Wake Forest Baptist Medical Center	**Chromogenic Factor VIII CL-CO26**	**Dept:**	Clinical Core Lab-Hematology Section
		Effective Date:	4/24/19
		Revised Date:	
		Contact:	Clinical Core Lab-Hematology Management
Name & Title: Gregory J. Pomper, MD Medical Director of Pathology Laboratories		**Date:**	4/24/19
Signature: GJP			

1) General Procedure Statement:

a. **Scope:**
To provide laboratory testing personnel with instructions for performing laboratory procedures as deemed appropriate by industry practices and regulatory agencies to assist in quality patient care.

b. **Responsible Department/Party/Parties:**

i. Procedure owner:	Clinical Core Laboratory Management-Hematology
ii. Procedure:	Clinical Core Laboratory Personnel
iii. Procedure prepared by:	Catherine Gilkey
iv. Supervision:	Clinical Core Laboratory Management-Hematology Clinical Core Laboratory Specialist and Designees Medical Director Clinical Hematology
v. Implementation:	Clinical Core Laboratory Management-Hematology Clinical Core Laboratory Specialist and Designees Medical Director Clinical Hematology

2) Definitions:
N/A

3) Procedure:

PRINCIPLE
Hemophilia is a sex-linked hemorrhagic disease caused by circulating Factor VIII or Factor IX deficiency. Hemophilia A is the term used for Factor VIII deficiency, and is more common than Hemophilia B (Factor IX deficiency). Approximately 10-40% factor VIII is required for normal hemostasis; below this, a tendency towards bleeding is apparent. In this chromogenic assay, the factor VIII in the sample is activated by thrombin. The FVIIIa (activated VIII) accelerates the conversion of Factor X into Factor Xa in the presence of activated Factor IX, phospholipids and calcium ions. The Factor Xa activity is assessed by hydrolysis of a substrate specific to FXa. The rate of release, read at 405nm, is proportional to the factor FVIII chromogenic activity in the sample.

SPECIMEN
Type:
Mix nine parts of freshly collected blood with one part of 3.2% sodium citrate anticoagulant. 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. Routine coagulation samples should be the first tube collected, unless sterile samples or a plain red top tube is to be collected. Special Coagulation tests should have a plain red tube before light blue. If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 mL saline and the first 5 mL of blood

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:

See "Coagulation Blood Collection Policies," CL-CG01, for detailed information regarding processing of coagulation samples.

Specimen Preparation:

Per the reagent manufacturer, Heparin concentrates of up to 10 U/mL do not interfere with the Factor VIII Chromogenic Assay. No additional sample preparation is needed.

SAFETY

- Personal protective equipment for this procedure:
  - Gloves worn at all times
  - Impermeable lab coats, worn closed at all times.
  - Shield when removing sample caps and pushing smears and any time there is a risk of sample or reagent splashing.
  - Approved Protective Eyewear when there is a risk of reagent splashing, pressurized air or instrument parts detaching and becoming airborne.

CLIA COMPLEXITY: High Complexity

REAGENTS AND MATERIALS

Equipment:

CS-5100® Coagulation Analyzer
Cuvettes
Waste, rinse and disinfectant containers
Sample Cups

Materials:

Chromogenic Factor VIII reagent FX
Chromogenic Factor VIII reagent IX
Chromogenic Factor VIII Substrate
Chromogenic Factor VIII Substrate Buffer
Standard Human Plasma
Control material: Control Plasma N, Control Plasma P, SLD mini cup
Preservative-free distilled or deionized water
CA Clean I
CA Clean II
OV Buffer

Preparation:

Reconsitute 1 bottle of Factor VIII Chromogenic Substrate with 1 mL deionized water and equilibrate for 30 minutes at 15-25°C. Add 7mL Factor VIII Chromogenic Substrate Buffer into the Factor VIII Chromogenic

Substrate bottle, yielding 8mL of ready for use Substrate Reagent and put the Factor VIII Chromogenic Substrate bottle on the reagent table. Reconsitute Reagent FX with 2 mL of deionized water. Reconstitute Reagent IX with 2.0mL of deionized water and allow to equilibrate for 30 minutes.

(a) Reconstitute Standard Human Plasma, Control Plasma N and P with 1 mL dH_2O.
(b) Restopper vial and allow to stand 15 minutes until dissolved.
(c) Invert gently to mix. **Do not shake.**

Indication of deterioration: Normal plasma or controls will show deviations in results from the established range.

ONBOARD STABILITY

Factor VIII Chromogenic Reagent FX, reagent table, 18 hours
Factor VIII Chromogenic Reagent FIX, reagent table, 18 hours
Factor VIII Chromogenic Substrate, reagent table, 18 hours
Factor VIII Chromogenic Substrate Buffer, n/a (does not go on board)
Control N, reagent table, 13 hours
Control P, reagent table, 7 hours

STORAGE AND STABILITY

The reagents may be used up to the expiry date indicated on the label if stored at 2-8°C. Stability after reconstitution is 8 hours at 15-25°C, or 3 days at 2-8°C.

Controls N, P and SHP are good for 4 hours at 15-25°C.

QUALITY CONTROL: Control Plasma N, Control Plasma P

(1) For specific control plasma values see "Table of Assigned Values" for the respective lot numbers.
(2) Controls should be tested once per shift of use.
(3) Control values are good for up to **three hours** only.
(4) Controls should also be tested upon reagent changes, and after each new calibration curve
(5) Controls should be run in the same manner as the test samples.
(5) Corrective action when tolerance limits are exceeded:
(a) Recalibration may be necessary if control values are outside the target range.
(b) Verify reagent performance.
(c) Check instrument performance.
(d) Document actions taken to identify and correct the problem before reporting any patient data.

CALIBRATION:

1. Enter reagent and calibrator lot information in Reagent Lot Master if not already done.
2. Load SHP and factor deficient reagent in the refrigerated reagent wheel. Load the buffer on the room temp reagent holder.
3. Press Order.
4. Press Switch Order
5. Select desired factor for calibration.
6. Press Change and select the correct reagent lot number.
7. Press OK.
8. Select the correct calibrator lot from the list on the right. Assay values automatically display if the lots are loaded in Reagent Lot Master. If not, locate the assay value from the package insert from the SHP lot being used to calibrate the reagent.
9. Press OK.

10. Press Start.
11. Once calibration is finished, press Calib. Curve.
12. Press Change.
13. Select the Assay key for desired factor.
14. Select the lot number
15. To compare to the previous lot, press Detailed Display.
16. Press Select Compared Calib. Curve
17. Select a calibration curve to compare and press Load.
18. View the curves, and if appear to be acceptable, click Print.
19. Press Close.
20. Press Validate to validate the calibration curve (MUST do this prior to ordering QC).
21. Order QC to verify calibration is acceptable.
22. File curve in the Calibration Logbook and document QC.

LOADING PATIENT SAMPLES ON THE CS-5100

Factor samples are not interfaced and will not download. Follow the steps below to order a factor.

1. Load barcoded samples into any sample rack with the barcode label visible.
2. Load the rack on the right side of the analyzer.
3. Click Order.
4. Click Switch Order.
5. Select Rack Order.
6. Enter the rack number
7. Click Order Entry from the tabs on the right.
8. Type in the CID or scan the barcode.
9. Order the PT/PTT and the factor.
10. Press the start button on the computer screen to send the order to the instrument.

II. BAR-CODED SAMPLES WITHOUT HOST – LIS Downtime Method

Same as above.

LOADING AND PROGRAMMING CONTROLS ON THE CS-5100

Control N and Control P are the QC materials for all factor assays.

1. Place control bottles into a "C" rack with the control material in an SLD mini cup.
2. Press Order
3. Press Switch Order
4. Press Holder QC Order
5. Press Order Entry.
6. Select the QC File radio button on the left to bring up the list of QC files. Select Control N and Control P from the list and order the desired factor for each.
7. Press Ok.
8. Press Start.

REPORTING RESULTS:

Results of the factor assay testing should be reported in % normal. Print the patient results from the coagulation analyzer. Please observe the analyzer job list closely for values that exceed the linearity of the method (values above or below measurable range of curve will be displayed with a "<", or ">" symbol).

LIS CODES:

PROCEDURE NOTES:

We do not run the MDA standard for this assay. Report the single result.

To Result in LIS:

1. From the Outstanding List, double click on the sample. This opens the Result Entry Activity window.
2. Click on Edit. Enter the result.
3. Click on the paper icon to the right of the result. This opens the Component Comment window, if any comment needs to be appended.
4. Click Save →Verify →Final Verify

NORMAL RANGES

The normal range is 70-150%, as indicated by the package insert from Siemens.

CRITICAL/PANIC VALUE

None.

LIMITATIONS

MEASURING INTERVAL:

11.2-149.0%

Report any value under 11.2 as <11.2% and any value over 149 as >149%. Do not dilute.

Review/Revision/Implementation:

a. Review Cycle: 2 years
b. Office of Record: Department of Clinical Core Laboratory-Hematology
c. All new procedures and procedures that have major revisions must be signed by the Laboratory Director.
d. All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.

Related Procedures:

References, National Professional Organizations, etc.:

Attachments:

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Related Procedures:

References, National Professional Organizations, etc.:

Attachments:

Revision Dates:

Review Date	Revision Date	Signature
	4/24/19	Cathy Gilley
4/24/19		Heather Lawson