**PURPOSE:**

The STA® - Liquid Anti-Xa Assay kits, are 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.

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**SCOPE:** Staff Medical Technologists

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**SPECIMEN REQUIREMENTS:**

Exercise great care in collecting and handling plasma to avoid the release of platelet factor 4 (PF4) which is a potent inhibitor of heparin (UFH). Heparin testing is performed on platelet poor (<10 thous) plasma.

Blood samples are collected in 3.2% sodium citrate anticoagulant tubes (blue top). The collection tube must be completely filled to ensure a 9:1 ratio of blood to anticoagulant. Invert the tube gently several times immediately after venipuncture to ensure proper mixing of blood and anticoagulant.

Partially filled or overfilled tubes are not acceptable and must be redrawn (refer to the tube fill level chart located near the coagulation centrifuge).

Specimens that are clotted, or have hemolysis must be redrawn. If unable to redraw, add result comment indicating the result should be viewed with caution noting that hemolysis and/or icterus are present.

Grossly lipemic specimens that give an error or no result, may be cleared using the Mikro 200 Centrifuge:

**Handling Conditions:**

Prior to centrifugation, check for clot formation by gentle inversion. Centrifuge blood for 3 minutes at 7200 RPM. Specimens centrifuged with the plasma remaining on the top of the cells in the unopened tube remains stable for 4 hours at 20 ± 5˚C on the Evolution. If on heparin therapy, plasmas remain stable for 2 hours at 20 ± 5˚C on the

Evolution. Plasma separated from the cells may be stored frozen for up to 2 weeks. Thaw specimen in 37ºC water bath.

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**EQUIPMENT:**

Centrifuge
Pipettes
Pipette tips
TYPE –I Reagent Grade Water
STA® - Cuvettes
STA–R®/STA-R Evolution® Analyzer
STA® - maxi Reducers

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**SAFETY REQUIREMENTS :**

W:\NMH\Pathology\Manuals\General\Facilities & Safety\Protection from Biological or Chemical Substances.doc

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**REAGENTS/SPECIAL SUPPLIES:**

1. **STA® - Multi Hep Calibrator :** 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. The UFH calibrators are standardized to the 07/328 International Standard for UFH from 2009. The LMWH calibrators are standardized to the 01/608 International Standard for LMWH from 2003.

   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 each vial is completely mixed. Reconstituted stability on the STA-R Evolution® is 4 hours.

   - 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 3: **STA® - Multi Hep Calibrator 7**: human plasma containing a well defined quantity of LMWH (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
   - Reagent 5**: STA® - Multi Hep Calibrator 18**: human plasma containing a well defined quantity of LMWH that is greater than that of Reagent 3 (see enclosed Assay Value Insert), lyophilized.

2. **Reagent 1**: **Ready to use**: chromogenic substrate CBS 02.44, approximately 9.0 µmoles per vial. Allow the reagent to sit **30 minutes at room** temperature (18-25 °C) 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 the vial. Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R1**.

   Reconstituted stability: 7 days on analyzer in its original vial with the STA® - Reducer and perforated cap in place for 8 ml vials

3. **Reagent 2**: **Ready to use**. Bovine factor Xa, approximately 2.0 IU per vial. Allow the reagent to sit for **30 minutes at room temperature** (18-25 °C) 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 the top. Request the drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the regents into the product drawer, **R2** .

Reconstituted stability: 7 days on analyzer in its original vial with the STA® - Reducer and perforated cap 8 ml vials

4. **STA® - Owren-Koller Buffer**: Ready to use buffer. Used by the STA-R Evolution® to perform dilutions of controls and patients' plasmas. Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R0**. Stability is 72 hours.

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**PROCEDURE:**

Refer to the START-UP procedure for the STA-R Evolution® before running patient specimens on STA-R Evolution® at the start of each shift. Run calibration if needed.

1. Load patients' samples: Barcode labels with LIS downloading of tests

- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded, their barcode labels read and the job list will be fed to the host computer for each tube. The tests will be automatically carried out by the STA-R Evolution®

2. Reagent Forecast

- When patient samples are loaded, if a product is missing or insufficient to run tests  icon will blink in the SYSTEM PANEL ALARM BOX
- Double click on  icon, the  tab will display
- The missing reagent will be listed with **RED triangle** 
- Load the necessary product and the tests will resume

3. All patient results are displayed on the TEST PANEL screen and automatically printed out and transmitted if selected.

4. Operator intervention of sample(s)

**Re-Run Result**

- Double click result in question
- Click **Rerun**
- Confirm by clicking on 

***Add (insert) Test to Sample***

- Scroll on the file identity (accession number…)
- Double click test box to be added for accession number
- Confirm by clicking on 

***Change Priority of Loaded Sample***

- Double click on the file identity (accession number…)
- Click STAT  icon

5. All dilutions of controls and patients' samples are automatically prepared by the STA-R Evolution® according to the parameters entered in the Test Set-up.

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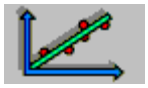
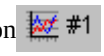
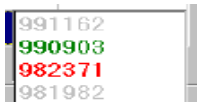
**CALIBRATION:** Calibration will be performed every six months. Calibrations shall also be performed if controls are not within stated ranges.

**Allow all reagents, once loaded to equilibrate on board the instrument for 10 minutes prior to starting the Calibration.**

A. **STA®-Multi Hep Calibrator**: After the reconstitution period, request the product drawer to open by clicking the above icon, select load products, bar code each vial of calibrator and place each vial of calibrator into the product drawer, **RD0**. The assay value for each Heparin Level is transferred to the STA–R® Evolution when each calibrator is loaded and prompted for the bar code information.

B. All standard dilutions are automatically prepared by the STA–R® Evolution by diluting the **STA®-Multi Hep Calibrator** - with STA® Owren-Koller Buffer according to the parameters entered in the Test Set-up.

C. To order the calibration:

- Click on Calibration menu  icon
- Click **LIQHYB1**
  - ❖ Calibration screen includes current calibration curves and product information
  - ❖ Position for two different lots of reagent are available
- Click on calibration icon  or calibration icon  (if both positions are in use, delete one calibration)
- Double click **LIQHYB1**
- Confirm by clicking on **Calibrate…..**
- Select the lot# to be calibrated  (usually the one highlighted in green).
- Confirm by clicking on  .

D. Examine and re-run a point on the calibration curve:

- Click the Calibration menu  icon
- Single click **LIQHYB1**
- Calibration screen includes current calibration curves and product information
- Select the calibration icon
- Select the calibration icon  or the calibration icon 
- If a data point on the calibration curves needs to be repeated, double click the calibration point to rerun .
- Confirm by clicking on  .

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**CALCULATIONS and INTERPRETATION of RESULTS:**

1. The STA-R Evolution® automatically converts the results from a linear standard curve (log-lin) to IU/ml.
2. The STA-R Evolution uses a dilution of 1:4, sample to buffer. No **auto re-dilution is allowed with this assay.** See Notes section below for directions on how to handle a patient sample with higher values.

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**QUALITY CONTROL:**

A combination of three controls will be used to cover the entire hybrid curve and be representative of both UFH and LMWH. The following is the combination that will be used.

- **STA® - Quality HNF/UFH Control 2 and 7**
- **STA® - Quality HBPM/LMWH 14**

1. **STA® - Quality HBPM/LMWH:** Two plasmas containing different levels of low molecular weight heparin (LMWH) intended to be used for quality control of LMWH assays using the anti-Xa activity. The kit contains 2 controls. The control that will be used is the **LEVEL 2** control for this method. The control is standardized to a secondary standard for the 01/608 International Standard for LMWH from 2003.
   - **STA® - Quality HBPM/LMWH Control 14**: plasma containing a well defined quantity of LMWH (see enclosed Assay Value Insert), lyophilized. Labeled Level 2.

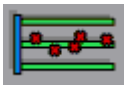
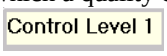
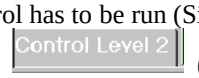
Tap vial gently on counter top. Reconstitute each vial with 1.0 ml reagent grade water. Let sit **30 minutes** at room temperature. **Swirl** gently to ensure each vial is completely mixed. Reconstituted stability on the STA-R Evolution® is 4 hours.

2. **STA® - Quality HNF/UFH:** 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. The kit contains 2 controls and both are to be used as controls for this method. These controls are standardized to a secondary standard for the 07/328 International Standard for UFH from 2009.
   - **STA® - Quality HNF/UFH 2**: human plasma containing a well defined quantity of UFH (see enclosed Assay Value Insert), lyophilized.
   - **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 the vial is completely mixed. Reconstituted stability on the STA-R Evolution® is 4 hours.

**Running Controls:**

1. To load STA® - Quality HBPM/LMWH **14** control and STA® - Quality HNF/UFH **2** and **7** control: After the reconstitution period, request the product drawer to open by clicking the Products menu  icon, select load products, bar code the controls and place the controls in the product drawer, **R0**.

2. To order QC manually:
   - Click the Quality Control menu  icon
   - Select the test abbreviation for which a quality control has to be run (Single Click only)
   - Select the Control Level tab #  or  (#corresponds to the number of the desired level – Single Click only)
   - Single Click on  icon.
   - Confirm by clicking on OK.
   - All control ranges are monitored automatically by the STA-R Evolution®. If any controls are outside of the bar coded ranges or the site specific QC range, the STA-R Evolution® will audibly and visually alert the operator by the Quality Control menu icon blinking at the System Panel Area.

3. Control results are automatically filed in the STA-R Evolution® QC file. All results for a 24-hour period will be converted to a "mean" value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean. Each point can be viewed on the Levy-Jennings Daily control chart by clicking the left arrow. To print all the QC data points for any test used to calculate the mean for a specific day, perform the following procedure.
   - Click the Quality Control menu icon.
   - Select the test abbreviation for which a quality control has be run (Single Click only)
   - Select the  or  tab (#corresponds to the number of the desired level – Single Click only)


- Select the date on the Levy-Jennings chart
- Click the Print icon.
- Then select the appropriate button,
- Confirm by clicking **Validate.**

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**LIMITATIONS:**

1. 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.
2. 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. Every month each of the Stat Spins are tested by running platelet counts on plasma that has completed the three minute spin.
3. The STA® - Liquid Anti-Xa assay relies on the Antithrombin level of the patient. Low levels [less than 60%] may reflect an underestimation of the heparin level result.
4. The STA® - Liquid Anti-Xa assay (UFH and LMWH protocols) is insensitive to the following substances:
   - Hemoglobin: up to 1.5 g/l
   - Conjugated bilirubin: up to 288 mg/l – 342 umol/l
   - Unconjugated bilirubin: up to 138 mg/l – 236 umol/l
   - Triglycerides: up to 6.9 g/l – 8 mmol/l

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**REFERENCE INTERVALS (NORMAL VALUES) :**

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

The therapeutic range is specific for each lot of LMWH. Refer to ranges recommended in package insert from the heparin manufacturer.

An interpretive comment is added automatically to every heparin result:
**Heparin Anti-Xa UFH**
*Therapeutic Range: 0.3 - 0.7 U/mL*
*Recommended prophylaxis levels are 0.2 - 0.4U/mL*
**Hep Xa LMWH**
*Therapeutic Range:*
*BID Dose: 0.5 - 1.1 U/mL*
*Once Daily Dose: 1.0 - 2.0 U/mL*
*Recommended prophylaxis levels are 0.2 - 0.4 U/mL*

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**IMMINENTLY LIFE-THREATENING (CRITICAL) TEST RESULTS:**

**Critical Heparin Anti-Xa UFH: Greater than 1.0 IU/mL.**

Any results greater than 1.0 must be phoned to the patient's nursing unit or physician as a Critical Value. Indicate using Result Comment window the following: *Critical result called to* (RN name), *at* (Notification date and time). You may use the template "CRITV" (for critical results that are verbally read back) for Critical result documentation.

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**DOCUMENTATION/REPORTING:**

The results for the Heparin Assay are reported out to the nearest 0.10 IU/ml.

**DETECTION THRESHOLD and LINEARITY RANGE:**

The detection threshold for STA® - Liquid Anti-Xa (UFH and LMWH) on the STA® System 0.10 IU/mL**.** (Reported as <0.10 IU/ml).

The linearity range extends to 1.1 IU/ml for UFH and to 2.00 anti-Xa IU/ml for LMWH.

See **NOTES** for dilution procedure.

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**COURSE OF ACTION:**

The two Stago Evolutions are used as back up to each other. All tests can be run on either instrument.

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**NOTES:**

1. The assay's linearity extends to 1.1 for UFH and to 2.00 IU/ml for LMWH. In order to determine a heparin level on a sample with a higher level, the operator must:
   - mix the patient sample 1:1 with 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 for UFH = 2.2 IU/ml
   - extended linearity for LMWH= 4.0 anti-Xa IU/ml

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**REFERENCES:**


1. 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.
2. 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.
3. STA®- Quality HNF/UFH (**Cat. No. 00381):** Control Plasmas for Assays of Unfractionated Heparin (UFH) Using Anti-Xa Method on STA® Analyzers. Package insert 24208 02 – December 2010.
4. STA® - QUALITY HBPM/LMWH **(Cat. No. 00686)**, Control Plasmas for Assays of Low Molecular Weight Heparins (LMWH) using Anti-Xa Method on STA® Analyzers. Revised January 2005.
5. STA–R® and STA-R Evolution® Operators Manual.
6. STA® - Owren-Koller Buffer **(Cat. No. 00360)** Buffer Solution for Coagulation Testing. Package insert 23070 06 – June 2011.
7. CLSI guidelines H3 – A6 and H21 – A5, or latest revision
8. Woodhams B *et al*. Stability of Coagulation Proteins in Frozen Plasma. *Blood Coag Fibrinol.* 2001; 12(4):229-236.
9. Clinical and Laboratory Standards Institute (CLSI). Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline- Fifth Edition. H21-A5 vol. 28 No. 5 or latest revision.
10. Clinical Laboratory Standards Institute. Collection and Processing of Blood Specimens for Testing Plasma Based Coagulation Assays and Molecular Hemostasis Assays: Approved Guideline. Fifth Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2008. Document H3 – A6 or latest revision.


*For additional information, please refer to the manufacturer's package insert.*

STA – R Evolution® Software Version 2.42.01
STA – R Evolution® Software Version 3.03.05

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**SIGNATURES:**

| **APPROVED BY:** | **AUTHORED BY:** |
|---|---|
| Dr. Gene Herbek, Section Medical Director | Barbara Flynn, MT(ASCP)H |

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