Heparin Anti-Xa Assay (UFH)

PURPOSE

This procedure provides instructions for performing the Heparin Anti-Xa Assay. This assay determines the plasma activity levels of unfractionated (UFH).

DILUENT /EQUPMENT

- STR-R coagulation analyzer
- STA Owren-Koller Buffer
- STA mini Reducer
- Pipettes
- Reagent grade water (NERL).

SPECIMEN

- Blue top tube-citrated plasma.
- Centrifuge within one hour of collection.
- Test must be performed within 2 hours of collection; otherwise FREEZE plasma until analysis can be performed.
- Samples that are less than 80% full, clotted or grossly hemolyzed should be rejected.

REAGENTS: CALIBRATORS

STA Multi Hep Calibrators for **UFH**:

- **Reagent 1**: STA®- Multi Hep Calibrator **0**
- **Reagent 2**: STA®- Multi Hep Calibrator **4**
- **Reagent 3**: STA®- Multi Hep Calibrator **7**
- **Reagent 4**: STA®- Multi Hep Calibrator **10**
- **Reagent 5**: STA®- Multi Hep Calibrator **18**

REAGENTS: CONTROLS

STA® Quality HNF/UFH Controls for **UFH**:

- **Reagent 1**: STA® Quality HNF/UFH Control **2**
- **Reagent 2**: STA® Quality HNF/UFH Control **7**

Heparin Anti-Xa Assay (UFH), Continued

CONTROL & CALIBRATOR RECONSTITUTION

Reconstitution of reagents and controls is very critical to getting your controls within range. Wait times are very important.

Step	Action
1.	Always use the pipette provided for reconstitution.
2.	Use freshly poured reagent grade water. Do not pipette directly out of the bottle.
3.	Before opening, tap bottle on hard surface to make all the particles fall to the bottom of the vial.
4.	When opening the vials, pop the vacuum seal by slightly lifting the rubber seals. Tap on the rubber stoppers to dislodge the lyophilized reagent on the caps so it goes back into the bottles and not on the counter. Remove the rubber stoppers.
5.	Add one (1) mL reagent grade water. Swirl to mix. Replace rubber stoppers.
6.	Let them sit for five minutes at room temperature, remove and discard the rubber stoppers.
7.	Replace the white caps on the bottles. Swirl again to mix.
8.	Allow controls/calibrators to stand at least another 25 minutes at room temperature.
9.	Load on instrument and let sit 10-15 minutes prior to calibrating or running controls.

REAGENTS STABILITY

STA® Multi Hep Calibrators for **UFH**:

- Reconstituted stability on the STA-R is **4 hours.**

STA® Quality HNF/UFH Controls for **UFH**:

- Reconstituted stability on the STA-R is **4 hours.**
- **7 days** at 2 – 8 °C in the original **capped** vials.

STA® LIQUID ANTI – Xa

- **Reagent 1** (Substrate) & **Reagent 2** (F. Xa)
  - **7 days** on STA-R (with Reducer)
  - **3 months** at 2 – 8 °C in the original **capped** vials (without Reducer)

Heparin Anti-Xa Assay (UFH), Continued

REAGENTS TESTING

STA® LIQUID ANTI - Xa

A bar-code insert is provided in each box of reagents. This contains the lot number, kit code number, and reagent code numbers.

Reagent 1: Substrate (ready to use)

- Allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use.
- Then install a mini Reducer in the vial and replace the perforated plastic cap on top.
- Request the product drawer to open by clicking the Products menu icon, select load products, bar code the reagent and place the reagent in product drawer R1.
- Equilibrate on instrument 10-15 minutes before using.

Reagent 2: F. Xa (ready to use)

- Allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use.
- Then install a mini Reducer in the vial and replace the perforated plastic cap on top.
- Request the product drawer to open by clicking the Products menu icon, select “open”, barcode the reagent and place the reagent in product drawer, **R2**.
- Equilibrate on instrument 10-15 minutes before using.

Heparin Anti-Xa Assay (UFH), Continued

CALIBRATION	See P&P STA-R Calibration procedureCOAG-03-0040 for instructions
QUALITY CONTROL	See QC section of Coagulation P&P STA-R Startup and Specimen Processing procedure COAG-03-0010.
PATIENT TESTING	See Load Reagents and Running Patients section of Coagulation P&P STA-R Startup and Specimen Processing procedure COAG-03-0010
THERAPEUTIC RANGE	See Coagulation P&P COAG-01-0030
CRITICAL VALUE	See Laboratory P&P 05-090-01 If value exceeded the reportable range see Linearity section of Coagulation P&P Heparin Anti-Xa Assay COAG-01-0020

Heparin Anti-Xa Assay (UFH), Continued

LINEARITY (UFH)

The acceptable linear range for this test is 0.10 to 1.10 IU/mL. A result of less or greater than this range must be acted upon. If the result is > 1.10, the STA-R will send a result of 1.10 to merge in CERNER. **All results of 1.10 must be reviewed on the instrument as follows**:

IF UFH RESULT IS 1.10 THEN CLS MUST TAKE ACTION:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1.</td><td>Double click the patient ID. Patient test screen appears.</td></tr>
<tr><td>2.</td><td>Select the UFH test.</td></tr>
<tr><td>3.</td><td>Review the RAW DATA (IU/mL) in lower right corner of window.</td></tr>
<tr><td></td><td>
<table>
<tr><th>IF</th><th>THEN</th></tr>
<tr><td>Result is 1.10</td><td>Repeat & if result duplicates, report result.</td></tr>
<tr><td>Result is >1.10</td><td>Dilute patient plasma 1:2 with normal pooled plasma[1], then retest.
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Result is <=1.10</td><td>Multiply x 2, report result if it is 1.10 or less.</td></tr>
<tr><td>Result is >1.10</td><td>If result is >1.10, report “>1.10”</td></tr>
</table></td></tr>
</table>
<u>IMPORTANT</u>:

DO NOT “Convert Results” to “Free text”, just type in < or > and the value, it will default to what is the linearity in Cerner and trigger the critical notification pop up if applicable.</td></tr>
<tr><td>4.</td><td>Click on the Bell icon (lower left corner) to view all error codes pertaining to this result.</td></tr>
</table>

[1] Use NPP (normal pooled plasma), located in -20°C freezer.

Heparin Anti-Xa Assay (UFH), Continued

LINEARITY (UFH), Continued

IF UFH RESULT IS LESS THAN 0.10, THEN:

Step	Action
1.	Double click the patient ID on the Star monitor.
2.	Select the UFH test.
3.	Review the **RAW DATA** (lower right corner of window). Verify that a test was completed and not a "QNS" or error. Then report as "<0.10".
	IF / **THEN** Result is 0.10 → Report patient result Result is <0.10 → Report the patient result as "<0.10" Result is 0.00 → Make certain the instrument did not get "QNS" or test error before reporting result as "<0.10"
4.	Click on the Bell icon (lower left corner) to view all error codes pertaining to this result.

LIMITATION

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

Heparin Anti-Xa Assay (UFH), Continued

Controlled Documents

The following controlled documents support this procedure.

Reference
Diagnostica Stago product insert: April 2012 STA-R Operator Manual

Form

Author(s)

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Imp.
Major	1) Removed procedure for LMWH. Test not done in medical center. 2) Revised procedure to reflect the new Liquid Anti-Xa reagent that is now being used for UFH.	Julius Salomon 03/28/13			3/28/13
Minor	Reviewed and revised Calibrators section.	Julius Salomon 09/12/14			
Major	Page 5: Revised reporting section if result is >1.10 or <0.10 to reflect current policy.	Julius Salomon 09/01/15			
Minor	1. Updated P&P format and Index number 2. Updated the referenced P&P number for QC, Calibration and Patient run	Marlon Esguerra 7/12/18			
Major	Revised the critical value section to reflect only value that exceeded reportable range	Marlon Esguerra 7/12/18			

Imp. =Implemented