|  |
| --- |
| **Factor IX Chromogenic Assay** |
| **Purpose** | This procedure provides instructions for Factor IX Chromogenic Assay in plasma.  |
| **Principle** | Factor IX in the sample is activated by human XIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Factor VIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined by the hydrolysis of a chromogenic FXa substrate. The FIX activity of the sample is assigned vs. a FIX plasma or FIX concentrate standard with FIX potency expressed in international units (IU). F.IX + F. XIa FIXa F.VIII + Thrombin F.VIIIa  F.X F.VIIIa F.Xa FIXa, PL, Ca²   F.Xa Z-D-Arg-Gly-Arg-pNA Z-D-Arg-Gly-Arg-OH+pNA (yellow) The Sysmex CS-5100 is a fully automated coagulation analyzer. The CS-5100 can analyze samples using clotting, chromogenic and immunoassay methods. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist.
 |
| **Materials** | **Equipment** | **Reagents** | **Supplies** |
|  | * **Sysmex CS-5100 System**: analyzer, personal computer, printer and associated non-disposable parts.
* **Reaction Tubes Sysmex CS**

PN 10488059• **Plastic transfer**  **pipettes**• **4ml sample cups** PN 10446526• **SLD Mini Cups** PN 10709524 | Factor IX Chromogenic Test Kit containing;**Reagent A** - lyophilized preparation containing human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor. Reconstitute with 1.4ml of water. Allow to stand 5 min.at room temperature with intermittent mixing for complete reconstitution.Stability is 72 hours at 2-8°C or 12 months at -70°C.**Reagent B** - lyophilized preparation containing human FXIa, human FII, calcium chloride and phospholipids.Reconstitute with 8.0ml of water. Allow to stand 5 min.at room temperature with intermittent mixing for complete reconstitution.Stability is 72 hours at 2-8°C or 12 months at -70°C.**FXa Substrate** -Liquid solution of chromogenic Xa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor. Ready for use.Opened vial is stable for 1 month at 2-8°C.**FIX Diluent Buffer, Stock Solution** – Liquid stock solution of diluents buffer, containing a heparin antagonist. For working solution prepare 1 part stock solution with 9 parts water.Open vial stability of stock solution is 1 month at 2-8°C.Working solution should be used the same day of preparation.**Standard Human Plasma (SHPL)** PN 10487098- lyophilized preparation of pooled human, normal citrated plasma and HEPES buffer solution (12 g/L)Reconstitute lyophilized SHPL with 1.0 ml distilled or deionized water.Mix carefully, let stand at 15-25°C for at least 15 minutes, mix again carefully before use.Stability 4 hours at 15 - 25°C.**Control Plasma N (BEN)**PN 10446235 **Control Plasma P (BEP)** PN 10446472 - lyophilized preparation of pooled normal plasma stabilized with HEPES buffer solution (12 g/L). Used for Quality Control (Normal and Pathological).Reconstitute lyophilized BEN and BEP with 1.0 ml distilled or deionized water.Mix carefully, let stand at 15-25°C for at least 15 minutes, mix again carefully before use.Stability: 16 hours on board analyzer.• **AFAC (Abnormal Factor Control)**Dilute Control Plasma P 1:5 or add 4 ml of water to a vial of Control Plasma P, pour into a sample cup, load in a rack. order the test factorVIII or IX low and load on analyzer. Result manually in Sunquest asC – AFAC. This control is only run with the lowcurve. | * **Type I deionized water,**

Available in canisters used to collect Type I water from the Millipore system. Stable seven (7) days.• **Owrens Veronal Buffer (OVB)** PN 10445724, (10 x 15 ml). **Stability:** 24 hours on board analyzer.• **CA System Buffer** PN 10873440 (8 x 250 ml)  **Stability**: 4 days on board analyzer.• **CA Clean I**  PN 10445689, (50 ml) **Stability:** 5 days on board Analyzer, 1 month 2-8°C.* **CA Clean II** PN 10708787,

(45mL) or CA Clean II PN 10445688 (500mL)**Stability**: 5 days on board analyzer, 2 months 5-35°C |
| **Special Safety Precautions****Sample** | 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.[MSDS Search | MSDSonline](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True) Childrens Star Net1. Collect blood from a clean venipuncture; avoid foaming.
2. Mix nine parts of freshly collected blood with one part 3.2% (0.105 M) sodium citrate:
3. Add 1.8 mL whole blood to 0.2 mL 3.2% sodium citrate (blue-top Vacutainer tube)

- or -1. Add 2.7 mL whole blood to 0.3 mL 3.2% sodium citrate (blue-top Vacutainer tube)

- or -1. Special tubes must be prepared for patients whose hematocrit is > 55%. See procedure entitled *Citrate Concentration Adjustments.*
2. Invert to mix well; transport to lab at room temperature.
3. Check sample for clots with applicator sticks.
4. Centrifuge in Stat Spin for five minutes or ten minutes at 3000 rpm at room temperature.

 1. Sample for testing: Remove plasma from RBCs and place in a 4 mL plastic

Cup, spin again and remove plasma leaving a small amount in the bottom of the first cup. Allow for 100 l of dead space in the sample cup for testing.1. Specimen Stability:
2. Plasma must be frozen if testing cannot be completed within four (4) hours.
3. Plasma two (2) weeks when stored -20°C.
4. Plasma six (6) months when stored -70°C (rapidly frozen).
5. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
6. If there is a delay in sample transport:
7. Notify supervisor or pathologist.
8. If approval is given to run test, append one of the following to the result:
* “-DELA” (transport delayed)
1. Reject specimen if:
2. Clotted
3. Tubes insufficiently filled (tubes may vary by no more than +/-10%, see comparison tubes by centrifuge).
4. Incorrect ratio of anticoagulant to blood.
5. Grossly hemolyzed specimens should be rejected unless a new specimen cannot be drawn without causing the patient trauma or a non-hemolyzed sample is unobtainable (post-op heart, ECMO, etc.).

**If a hemolyzed sample is tested, add one of the following comments to the result depending on the amount of hemolysis:*** + “-HP” (hemolysis present may affect results)

 or – * + “-GRH” (gross hemolysis may interfere with testing)
1. Notify unit or physician of unacceptable specimens; enter appropriate comment in the computer.
 |
| **Calibration****Quality Control** | Calibration is done using SHPL as calibrator, one vial per calibration. 1. A calibration **must** be done every time a new lot of reagents is opened. Dilute and prepare reagents according to directions.
2. Enter reagent and calibrator lot information in the Reagent Lot Master.
3. Load reagents. Slowly dispense the entire volume of the calibrator into a SLD Mini cup.
4. Insert the vial into a C-Rack and place back into the reagent Table.
5. Close the cover and press O.K. to read the barcode.
6. On the Reagent screen, highlight the vial just loaded and press Change to update the date and time.

Refer to the Supply and Reagent Management section of the System Training  Workbook pages 14-22 for more details on steps 2-6.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)1. Order the calibration curve.

**Press Order / Switch Order / Holder Calib Curve Order / Select the desired assay to be calibrated / Press Change / Press O.K. / Select Calibrator / Press O.K. / Press** **Start / to view calibration status press job list.**1. When calibration is complete view the new calibration curve.

**Press Calib. Curve / Press Change / Select correct assay / Select lot number.**1. To compare new versus current calibration curve.

**Press Calib. Curve / Press Detailed Display on the Operation Panel / Press selct Compared Calib. Curve / Select a curve to compare, press Load / Compare curves / Press Close.**1. Validate or Delete the new Calibration Curve.

**Display the desired calibration curve / Press Validate to validate the curve or Delete to delete the curve / Press O.K. / Press Print** Note: Validate the new calibration curve by performing QC.1. Restoring old Calibration Curves.

**Display the calibration curve / Press restore on the Operation Panel, if Restore is not displayed, press More / Select the desired curve to restore / Press O.K. / Press Validate.**Refer to the Calibration section of the System Training  Workbook pages 42-46 for more details on steps 7-11.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)Control Plasma N (BEN) and Control Plasma P (BEP) are assayed controls with ranges that are verified by our laboratory before test results can be reported.If it is necessary to report a value from the low curve, AFAC control should be run.1. Control Plasma N (BEN) and Control Plasma P (BEP) are run:
	1. Each time a patient sample is run up to once per eight hour shift.
	2. Each time a reagent is changed.
2. Patient results cannot be reported unless control values are within expected tolerance limits.
3. If values do not fall within the expected range, test new controls then new reagents.
4. If QC is still out of range, notify the supervisor.
5. Control values are recorded each day they are performed.
6. All control values must be entered into Sunquest (method code; CS5M1, CS5M2) whether in or out of control range.
* Out of control values must have an appropriate modifier appended.
1. When QC data is entered, it is reviewed using Westgard rules.
* If a Westgard rule fails in Sunquest, the computer displays the result’s standard deviation from the mean.
1. If action is taken to get a control value in range, enter an appropriate comment in Sunquest from

[Table P - Exclusion Codes](https://starnet.childrenshc.org/References/labsop/heme/res/table-p-exclusion-codes.pdf) |
| **Procedure** | Follow the activities in the table below for FIX.CH or FIX.CH.Low (Factor IX, CHROMOGENIC) IN PLASMA. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Reagent A, Reagent B, Factor Xa Substrate, Factor IX Diluent (working solution) in any reagent rack.Place controls and appropriate deficient into a C-Rack using SLD Mini cups.Load the Owrens Veronal Buffer (OVB) or CA System Buffer on the Buffer Table. | Training Workbook Pages 20-22.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | 2 | To load patients, follow the procedural steps below that match the situation. |  |
|  |  | **If** | **Then** |  |
|  | Manual Order Processing | 1. Place rack with sample tubes on the sampler.
2. Press **Order**.
3. Enter the Rack number.
4. Select a tube position to input an order.
5. Press **Order Entry** on the Operation Panel.
6. Select **Ordinary Sample**.
7. Place the cursor in Sample No. and input the sample ID if the sample does not have a barcode. If the sample has a barcode, the 2D barcode reader can be used to input the sample ID.
8. Select the assays to be analyzed.
9. Use the down arrow to order the next sample.
10. Press **O.K**.
11. Press **Start**.
12. Confirm the sample order status on the Joblist screen.
 | Training Workbook, page 27.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  |  | LIS Order Processing (Sample with barcode | 1. Place rack with barcoded sample tube on sampler.
2. Check the host connection status. The host connection status icon must be green or orange.
3. **Press Start**.
4. After the barcodes have been read, confirm the sample order status and progress on the Joblist screen.
 | TrainingWorkbook,page 26.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | Micro Mode Sampling | 1. Follow the Manual Ordering Processing steps.
2. Select the **Mc** column on the Order screen.
3. Load the un-capped tube onto the system.
4. Press **Start**.

Note: Reflex testing is not available in the Micro Mode. |  |
|  | 3 |  | Job analysis progress will be displayed on the Joblist; |  |
| **Procedure Notes** |  | Additional Notes:1. Linearity: Approximately 10.0 - 150.0 (lowest and highest points on the calibration curve). Factor IX values below 10.0 should be measured on the low curve which measures between 1.0 – 25.0. 2. Heparin concentrations of up to 2 IU/ml do not interfere with the Factor IX Chromogenic assay.3. Elevated Factor IX values should be diluted with saline, tested and the result multiplied by the appropriate dilution factor. 4. Lupus anticoagulants (LA) do not interfere with the chromogenic assay. In the presence of a LA, markedly falsely decreased FIX activity may be evident with a one stage clotting assay, but the FIX activity appears normal with the chromogenic assay. |  |
| **Interpretation/****Results/Alert Values** | 1. Certain modified recombinant FIX replacement products demonstrate variable and clinically significant differences in post infusion recovery (that is, the amount of factor measured vs. the actual concentration present), based on the activated partial thromboplastin time (APTT) reagent used in the one stage clotting assay. Overestimation of post infusion plasma factor activity can lead to underdosing of the replacement factor and an increased risk of bleeding. Conversely, underestimation of factor activity in a post infusion sample may lead to overdosing of the replacement factor, which not only has cost implications but may also place the patient at risk for thrombosis. Most recombinant FIX products may be accurately measured using a chromogenic assay, even when this is performed with a plasma calibrator rather than a product specific calibrator.2. All results will be appended with the coded comment “ASR” in Sunquest translated as the following:"This test was developed and its performance characteristics determined by Children's Hospitals and Clinics. It has not been cleared or approved by the U.S. Food and Drug Administration. Analyte Specific Reagents(ASR's) are used in many laboratory tests necessary for standard medical care andgenerally do not require FDA approval." |
| **Reference Intervals****Result Reporting** | 1. [Table - QQ Factor Assays Reference Intervals](https://starnet.childrenshc.org/References/labsop/coag/res/table-qq-factor-assays-reference-intervals.pdf)Sunquest:1. On-line mode (OEM): MPLS- See procedure “Autoverification of Coagulation”

Function: OEM <CR>Device: CS5M1 or CS5M2 <CR>Workload data for - <CR>Last Cup Received = xxxx Last Cup Processed = xxxxxStart at Cup Enter cup # if appropriate (same as sequence #)WAITING (ENTER \* TO EXIT ‘OE’)Accession numbers appear as results are transmitted. Check flagged results on the CS-5100, if all results are acceptable:Accept (A), Modify (M), or Reject (R): A <CR>If results are unacceptable:Accept (A), Modify (M), or Reject (R): R <CR>1. Manual entry mode (MEM):

Function: MEM <CR>Worksheet: FAC <CR>Test-1: <CR>Test-2: <CR>CAP Method: M <CR>Lots of tests appear one at a time Enter CS5M1 or CS5M2(A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>F9C Enter results (xxx.x) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
| **References** | 1. BCS®XP System Instruction Manual 1 000 767.0506 Manual Version 1.0, Siemens Diagnostics Inc., Marburg Germany, Copyright 2006.
2. Rossix Chromogenic Factor IX product insert, ROX FACTOR IX – 90 00 20,

Rossix AB SE-431 53 Molndal, Sweden Revision 04/2014.1. Control Plasma N package inserts, Siemens Healthcare Diagnostics, Newark, DE, December 2007.
2. Control Plasma P package inserts, Siemens Healthcare Diagnostics, Newark, DE, November

2007.1. Standard Human Plasma package insert, August 2008.
2. ROX FIX Test Definition, BCS-XP, ML-00-00172 Rev01.

7. The value of the chromogenic activity assay in diagnosis and therapeutic monitoring of hemophilia. By Dorothy Adcock, Stefan Tiefenbacher, Rajiv Pruthi 01/23/2017.   |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-5100 application |