|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Factor VIII, IX, XI, XII Assays** | | | | | | | | | |
| **Purpose** | This procedure provides instructions for performing FACTOR VIII, IX, XI, XII ASSAYS ( F8, F9, F11, F12 ). | | | | | | | | |
| **Principle** | The Sysmex CS-5100 is a fully automated coagulation analyzer. The CS-5100 can analyze samples using clotting, chromogenic and immunoassay methods. This method is a clotting assay used to perform factor assays using the basic prothrombin time (aPTT). The percent of factor activity present in plasma can be determined by the degree of correction obtained when the test plasma is added to severe factor deficient substrate plasma. The degree of correction is determined by the aPTT. Results are compared to the degree of correction obtained when dilutions of normal plasma are added to the same severe factor deficient substrate plasma. | | | | | | | | |
| **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 | | | | * **Dade Actin® FSL** PN 10445714 10x10 mL. Purified soy phosphatides and rabbit brain phosphatides in .0001M ellagic acid with added buffer, stabilizers and preservative. A reagent for the determination of the activated partial thromboplastin time in citrated plasma.  1. One year’s worth of reagent is sequestered, reorder by using lot number. 2. Ready for use; mix 5-8 times, place on instrument in either cooler rack with bar code facing left. 3. Stability:   • 72 hours (3 days) onboard analyzer.   * Until date on label when stored at 2 to 8°C, unopened * Seven (7) days when stored at 2-8°C, opened.   .  • **Calcium Chloride**  **0.025 M**:  PN 10446232. Ready for  use.  Stability :  • 72 hours (3 days) on  board analyzer.  • Until date on label when  stored at 2-8°C,  unopened.  • 8 weeks when stored at  2-8°C opened.   * **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.   * **Factor Deficient Substrates:**   Factor VIII PN 10446411,  **(**8 x 1 ml),  Factor IX PN 10446414  (8 x 1 ml),  Factor XI PN10446316  (3 x 1 ml)  Factor XII PN 10446330  (3 x 1 ml).  Dilute with 1ml Type I deionized water. Invert gently, let stand 15 minutes before use.  **Stability:** 24 hours  onboard 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.   * **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 factor  VIII or IX low and load  on analyzer. Result  manually in Sunquest as  C – AFAC. This control is  only run with the low  curve.  (Factor VIII and IX only). | | | * **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 | |
| **Sample** | 1. 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 - 4. Add 2.7 mL whole blood to 0.3 mL 3.2% sodium citrate (blue-top vacutainer tube)    * or - 5. Special tubes must be prepared for patients whose hematocrit is > 55% or < 20%. See procedure entitled *Citrate Concentration Adjustments.* 6. Invert to mix well; transport to lab at room temperature. 7. Check sample for clots with applicator sticks. 8. Centrifuge in Stat Spin for five minutes – or - 10 minutes at 3000 rpm at room temperature. 9. Remove plasma and place in a 4mL plastic cup; centrifuge again. 10. Specimen Stability: 11. Four (4) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C. 12. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C. 13. Two (2) weeks when stored -20°C. 14. Six (6) months when stored -70°C (rapidly frozen). 15. Plasma must be frozen if testing cannot be completed within four (4) hours. 16. Frozen plasmas are thawed at 37°C for three (3) minutes, test immediately. 17. Delay in sample transport: 18. Notify supervisor or pathologist 19. 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:   * **Mpls: “-HP” (hemolysis present may affect results)**   **“-GRH” (gross hemolysis may interfere with testing)**   1. Notify unit or physician of unacceptable specimens; enter appropriate comment in the computer. | | | | | | | | |
| **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.   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 PERFORMING FACTOR VIII, IX, XI, XII ASSAYS ( F8, F9, F11, F12 ). | | | | | | | | |
|  | **Step** | **Action** | | | | | | | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Actin FSL and Calcium Chloride in any reagent rack.  Place controls and appropriate deficient substrate 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-](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  [5100 System](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  [Training](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  [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 | Evaluating Results | | 1. The result displayed on the Joblist is a mean result calculated from three dilutions. 2. Check the calibration curve to verify that there is a linear relationship between the clotting times and the dilution ratio values. The SCr value should be >0.900. 3. Check the test sample dilutions to verify the results are within the calibration curve linear range. The MDA average will not be displayed if the test samples dilutions are not within the linear range. 4. Measure additional dilutions or MDA dilution sets if test samples are not within the linear range by selecting **Order**, **Detailed Settings** and **Dilution Ratio** in the CS analyzers software.   Factor results with low activity should be  re-measured using the MDA low dilution set.  Factor results with high activity should be re-measured using the MDA high dilution set. | | | | |  |
|  | 4 | Job analysis progress will be displayed on the Joblist; | | | | | | |  |
| **Procedure Notes** | 1. Results with flags or markings are to be examined in more detail. Refer to the System Training Workbook, Sample Processing Section pages 29-37. [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) 2. Multi-Dilution Analysis (MDA) on the CS-5100 allows users to perform dilutions automatically. Samples are analyzed using multiple dilution ratios that are compared to the graphically displayed calibration curve. The MDA sample analysis screen is used to evaluate parallelism. This includes a line parallel to the calibration curve, the results of the individual test sample dilutions, and the mean of the results. 3. The CS analyzers provide the calibration curve correlation coefficient (SCr) which demonstrates the relationship between the clotting times and the dilution ratio values. According to CLSI H48, values of >0.990 should be achievable, and curves with r - values <0.980 should be rejected. 4. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis. 5. Repeat patient samples with an invalid or questionable result flag. 6. Greatly abnormal or non-reproducible results can be encountered with reagents and samples that contain air bubbles at the surface; remove all bubbles in reagents and samples. 7. For an autosomally inherited hemorrhagic disorder (XI or XII), severe bleeding occurs in the homozygous state, whereas only mild bleeding occurs in the heterozygous state, unless challenged by significant surgery or trauma. 8. For X-linked hemostatic disorders (VIII or IX), the severity of the hemorrhagic disorder depends on the type of mutation. 9. Severe (<1%) and moderate (1 to 5%) deficiencies of VIII and IX, can be easily distinguished from physiologic values. If the % activity is lower than the lowest point on the reference curve, i.e., 1.00<, report <1%. Only Factor VIII and IX are run on a low curve, Factors XI and XII would be reported as less than the lowest point on the routine curve (usually around 10.0). 10. [Table R - Laboratory Diagnosis of Classic von Willebrands Disease (Type IA) and Variants.doc](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200685.pdf) 11. [Table S - Comparison of Hemophilia A and Classic von Willebrands Disease.doc](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200686.pdf) 12. High levels of Factor VIII, sometimes IX, can be observed in infection, or inflammatory processes. 13. Extremely low levels of Factor VIII can be seen in classic hemophilia A, von Willebrand’s Disease and consumptive coagulopathy (DIC). 14. Low levels of Factor IX can be seen in hemophilia B, glomerulonephritis (small molecules such as factor IX leak through the kidney) amyloidosis, vitamin K deficiency and liver disease. 15. Low levels of Factor XI are seen in hemophilia C, most often found in Ashkanazi Jewish families. 16. A class of anticoagulants referred to as *Direct Thrombin Inhibitors* such as Hirudin (Refludan) and argatroban (Novastan®) may cause a falsely decreased factor level. 17. Low levels of Factor XII will present with an extremely prolonged aPTT:   Patients do not present with clinical bleeding problems.  In later life the patient often develops thrombotic events. | | | | | | | | |
| **Interpretation/**  **Results/Alert Values** | 1. Plasma concentrations of any coagulation factor must be interpreted in context with the age-specific values.   2. Various anticoagulants may affect factor assay results [Effect of various anticoagulants on commonly used coagulation assays](https://starnet.childrenshc.org/References/labsop/coag/res/effect-of-various-anticoagulants-on-commonly-used-coagulation-assays.pdf) | | | | | | | | |
| **Reference Intervals** | See [Table QQ - Factor Assays Reference Intervals.doc](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200684.pdf)  Critical Values – All Ages:   1. Call results to the patient’s caregiver within 10 minutes if results are <1%. 2. Report extremely low results, e.g., < .25 as < 1%. | | | | | | | | |
|  | The results must be interpreted in conjunction with the physical condition of the child.  1. Sample decomposition (especially FV) occurs more rapidly in stored samples that are not  refrigerated or frozen.  2. If the % activity is lower than the lowest point on the reference curve, i.e., <1.00, report <1%.  Only Factors VIII and IX are run on a low curve, Factors IX and XII would be reported as  less than the lowest point on the routine curve (usually around 10.0).  3. Suspect an inhibitor if the % activity of each dilution is one and a half (1½) to two (2) or more  times the previous % activity.  Check with unit or physician to determine method of sample collection before reporting an  inhibitor.  **If an inhibitor is suspected append results with the comment PIP (Possible Inhibitor**  **Present) in Sunquest.**   1. Collecting a sample through a heparinized line can easily contaminate the test plasma.    1. Results from such a contaminated sample will appear to contain an inhibitor.    2. When heparin contamination is suspected, treat the plasma with Hepzyme®.    3. DO NOT run factor assays on plasma treated with any other neutralization product.    4. Append comment "-;plasma treated to remove heparin" to treated plasma. | | | | | | | | |
| **Result Reporting** | 1. Online mode (OEM):   Function: OEM <CR>  Device: CS5M1 or CS5M2 (Mpls) <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. Mpls (Sunquest):   Function: MEM <CR>  Worksheet: FAC <CR>  Test-1: Enter specific factor number (F#) <CR>  Test-2: <CR>  CAP Method: Accept (A)  Workload data for - <CR>  Acc. No.: Enter ##### <CR>  F(#): Enter results (xxx) <CR>  Accept (A), Modify (M), or Reject (R): A <CR> | | | | | | | | |
| **References** | 1. Andrew, M., et al., Development of the Human Coagulation System in the Full-Term Infant, Blood 70:165 -72, 1987. 2. Andrew, M. et al., Development of the Human Coagulation System in the Healthy Premature Infant, Blood 72:1651 -57, 1988. 3. Andrew, M., et al., Maturation of the Human Coagulation System During Childhood, Blood 80: 1998 - 05, 1992. 4. Actin® FSL Dade Behring product insert , Dade Behring Marburg GMBH, edition June 2006. 5. Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, 2nd edition, NCCLS Document H21-A2, Vol 11, No 23, December 1991. 6. Corriveau, D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107. 7. Geaghan S.H., Clinics in Laboratory Medicine, Diagnostic Pediatric Hematology, Vol 19, No 1, March 19,99, 39 – 63. 8. Harmening, D.: Clinical Hematology and Fundamentals of Hemostasis, 2nd edition, FA Davis Company, Philadelphia, 1992, pp. 427-437. 9. Lusher, J.: Acquired Bleeding Disorders in Children, Vol 3, Masson Publishing, New York, pp. 13-25, 1981. 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)   1. Multi-Dilution Analysis (MDA) FOR Factor Assays, Automated Blood Coagulation Analyzers CS-5100 , CS-2500. [Multi-Dilution Assay (MDA) For Factor Assays](https://starnet.childrenshc.org/References/labsop/coag/res/multi-dilution-assay-(mda)-for-factor-assays.pdf) | | | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | | **Effective Date:** | **Summary of Revisions** | | |
| 1 | | Al Quigley | | | 9/19/22 | Initial Version,CS-5100 application | | |