| **Ridgeview Medical Center Laboratory Services** | **Department of Origin****COAG** | **Effective Date** |
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| **Purpose** To provide instruction for calibration of the Berichrom Heparin Xa Assay, including supplies & reagents, reconstitution, handling & stability of reagents, registering new lot# reagents, loading reagents and calibrator on analyzer, performing standard calibration and accepting/updating standard curve. Also included are calibration notes and limitations of the procedure.**Policy**The reference curve is valid for the respective lot of the reagent employed. A new curve should be prepared with a new lot of reagent or calibrator, every six months and if indicated by any change in analytical conditions. The calibration curve can be used as long as the assay-dependent control values are within the corresponding acceptance ranges.  |
| **Materials** |
| **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
* Berichrom®Heparin Unfractionated Control 2
* Standard 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
 |
| Preparation: 1. **Dextran Sulfate (Diluent):** Lyophilized; concentration in the working solution: 0.02 g/L.
* **Dissolve in 10.0 mL of distilled water,**
* **Let sit for 15 minutes** to allow water to absorb into lyophilized cake in the bottom of the vial.
* **Swirl after 15 minutes** to mix.
1. **Factor Xa Reagent:** Lyophilized 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).
* **Pour 10.0 mL** of reconstituted **Dextran Sulfate (diluent) into Heparin** **Xa Reagent** vial.
* **Let sit 30 minutes** to allow lyophilized reagent to come into solution.
* **Swirl to mix.**
1. **AT III Reagent (HUMAN):** Lyophilized. Concentration in working solution: 1 IU/mL. Preservatives: Sodium Azide (<1 g/L).
* Dissolve in **1.0 mL of distilled water.**
* **Mix gently and let sit at least 15 minutes.**
1. **Substrate Reagent**: Lyophilized; concentration in the working solution: 4 mmol/L Z-D-leu-gly-arg-ANBA-methyl amide.
* Dissolve in **2.0 mL of distilled water.**
* **Mix gently and let sit at least 15 minutes.**

**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 (SHP.DIL—Used only for standard curve calibration)**Lyophilized preparation of pooled human, normal citrated plasma and HEPES buffer solution (12 g/L). Used for the calibration of coagulation and fibrinolysis tests.
	* Reconstitute lyophilized Standard Human Plasma with 1.0 mL deionized water.
	* Shake carefully to dissolve (without foam formation).
	* Let stand at +15 to +25 °C for at least 15 minutes.
	* Before use, again shake carefully.

**Stability after reconstitution: 15-25°C 4 hours**  -20°C 4 weeks**Note: Do not store** reconstituted Standard Human Plasma **at 2-8ºC.** It may be quick frozen and thawed once within 10 minutes at 37ºC.1. **Berichrom® Heparin Unfractionated Calibrator**

Contains unfractionated heparin from porcine intestine (≤ 1.3 IU/mL) and buffered human plasma. The calibrator is lyophilized. Used for calibration with unfractionated heparin.* Reconstitute HepUF with **1 mL** of deionized water.
* Mix carefully to dissolve (without foam formation)
* Allow to stand at +15 to +25°C for at least 30 minutes.
* Mix gently once more before use.

**Stability after reconstitution:**

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| --- | --- |
| **Temperature** | **Heparin UF Cal.** |
| +15 to +25°C | 24 hours |
| **+2 to +8°C** | **48 hrs** |
| -18°C | 4 weeks |
| Onboard | 24 hours |

**CONTROLS**1. **Berichrom® Heparin Unfractionated Control 1**

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

Contains unfractionated heparin from porcine intestine (≤ 0.7 IU/mL) and buffered human plasma. The control is lyophilized.* **Prepare controls as follows:**
* Reconstitute all controls with **1 mL** of deionized water.
* Mix carefully to dissolve (without foam formation)
* Allow to stand at +15 to +25°C for at least 15 minutes.
* Mix gently once more before use.

**Stability after reconstitution:**

|  |  |  |
| --- | --- | --- |
| **Temperature** | **UF Ct. 1** | **UF Ct. 2** |
| +15 to +25°C | 24 hours | 24 hours |
| **+2 to +8°C** | **48 hours** | **48 hours** |
| -18°C | 4 weeks | 4 weeks |
| Onboard | - | - |

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**.**These products are for in vitro diagnostic use only.**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.* |
| STANDARD CALIBRATION*(Check the Reagent Info in Settings to make sure new-lot-numbers are registered and reagents are loaded on CA1500.)*Registering New Lot Reagents (Before Calibration of New Lot)Main Menu → Settings → Analysis Settings → Reagent Info (Type Selection\*\*) → Reagent (AT3Reag, FXaReag, HepSubs) → Add → Other → OK → copy name/lot#/o.d. (enter 1st 4 digits of lot#) → Enter → Change (name/lot#/Exp date) → Enter → Vial type → change → Select vial type ↓ → OK → Return (or Select type for additional rgts) → Set → Return. *\*\*Type Selection: Control Plasma=HepU.1, Hep.2. Calibrator= UF.CAL & (HepL.CAL)* |
| **Step** | **Action** |
| **I.** | Load Reagents |
| 1 | Press [Set Reagents] – Change Lot #, Select from Registration List – Place reagent into correct position/vial as follows:* B5 – AT3 – 4mL cup
* B6 – HepSub – 5mL vial
* C3 – FXa (Rgt) – 15mL vial
* D8 – Dil SHPL – 2mL cup
 |
| 2 | Close Lid—do not run HepU testing with the lid open. |
| **II.** | Load Calibrator  |
| 1 | Pour the reconstituted **HepCal UF** into a **2 mL** analyzer cup. |
| 2 | Place the analyzer cup into the first position of the sample rack.***Note:*** *If patients or controls are to be run, these samples must be loaded into the next sample rack and programmed in the next rack Work List.* |
| 3 | Load the sample rack(s) in the right rack pool (input tray). |
| **III.** | **Standard Curve Calibration**  |
| 1 | From the Main Menu, press [Standard Curve] to display the Standard Curve screen. |
| 2 | Press [HepU] to display the Standard Curve screen. |
| 3 | Press [Analysis Setting] to display the Standard Curve Analysis Setting screen. |
| 4 | Verify **Sampler** is displayed for the calibrator position.***If not****, press [Sampler/Holder] to change the position.* |
| 5 | Verify **Auto Dil. & Analyze** is displayed for the mode. ***If not****, press [Change Mode] to toggle between the modes.* |
| 6 | Verify that **HepCal U** is displayed as the calibrator. ***If not:**** *Press [Select Reagent]*
* *Use the up or down arrow to select appropriate calibrator*
* *Press [OK]*
 |
| 7 | Use the up [↑] or down [↓] arrow to highlight the **Assay Sheet Val**. position. A keypad will be displayed. |
| 8 | Enter the assay value of the calibrator from the **HepCal U** lot number package insert.  |
| 9 | Press **[Enter].**  |
| 10 | Verify that the dilution ratios are correct. They should be **1/1, 4/5, 3/5, 2/5, 1/5, 0/1.*****If not:**** + *Use the arrow keys to highlight the incorrect dilution ratio*
	+ *Press [Select Dil. Rat.]*
	+ *Use the arrow keys to highlight the correct dilution ratio*
	+ *Press [OK]*
 |
| 11 | Verify the replication for each dilution ratio is set to **2**. ***If not:**** *Use the arrow keys to highlight the incorrect replication field*
* *Enter on the keypad "****2****"*
* *Press Enter on the keypad*
 |
| 12 | Press [Return] or [Quit], whichever is highlighted. The **Update Settings?** box will be displayed.  |
| 13 | Press [OK]. Press [Main Menu]. |
| **IV.** | **START the calibration from a sample rack** |
| 1 | From the Main Menu, press [Work List] to display the Work List screen. |
| 2 | Verify <Register Rack> is displayed on the Work List screen.***If not:*** *Press [Rack] to choose the calibrator position.* |
| 3 | Press [Standard Curve] to display the Standard Curve Parameter Settings screen. |
| 4 | Press [HepU] to register the calibration. The symbol "⭘" will be displayed, indicating the assay has been selected for standard curve analysis. |
| 5 | Press [Return] to display the Standard Curve Work List screen. |
| 6 | Press [Start] to begin the analysis. |
| 7 | Press [Main Menu] to display the Main Menu screen. |
| **V.** | Accept/Update the Standard Curve  |
| 1 | When the standard curve analysis is complete, press [Standard Curve] at the Main Menu to display the Standard Curve screen. |
| 2 | When the **Update the Standard Curve?** box appears, press [OK]. |
| 3 | Press [HepU]. |
| 4 | Press [Update].  ***Note:*** *This does not update the curve; it causes the CA-1500 to display the ‘Standard Curve Update’ menu.* |
| 5 | View the new standard curve to determine the appropriate key to press: * **[Continue]** returns to the previous menu when decision to accept has not been made.
	+ **[Reject Both]** rejects the new standard curve and no calculations are made for samples processed behind the Standard Curve Analysis, even if a previous standard curve exists.
	+ **[Update]** accepts the new standard curve.
	+ **[No-update]** rejects the new standard curve.
 |
| **VI.** | **Printing the standard curve** |
| 6 | From the Main Menu press [Standard Curve]. |
| 7 | Select [HepU]. |
| 8 | From the Standard Curve screen, press [More].  |
| 9 | Press [Output Input] to display the Standard Curve Data Output menu.* + Press [GP Print] to display the **Print Confirmation** message window.
	+ Press [OK] to start printing.
	+ Press [OK] to return to the Main Menu or press [Return] when printing is complete to remain at the Standard Curve screen.
 |
| 10 | Run controls. If QC is acceptable, proceed with patient testing. |

**CALIBRATION NOTES**

A new reference curve should be established with each change of reagent lot and change of instrument or with any deviation from control or proficiency testing limits. A new reference curve should also be established every 6 months regardless of reagent lot number.

**LIMITATIONS OF THE PROCEDURE**

Fresh Normal Pooled Plasmashould be free of platelets (<10,000 platelets/uL.) When removing plasma from the cells, the plasma should be re-centrifuged (double-spun) to ensure that it is platelet free. If frozen Normal Pooled Plasma is used, thaw and centrifuge before using.

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.

**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)
* HepU (anti-Xa) QC

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

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| **Annual Review** |
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