1. **PRINCIPLE:**

The STA - Liquid anti-Xa Assay kit Cat.# 00011US is intended for the quantitative determination of the plasma levels of unfractionated (UFH) and low molecular weight heparin (LMWH) by the measurement of their anti-Xa activity on antithrombin in a competitive system using a synthetic chromogenic substrate.

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. Unfractionated Heparin (UFH) and Low molecular weight heparins (LMWH) are used for the prevention and treatment of thromboembolic diseases. The quantitative determination of plasma UFH and LMWH levels are useful for monitoring treatment efficacy.

The heparin-antithrombin complex is made up from the heparin and the antithrombin (AT) from the patient.

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. STA - Multi Hep Calibrator
10. STA - Quality HNF/UFH Control
11. **REAGENTS:**
12. **STA - Multi Hep Calibrator (Cat. No. 00348):** A set of calibrator plasmas intended to be used for the calibration of heparin (unfractionated heparin (UFH) and low molecular weight heparin (LMWH)) activity assays by measuring the anti-Xa activity.
    * **Reagent 1:** STA - Multi Hep Calibrator 0: human plasma free of heparin (see enclosed Assay Value Insert), lyophilized.
    * **Reagent 2:** STA - Multi Hep Calibrator 4: human plasma containing a well defined quantity of UFH (see enclosed Assay Value Insert), lyophilized
    * **Reagent 4:** STA- Multi Hep Calibrator 10: human plasma containing a well defined quantity of UFH that is greater than that of Reagent 2 (see enclosed Assay Value Insert), lyophilized
13. **STA - Quality HNF/UFH Control** (Cat. # 00381): Two plasmas containing different levels of unfractionated heparin (UFH) intended to be used for quality control of UFH assays by measuring the anti-Xa activity.

* **Reagent 1:**STA - Quality HNF/UFH 2: human plasma containing a well defined quantity of UFH (see enclosed Assay Value Insert), lyophilized.
* **Reagent 2:**STA - Quality HNF/UFH 7: human plasma containing a well defined quantity of UFH that is greater than that of Reagent 1 (see enclosed Assay Value Insert), lyophilized.

Tap vial gently on counter top. Reconstitute each vial with 1.0 ml reagent grade water. Let sit 30 minutes at room temperature. Swirl gently to ensure that each vial is completely mixed. Reconstituted stability on the STA - Compact is 4 hours.

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 STAef 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. STA - Multi Hep Calibrator: After the reconstitution period, request the reagent drawer to open through the MAIN MENU under LOADING and bar code each vial of calibrator. Place each vial of the calibrators into the drawer after it has been bar coded. The assay value for each Heparin Level is transferred to the STA® - Compact® when each calibrator is loaded and prompted for the bar code information.
6. All standard dilutions are automatically prepared by the STA® - Compact® by diluting the STA- Multi Hep Calibrators with STA - Owren-Koller Buffer according to the parameters entered in the Test Set-up.
7. To order calibration: Through the MAIN MENU select CAL/CONTROL. Select CALIBRATION and press Enter . Place the cursor on UFH ASSAY.
8. Select UFH ASSAY by pressing F1 and then F10. Type in your Access Code. **Enter** **⮠** to run the calibration.
9. To examine the calibration curve: Through the MAIN MENU under CAL/CONTROL select CALIBRATION and press Enter . Place the cursor on UFH ASSAY and press Enter . The calibration curve will appear on the STA - Compact screen.
10. To verify the calibration curve is valid: Check that the correlation coefficient(r value) of the curve is 0.980 or greater. If any controls are outside the expected range, the control must be repeated. If the value is still outside the expected range, a new calibration must be run along with fresh controls.
11. To print the calibration curve: While examining the calibration curve on the STA -Compact screen, press ESC key for options. Select PRINT, Select Execute. Press **Enter** **⮠** . The STA - Compact cannot print a calibration curve while the STA - Compact is running.
12. **QUALITY CONTROL:**

STA-Quality HNF/UFH Control 2 and 7 is used :

To load STA - Quality HNF/UFH 2 and 7 control: After the reconstitution period, request the reagent drawer to open through the MAIN MENU under LOADING and bar code the controls. Place the controls into the drawer.

1. QC will automatically be run when a new calibration curve has been requested. If using a stored curve, the STA - Compact will automatically run QC when the patients’ samples have been loaded. The QC can also be ordered manually before loading patients’ samples.
2. All control ranges are monitored automatically by the STA/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 AUTO CONTROL file and the individual QC files. Control results are automatically filed in the STA/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.
3. **PROCEDURE:**
4. 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.
5. Run the calibration if needed.
6. Request quality control if using a stored curve: Through the MAIN MENU under CAL/CONTROL select QUALITY CONTROL and enter. Place the cursor on UFH ASSAY. Select UFH ASSAY by pressing F1 and then F10 and then press  **Enter** **⮠** type your Acess Code , press **Enter** **⮠** to run QC.
7. 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.
8. In MANUAL MODE, the operator must order the test from the menu, and then press F10 to save.
9. In AUTO MODE, the STA/STA Compact will automatically order the test if it is selected in the AUTO MODE profile.
10. 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.
11. 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.
12. 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.
13. All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected.
14. 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.
15. **CALCULATIONS:**
16. The STA/STA Compact automatically converts the results off of a standard curve (log-lin) to IU/ml.
17. The STA/STA Compact uses a dilution of 1:2, sample to buffer. No auto re-dilution is allowed with this assay.
18. **REFERENCE RANGE:**

* The therapeutic range for UFH is 0.3 – 0.7 IU/ml.

1. **REPORTING RESULTS:**

The results for the Heparin Assay are manually reported out to the nearest 0.10 IU/ml. Post results from the Compact into Soft, with reference to Soft manual if necessary. (Example: 0.5 IU/ml). For a result below the detection threshold report **“0” U/mL**.

1. **DETECTION THRESHOLD AND LINEARITY RANGE:**
   * The detection threshold of STA® - Liquid Anti-Xa (UFH and LMWH) on the STA® System is 0.10 IU/ml.
   * The linearity range extends to 1.1 IU/ml for UFH.
2. **NOTES:**
3. The assay’s linearity extends to 1.1 for UFH. 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
* re-test the heparin assay on this mixture
* multiply the result X 2 to correct for the dilutional difference.

extended linearity of UFH = 2.2 IU/ml.

1. **LIMITATIONS OF THE PROCEDURE**
2. **The lab must know which heparin is being administered**. In the case of intermittent administration of heparin, the time interval between sample collection and the previous, or next injection should be specified.
3. 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.
4. The STA - Liquid Anti-Xa assay relies on the Anti-thrombin level of the patient. Low levels [less than 60%] may reflect an underestimation of the heparin level result.
5. The APTT and the quantitative assay of heparin do not always correlate. As a test of overall coagulability of the patient, the APTT reflects the effect produced by several phenomenon, such as, the presence of heparin and the presence of an inflammatory syndrome. It is noted that for a given heparin level, different individuals may exhibit different APTT values as well as circadian variability.
6. Icteric plasma may render result lower than it should be, due to intense yellow coloration; the result, therefore, may be in0.0valid for monitoring purposes. Lipemic plasma may be ultracentrifuged prior to use on the instrument. Hemolyzed plasma may render results inaccurate and may need to be repeated on a fresh sample.
7. **REFERENCES:**
8. 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.
9. STA - Multi Hep Calibrator (Cat. No. 00684): Calibration Plasmas for Assays of heparins (UFH and LMWH) Using anti-Xa Method on STA®. Package inserts 24274 03 – January 2012.
10. STA - Quality HNF/UFH (Cat. No. 00381): Control Plasmas for Assays of Unfractionated Heparin (UFH) Using Anti-Xa Method on STA®. Package insert 24208 02 – December 2010.
11. STA - Owren-Koller Buffer (Cat. No. 00360) Buffer Solution for Coagulation Testing. Package insert 23070 06 – June 2011.
12. STA - Compact ® Operators Manual. CLSI guidelines H3 – A6 and H21 – A5, or latest revision
13. **HISTORY:**

H-1 This procedure was written by P.Bahel on 03-18-2013.

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