| **Ridgeview Medical Center Laboratory Services** | **Department of Origin****COAG** | **Effective Date****03/18/2013** |
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| **Purpose**To provide instruction for performing the Heparin Unfractionated (anti-Xa) assay, including specimen type and handling, supplies and reagents, reconstitution of those reagents, registration of new lots, loading reagents and consumables, sample processing, reporting results, therapeutic range and limitations of the procedure. **Principle** Heparin considerably accelerates the inactivation of coagulation Factor Xa and thrombin by antithrombin III. For this reason unfractionated and low-molecular-weight heparin preparations are widely used as therapeutic anticoagulants. Due to numerous influences, the effect of an identical dose of heparin varies from patient to patient. Using a Heparin anti-factor Xa protocol it is possible to monitor therapy both with low molecular weight (LMW) and unfractionated heparin preparations.Factor Xa is inactivated by ATIII during the incubation phase of the test. This reaction is catalyzed by heparin. Dextran sulfate (DS) releases heparin which has bound to interfering factors and thus makes it accessible to the assay. The quantity of FXa remaining after the incubation phase is determined via the increase in absorbance at 405nm, using a chromogenic substrate on a CA-1500 analyzer.heparinbound + DS DSbound + heparinfree heparinsampleF Xa + AT IIIexcess [F Xa-AT III] + F Xaresidue F XaresidueChromogenic substrate tripeptide + dye |
| **Specimen**Type:Mix nine parts of freshly collected blood with one part of 0.11 mol/L (3.2%) sodium citrate anticoagulant, avoiding the formation of foam. 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 with caution for collection. If multiple specimens are collected, the coagulation sample should be the first or second tube collected. If blood is drawn from a vascular access device (VAD), the line should be flushed with 5.0 mL saline and the first 5.0 mL of blood or six dead space volumes of the VAD discarded. The citrate concentration must be adjusted in patients who have hematocrit values above 55%. Specimens that are clotted, collected in the wrong tube, have visible hemolysis or have less than the expected fill should be rejected.Handling Conditions:The specimen should be transported at room temperature. The whole blood specimen is checked for clot formation by gentle inversion and centrifuged and tested within 8 hours from the time of specimen collection. Centrifuge the capped specimen tube for a minimum of 10 minutes at 1000 rcf (3600rpm) or StatSpin (2 minutes at 7200 rpm, S/P Brand Stat-60) to consistently produce platelet poor plasma (platelet count <10,000/uL ). To separate plasma, use a plastic transfer pipette; remove the plasma to a plastic tube without disturbing the buffy coat. If testing is not complete within 8 hours, specimens can be frozen at -20°C or below for short term storage (1month) or -70°C or below for long term storage. Frozen plasma samples must be rapidly thawed at 37°C while gently mixing and tested immediately.  **Mixing is critical before testing.** |
| **Reagents** | **Supplies** | **Equipment** |
| * Berichrom Heparin kit containing:
* Dextran Sulfate Reagent
* Factor Xa Reagent
* AT III Reagent
* Substrate Reagent
* Berichrom®Heparin Unfractionated Calibrator
* Berichrom®Heparin Unfractionated Control 1 & Control 2
* Stand Human Plasma (SHPL)
* CA Clean I
* CA Clean II
 | * 4.0 mL Sample Cups
* 2.0 mL Sample Cups
* Reaction Tubes
* Preservative-free distilled water
 | * Sysmex® CA-1500 System
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| Reagent Preparation: (See HepU Calibration Procedure for specific reconstitution directions.)1. **Dextran Sulfate Reagent:** **white** capLyophilized; concentration in the working solution: 0.02 g/L. Dissolve in **10.0 mL** **of distilled water**. (**15**min)
2. **Factor Xa Reagent:** **Orange** capLyophilized. Human plasma fraction with the additives: Tris (6 g/L), sodium chloride (12 g/L) and EDTA (0.74 g/L). Preservative: Sodium azide (<1 g/L). Dissolve in **10.0 mL** of reconstituted **Dextran Sulfate Reagent. (1. See above) (30min)**
3. **AT III Reagent (HUMAN):** **Green** capLyophilized. Concentration in working solution: 1 IU/mL. Preservatives: Sodium Azide (<1 g/L). Dissolve in **1.0 mL of distilled water**. (**15**min)
4. **Substrate Reagent**: **Yellow** capLyophilized; concentration in the working solution: 4 mmol/L Z-D-leu-gly-arg-ANBA-methyl amide. Dissolve in **2.0 mL of distilled water.** (**15**min)

**Storage and Stability**: Store the test kit unopened at 2 to 8 °C. Use before the expiration date given on the label.**After reconstitution:**

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| **Temperature** | **Factor Xa** | **AT III** | **Substrate Reagent** |
| +15 to +25°C | 3 days | 1 week | 2 weeks |
| **+2 to +8°C** | **2 weeks** | **2 weeks** | **6 weeks** |
| -20°C | 2 months | 2 months | 6 months |
| Onboard | 8 hours | 8 hours | 8 hours |

*In the original vial, the solutions can be frozen and thawed up to 3 times.*1. **Standard Human Plasma (DIL SHPL) *used for calibration only***Lyophilized preparation of pooled human, normal citrated plasma and HEPES buffer solution (12 g/L). Used for the calibration of coagulation and fibrinolysis tests.
2. **Berichrom® Heparin Unfractionated Calibrator *(See Calibration procedure)***

Contains unfractionated heparin from porcine intestine (≤ 1.3 IU/mL) and buffered human plasma. The calibrator is lyophilized. Used for calibration with unfractionated heparin. (let stand **30min**)1. **Berichrom® Heparin Unfractionated Control 1 *(See HepU QC procedure)***

 Contains unfractionated heparin from porcine intestine (≤ 0.3 IU/mL) and buffered human plasma. The control is lyophilized.1. **Berichrom® Heparin Unfractionated Control 2 *(See HepU QC procedure)***

Contains unfractionated heparin from porcine intestine (≤ 0.7 IU/mL) and buffered human plasma. The control is lyophilized.1. **CA Clean I** is liquid and ready to use.

 Store at 2-8°C. Do Not Freeze.  Stable unopened at 2-8°C until expiration date on bottle.  Opened bottle stable for 30 days at 2-8°C.1. **CA Clean II** is liquid and ready to use.

Stable unopened at 5-35°C until expiration date on cubitainer.Opened cubitainer is stable for 60 days.Reagent Integrity:Indication of deterioration: No evidence of vacuum in vial upon opening, difficulty in reconstituting reagent, control values outside of determined range**WARNING:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into a sink, flush with a large volume of water to prevent azide build up.  **These products are for in vitro diagnostic use only.**Loading Reagents: (when the instrument is not operating)1. Press the [Set Reagents] key from the Main Menu. The Consumable screen will appear.
2. Confirm that the lid signal (status of cover opening) is green, and then open the light shield lid.
3. Verify that the appropriate group (Group 1) is displayed on the Consumable screen. ***Note:*** *If the appropriate Group is not displayed, press [Select Group] to display the Change Group screen. Press the correct group key, then [Return].*

4. Press [AT3Reag] reagent holder position key 5. Place the vial of AT3 reagent in the appropriate reagent holder position (B5/4.0).1. Verify that the correct lot number is displayed. To change the lot number, press [Lot #], enter the correct information and press [Enter].
2. Verify that the correct expiration date is displayed. To change the date, press [Exp.Date], enter the correct date in the format mm/dd/yyyy, and press [Enter].
3. Verify that the correct vial type is displayed. To change it press [Vial], and select the correct type and press [OK].
4. Verify that the correct volume is displayed in the keypad for the reagent. To change it, enter the correct volume.

10. Press [ENTER] on the numeric keypad. The reagent’s holder position will be displayed with a green background. Repeat above procedure for remaining reagents [HepSubs] (B5/5.0), [FXaReag] (C3/15.0). 1. Repeat the above procedure to add CA Clean I into reagent holder positions A2 and E3. Pour CA Clean II into an appropriate vial. Place CA Clean II in position A1.
2. Close the light shield lid.
3. Press [Return] to display the Main Menu.

Loading Consumables and Discarding Waste Material: (do the following as necessary)**1. Replenish Sample Plates** * Confirm that the “lid” signal (status of cover opening) is green, and then open the light shield lid.
* Remove the used sample plate.
* Confirm on the screen that the sample plate has been replaced with a new sample plate by pressing [OK].
* Place the unused sample plate on the CA-1500. The sample plates are keyed to be loaded in one direction. ***Caution:*** *Do not load partially used sample plates since the instrument will not recognize partially used wells.*
* Close the light shield lid.

**2. Replenish Reaction Tubes*** Reaction tubes can be replenished at any time during operation. Open the reaction tube hopper on the top of the CA-1500 by pressing the front part of the reaction tube hopper lid.
* Replenish/Fill the reaction tubes to the red line inside the reaction tube hopper (Max. 300 reaction tubes).

**3. Replenish Rinse Solution*** Confirm that the instrument is not operating or has been interrupted due to insufficient rinse (error message “Replenish Rinse fluid”).
* Remove the cap with the float switch from the Rinse solution container by turning the cap counter-clockwise. **Caution:** Do not touch the float switch. Rinse the float switch thoroughly with deionized or distilled water if contact is made.
* Replace the Rinse solution with distilled or deionized water.
* Replace the float switch by tightening the cap clockwise to close.
* Verify that the tubing is securely connected and is not kinked.
* If the analysis was interrupted due to insufficient rinse, press [Resume] key.

**4. Dispose of Liquid Waste*** Waste is plumbed into a drain pipe (Heme/coag wall); make sure there is no leakage behind Workstation (Table 6.4).

**5. Dispose of Used Reaction Tubes from the Trash Box*** Confirm that the instrument is not operating.
* On the left side of the instrument, remove the reaction tube trash box.
* Discard the used reaction tubes. ***Caution:*** *Biohazard*
* Clean the reaction tube trash box if necessary
* Restore the reaction tube trash box.
* If the volume monitoring is set to monitor the number of used reaction tubes in the trash box, a message will appear asking the operator if a reset of the number of used reaction tubes is necessary. If the used reaction tubes have been discarded press [OK].
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| SAMPLE PROCESSING  |
| **Step** | **Action** |
| **I.** | Check that the calibration curve is current with the lot # of reagents in use. *[Main Menu–Standard Curve–HepU].* Check date of calibration, Rgt lot #s, exp dates. *(Calibrate every new lot Rgt/s, recalib every 6 mo).* *If required, perform a calibration curve with new lot of reagents. See HepU Calibration procedure.* |
| **II.** | Load HepU reagents. |
| **III.** | Load SamplesSample ID number (Barcoded sample) read by barcode reader / Automatic Inquiry of tests (host connection is operational) |
| 1 | When the host computer is connected using bi-directional communication, host inquiry takes place when the sample ID is read and the analysis parameters are automatically registered. |
| 2 | Load the barcoded sample in the respective rack position with the barcode facing the analyzer. |
| 3 | Place the sample rack into the right rack pool of the sampler (input tray). |
| 4 | Press [Start] to begin processing. |
|  | Manually enter sample ID (non-barcoded) / No automatic inquiry of tests (no host connection or the host is down) |
| 1 | From the Main Menu, press [Work List]. |
| 2 | Verify that <**Register Rack>** is displayed above the parameter keys. If not, press [Rack]. |
| 3 | Verify that the next available rack is displayed (white background). Use the [Prev] or [Next] key to locate the next available rack. |
| 4 | Use the up and down arrow keys to select the desired sample rack position, press [ID No. Entry]. |
| 5 | The numeric keypad is displayed. Enter the sample ID number. |
| 6 | Press the blue test key, [HepU]. Continue to select test to be performed on the sample if necessary.  |
| 7 | Press [Enter]. Each test selected should show the symbol “O”. * To perform the sample in the Micro mode: For samples to be performed in the Micro mode, press the [Micro] key for each sample. An “O” symbol will be displayed on the Micro key. Samples programmed to be performed in the Micro mode will display an “M” in the “opt.” column in the respective Work List position on the screen.
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| 8 | Load the sample in the respective sample rack position. |
| 9 | 1. Repeat until all sample ID numbers and tests are entered for the respective sample rack positions.
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| 10 | When all samples are programmed and loaded in the respective sample rack position, press [Quit] on the numeric keypad. |
| 11 | With all reagents and consumables on board, place the sample rack into the right rack pool of the sampler (input tray). |
| 12 | Press **[Start]** to begin processing. |

REPORTING RESULTS

 Results are reported as heparin concentration in IU/mL. Results that exceed the linearity range of the calibration curve can be diluted with Standard Human Plasma and rerun.

Hemolyzed, lipemic, or icteric samples must be noted with the result.

Therapeutic range:

The heparin therapeutic range is: **0.30 - 0.70 IU/mL**

**Abnormal results:**

Results of **0.009 or less** will be reported as **<0.01**

Results of **0.981 and greater** will be reported as **>0.98** (No dilution needed at this time)

**PROCEDURE NOTES**

The limit of detection for unfractionated heparin is 0.05 IU/mL.

The determination is not disturbed by platelet factor 4 (PF4).

Overall performance of Berichrom® Heparin testing is dependent on reagent and instrument performance. Acceptable variability (imprecision) should be such, that the total coefficient of variation (CV) of the analytic system is less than 15% on the same lot of control plasma.

**LIMITATIONS OF THE PROCEDURE**

* Disposable plastic materials should be used for sampling, measuring and making dilutions of standard materials.
* **The measuring range is defined by the concentration of the calibrator used.**
* Berichrom® Heparin is not suitable for the determination of heparin in samples of patients under heparin-neutralizing medication (i.e. protamine sulfate), as components of the reagent will compensate for the neutralizing effect in vitro and may thus lead to falsely high results. The incubation periods must be exactly observed in order to obtain reproducible results.
* FDA approved reportable range: 0.01 - 0.98 IU/mL or the limits of the calibrator

**SAFETY**

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| Follow RMC Policy-Laboratory Procedure: Infection Control and Laboratory Safety Hygiene Procedure.Dispose of all biohazard materials in marked biohazard containers. |

Human source material. *Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.*

Related Documents/ Backup Procedure

* HepU (anti-Xa) QC
* HepU (anti-Xa) Calibration
* Backup – Sendout to Methodist or Mayo Labs (HEPN)

REFERENCES

* 1. Berichrom® Heparin package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, May 2008
	2. Standard Human Plasma package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, August, 2008
	3. Berichrom® Heparin Calibrator and Controls package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, February 2009.
	4. Clinical Laboratory Standards Institute. Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays: Approved Guideline-Fifth Edition. CLSI Publication H21-A5. Wayne, PA, January, 2008.
	5. Application Sheet for Berichrom® Heparin Assay on Sysmex® CA-1500 System
	6. Sysmex® CA-1500 System Operator’s Manual