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| **F8C Factor VIII Chromogenic Assay** | | | | | | | | | |
| **Purpose** | This procedure provides instructions for Factor VIII Chromogenic Assay in plasma. | | | | | | | | |
| **Principle** | Factor VIII in the sample is activated by thrombin. Activated Factor VIII (F.VIIIa) then accelerates the conversion of Factor X (F.X) into Factor Xa (F.Xa) in the presence of of activated Factor IX (IXa), phopholipids (PL) and calcium ions. The F.Xa activity is assessed by hydrolysis of a p-nitroanilide substrate specific to F.Xa. The initial rate of release of p-nitroaniline (pNA) measured is proportional to the F.Xa activity, thus to the F.VIII activity of the sample.    F.VIII + Thrombin F.VIIIa  F.X F.VIIIa  F.Xa  F.IXa, PL, Ca²  F.Xa  CH3OCO-DCHG-Gly-Arg-pNA CH3OCO-D-CHG-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 VIII Chromogenic Test Kit, PN 10445729, containing;  **Factor IX Reagent** - lyophilized preparation containing approximately 0.6nmol bovine F.IXa, approximately 0.6 nmol bovine thrombin, approximately 0.06 nmol of calcium chloride, approximately 0.12 umol of phopholipids. Tris buffer pH 8.0, and stabilizers.  **Factor X Reagent** - lyophilized preparation containing approximately 2 nmol of bovine F.X, Tris buffer pH 8.0, and stabilizers.  Dissolve contents of a vial of Factor IXa and Factor X with 3.0 ml of distilled or  deionized water.  **( USE 3 mls EVEN THOUGH IT MAY SAY 2 mls ON THE VIAL ).**  **Substrate Reagent** -  lyophilized preparation containing approximately 3.4 umol of CH3OCO-D-CHG-Gly-Arg-pNA. AcOH, a F.Xa substrate, Nα-(Naphthylsulfonylglycyl)-D-phenylalanine piperidide (αNAPAP), a thrombin inhibitor, and stabilizers.  Dissolve the contents of a vial of substrate with 1 ml of distilled of deionized water and equilibrate for 30 minutes at room temperature (15-25°C). Mix 1 ml of Substrate Reagent with 10 ml of Stopping Buffer yielding 11 ml of ready for use Substrate Reagent.  **Stopping Buffer** - solution containing Tris, ethylendiamintetraacetic acid, sodium chloride and 0.02% sodium azide.  **Storage and Stability after reconstitution of Factor IXa, and Factor X Reagent, Substrate/Stopping Buffer;**  37°C 2 hours  15 - 25°C 8 hours  2 - 8°C 3 days  Onboard 18 hours   * **Control Plasma N (BEN):** PN 10446235   (10 x 1 ml).  Dilute with 1ml Type I deionized water. Invert gently, let stand 15 minutes before use.  **Stability:** 16 hours on board analyzer.   * **Control Plasma P (BEP):** PN 10446472,   **(**10 x 1 ml).  Dilute with 1ml Type I deionized water. Invert gently, let stand 15 minutes before use.  **Stability:** 16 hours on board analyzer.   * **Standard Human Plasma** (SHPL):   PN 10487098**,**  **(**10 x 1 ml).  Dilute with 1ml Type I deionized water. Invert gently, let stand 15 minutes before use.  **Standard Human Plasma (SHPL)** - 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), Control Plasma P (BEP)** -  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 4 hours at 15 - 25°C. | | | * **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 | |
| **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.   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.   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 FVIII.ch (Factor VIII, CHROMOGENIC) IN PLASMA. | | | | | | | | |
|  | **Step** | **Action** | | | | | | | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Factor IX Reagent, Factor X Reagent and Substrate Reagent 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. | | | | | Training  Workbook,  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**  **Interpretation/**  **Results/Alert Values** | Additional Notes:  1. Linearity:  Approximately 1.0 – 150.0 (lowest and highest points on the  calibration curve).  2. Heparin concentrations of up to 10 U/ml do not interfere with the  Factor VIII Chromogenic assay.  3. Elevated Factor VIII values should be diluted with saline, tested and  the result multiplied by the appropriate dilution factor.  4.The Chromogenic Factor VIII assay is less likely to show interference from direct thrombin inhibitors than the one-stage clotting assay.  5. Direct Factor Xa inhibitors may result in falsely decreased Chromogenic Factor VIII values.  6. Performing a rinse after testing is completed prevents carryover of Emicizumab ( Hemlibra ). This has been demonstrated to overestimate the values for factor VIII that are run using the low calibration curve with the factor VIII clotting assay following testing of a patient that is receiving Emicizumab ( Hemlibra ).  1. In mild hemophilia A patient populations approximately 20-30% of patients show discrepancies between the one stage clotting assays and chromogenic assays. Greater than 20 Factor VIII mutations to date have been described with discrepantly lower chromogenic activity results. The bleeding phenotype correlates with the lower chromogenic results and is similar to other patients with hemophilia A. Thus, the concern with using the one stage assay alone is that a subset of mild hemophilia A patients will be missed.  2. There have been a few reports describing discrepancies leading to higher chromogenic than one stage assay results. Bleeding in these patients is not generally significant.  3. Lupus anticoagulants (LA) do not interfere with the chromogenic assay. In the presence of a LA, markedly falsely decreased FVIII activity may be evident with a one stage clotting assay, but the FVIII activity appears normal with the chromogenic assay.  4. Certain modified recombinant FVIII 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 under dosing of the replacement factor and an increased risk of bleeding. Conversely, underestimation of factor activity in a post infusion sample may lead to over dosing of the replacement factor, which not only has cost implications but may also place the patient at risk for thrombosis. Most recombinant FVIII products may be accurately measured using a chromogenic assay, even when this is performed with a plasma calibrator rather than a product specific calibrator.  5. Hemlibra (Emicizumab) is a humanized mococlonal modified immunoglobulin G4 (IgG4) antibody  with a bispecific antibody structure produced by recombinant DNA technology in Chinese hamster ovary cells. Hemlibra bridges activated factor IX and factor X to restore function of missing factor VIII that is needed for hemostasis.  Prophylactic therapy with Hemlibra shortens the APTT and increases the reported factor VIII activity using one stage clotting assays or chromogenic assays that use human coagulation factors. Chromogenic factor assays containing bovine coagulation factors are insensitive to Hemlibra and can be used to monitor endogenous or infused factor VIII activity as well as factor VIII inhibitors. | | | | | | | | |
| **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 = xxxxx  Start 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>  F8C 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. Factor VIII Chromogenic Assay package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, September 2009. 3. Control Plasma N package inserts, Siemens Healthcare Diagnostics, Newark, DE, December 2007. 4. Control Plasma P package inserts, Siemens Healthcare Diagnostics, Newark, DE, November   2007.   1. Standard Human Plasma package insert, August 2008. 2. Application Sheets for Factor VIII Chromogenic on the BCS XP.   7. American Journal of Hematology, Karen A. Moser and Dorothy M. (Adcock) Funk  Am. J. Hematol, 89;781-784, 2014 @ 2014 Wiley Periodicals, Inc.    8. Hemlibra (Emicizumab) Laboratory Professional Guide  IE Version 1.0.1 Date of HPRA Approval May 2018  Copyright 2018 by Roche Products (Ireland) Limited. All rights reserved.  9. Siemens Healthineers Bulletin Potential Carryover of Emicizumab by patient samples  PH21-003.A.US March 17, 2021  10.Sysmex CS-5100 System Application Sheet RG\_39\_EN-U Rev. 2.10  11.SysmexCS-5100Training Workbook, EffectiveDate:14-Jan-2021JobAid HOOD05162003158941  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) | | | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | | **Effective Date:** | **Summary of Revisions** | | |
| 1 | | Al Quigley | | | 9/19/22 | Initial Version,CS-5100 application | | |