **F10** to run the calibration.

D. Controls are used by the STA/STA Compact to automatically validate the calibration. (Refer to the QUALITY CONTROL section).

 E. To examine the calibration curve:

 Through the MAIN MENU under CAL/CONTROL select CALIBRATION and enter. Place the cursor on ARIXROTA and enter. The calibration curve will appear on the STA/STA Compact screen.

 F. To verify the calibration curve is valid:

 Check that the correlation coefficient (r value) of the curve is 0.90 or

greater. If any controls are outside the expected range, the control must be re-run. If the value is still outside the expected range, a new calibration must be run along with fresh controls.

G. To print the calibration curve:

 While examining the calibration curve on the STA/STA Compact screen, press ESC for options. Select PRINT. The STA/STA Compact cannot print a calibration curve while the STA/STA Compact is running.

1. **QUALITY CONTROL:**

**Controls:**

1. After the reconstitution period, request the sample or the reagent drawer to open through the MAIN MENU under LOADING. Place the controls into the appropriate drawer, entering information as in the Test Setup.
2. QC will automatically be run when a new calibration curve has been requested, if it is in place on the instrument
3. If using a stored curve, the STA/STA Compact will automatically run QC when the patients’ samples have been loaded. The QC can also be ordered manually before loading patients’ samples.

3. Controls placed in the product drawer are monitored automatically by the STA Compact. If any controls are outside the 2 SD range, the STA/STA Compact will audibly and visually alarm the operator. Otherwise, the results can be found in the individual QC files. Control results are automatically filed in the STA Compact QC file. All results for a 24 hour period will be converted to a mean value at midnight. This mean value is used in the statistical data and is plotted on the Levy-jennings chart as a daily mean.

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

1. **PROCEDURE:**
2. Refer to the START-UP procedure for the STA/STA Compact before running patient samples on the STA/STA Compact at the start of each shift.
3. Run the calibration if needed.
4. Request quality control if using a stored curve. (Otherwise, calibrate and run controls).
5. Load patients’ samples: Access the sample drawer through the MAIN MENU under LOADING. After the drawer opens, identify the type of specimen, such as microsample, with **F8,** or stat, with **F12**. Identify the sample by bar coding or manually entering on the keyboard the patient identification number and then placing the sample into the drawer.
6. In MANUAL MODE, the operator must order the test from the menu, and then press F10 to save.
7. In AUTO MODE, the STA/STA Compact will automatically order the test if it is selected in the AUTO MODE profile.
8. In TELELOADING MODE, the STA/STA Compact will query the host computer and download the test as well as assign the status, i.e. stat.
9. As soon as the sample drawer closes, the TEST STATUS screen will appear. If there is not enough reagent(s) to run the test, the suspect reagent(s) will appear in red with the amount of deficiency. This deficiency will BLOCK the sample pipettor. When this occurs, add the deficient reagent(s) to run the samples.
10. All dilutions of the calibrators, controls and patients’ samples are automatically prepared by the STA/STA Compact according to the parameters entered in the Test Set-up. If the patients’ results fall outside the assay reportable range, the STA/STA Compact automatically re-tests the sample in question at an appropriate dilution provided that the option has been entered in the Test Set-up.
11. All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected.
12. For results in question, that need operator intervention, cursor to the identification number in the TEST PANEL screen and enter. This will bring up the FILE PROCESSING screen. Follow the options in the left-hand corner of the screen, i.e. re-run test.
13. **CALCULATIONS:**
14. The STA/STA Compact automatically converts the results off of a standard curve (log-lin) to IU/ml.
15. The STA/STA Compact uses a dilution of 1:2, sample to buffer.
16. **REFERENCE RANGE:**
17. The therapeutic range for Arixtra (fondaparinux), determined on a specimen approximately 3 hours post dose, is 1.20-1.26 mg/L per manufacturer’s information.
18. Prophylactic range is 0.39-0.50 mg/L per manufacturer’s information
19. Therapeutic / prophylactic levels needed may change due to clinical considerations specific to the patient’s condition.
20. Detection threshold for the assay is 0.10 mg/L

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1. **REPORTING RESULTS:**
2. The results for the Heparin Assay are reported out to two decimal places (Example: 0.5 mg/L) and can be posted from the instrument menu.
3. For a result below the detection threshold report **“0” U/mL**.
4. **DETECTION THRESHOLD AND LINEARITY RANGE:**
* The detection threshold of STA - Liquid Anti-Xa (UFH ) on the STA System is 0.10 IU/ml.

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1. **NOTES:**
2. The assay’s linearity extends to 1.00 mg/L. In order to determine a heparin level on a sample with a higher level, the operator must:
* mix the patient sample 1:1 with **Pooled Normal Plasma** example: 0.4 sample + 0.4 Normal Plasma( as the only source of ATIII, which is required for this assay).
* Re-test the heparin assay on this mixture
* Multiply the result X 2 to correct for the dilutional difference.

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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. Icteric plasma (bilirubin>6.6 mg/dL) may render result somewhat lower than it should be, due to intense yellow coloration; the results, therefore, may be invalid for monitoring purposes.
3. Lipemic plasma may be ultracentrifuged before testing, if results are in question. Hemolyzed plasma may be run, but reported with a comment.
4. **REFERENCES:**
* STA - Liquid Anti-Xa (Cat. No. 00311US or Cat. No. 00322US) Colorimetric Assay of Heparins (UFH and LMWH) by STA® Analyzers. Package insert 25536 01 – January 2012.
* STA - Multi Hep Calibrator (Cat. No. 00684): Calibration Plasmas for Assays of heparins (UFH and LMWH) Using anti-Xa Method on STA Analyzers. Package inserts 24274 03 – January 2012.
* STA - QUALITY HNF/UFH (Cat. No. 00381), Control Plasmas for Assays of unfractionated heparin(UFH) using Anti-Xa Method on STA Analyzers. Package insert24208 02-December 2010.
* STA - Compact Operators Manual.
1. **HISTORY:**

H-1 This procedure was written by P.Bahel on 03-10-2013.

H-2 This procedure was reviewed by P. Bahel on 09-29-2014.