|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Performing Factor V Leiden Screen**  **(Activated Protein C Resistance)** | | | | | | | | | |
| **Purpose** | This procedure provides instructions for performing FACTOR V LIEDEN SCREEN (ACTIVATED PROTEIN C RESISTANCE) testing. | | | | | | | | |
| **Clinical Significance/**  **Principle** | This Factor V Lieden Screen is a simple functional clotting test system intended for the screening of resistance to Activated Protein C (APC) in plasma from individuals with the Factor V Leiden defect. It can also be performed on plasma from patients on stabilized oral anticoagulant or heparin therapy.  Activated Protein C Resistance is associated with a point mutation in the Factor V gene (Factor V Leiden). The mutation results in the replacement of Arginine with Glutamine in the Factor V protein. This mutation slows the inactivation of Factor V by APC causing a hypercoagulable state. The presence of Factor V Leiden is the most common cause of inherited thrombophilia accounting for 20% to 50% of cases.  The Siemens Factor V Leiden Assay is based on the activation of endogenous Protein C by incubation of plasma with Agkistrodon contorix (Southern Copperhead) venom. A dilute Russell’s Viper Venom time (DRVVT) is then performed on the plasma. The DRVVT is sensitive to prolongation in the presence of APC. In normal individuals activation of their Protein C prolongs the result 2-3 fold, compared to plasma without activator. In individuals with Factor V Leiden the venom activation of Protein C induces only marginal prolongation of the result (usually less than 1.5 times the plasma without activator). To minimize the effect of other clotting variables, a ratio of clotting times obtained with and without venom activation should be determined.  Activated Protein C resistance may also be caused by other mutations in the Factor V gene (e.g. Factor V (Cambridge) and Factor V (Hong Kong). [Document O - APC Resistance, effect of Factor V Leiden](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200764.pdf)  The Sysmex CS-5100 is a fully automated coagulation analyzer. The CS-5100 can analyze samples using clotting, chromogenic and immunoassay methods. | | | | | | | | |
| **Test Code** | APCRB | | | | | | | | |
| **Policy Statements** | * This procedure applies to all clinical laboratory scientists performing coagulation tests, section supervisor and section 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 V Leiden Screen Kit**  PN 10459420  Activator Reagent, Whole Agkitrodon contorix venom at 0.1 g/L in stabilizing buffer solution.  Preservative: Sodium azide (0.01%).  Reconstitute Activator with 2.0ml purified water and let stand for 10 minutes at room temperature before use. The reconstituted product should be a clear, pale green solution with no apparent particles.  **Stability: 2 weeks at 2-8°C.**  PR3V Reagent, Dilute phospholipids rich Vipera Russelli venom FV reagent containing Russell’s Viper Venom at <0.001g/L, plant derived phospholipids at <10g/L, calcium at <0.1M and heparin inhibitor at <0.5g/L.  Preservative: sodium azide (0.01%).  Reconstitute the PR3V in 4.0ml of purified water and let stand for 10 minutes at room temperature before use.  **Stability: 2 weeks at 2-8°C.**  Both Activator Reagent and PR3V Reagent contain sodium azide (<0.1%) as a preservative.  Reagents containing sodium azide should be handled with caution, refer to package insert.     * **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.   * **ProC Control Plasma: ProC Control Plasma (AFVL)**   PN 10446097  is obtained from pooled plasma from selected healthy donors and is adjusted to a defined sensitivity value by adding rabbit plasma. Rabbit Factor V like Factor V Leiden, is not rapidly degraded by Activated Protein C (APC). This reduces the coagulation time in APC-dependent tests.  Dilute with 1.0ml of purified water and let stand at least 15 minutes at 15-25°C before use.  **Stability:**  **15-25°C 4hrs**  **-20°C 4weeks, can only be thawed once.** | | | | * **Type I deionized water,**   Available in canisters used to collect Type I water from the Millipore system. Stable seven (7) days.  • **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 -   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 - 10 minutes at 3,000 rpm at room temperature. 5. Remove plasma, place in 4 mL plastic cup, centrifuge again. 6. Sample for testing: Remove plasma and place in a 4 mL plastic cup; allow for 100 μl of dead space. 7. Specimen Stability: 8. Four (4) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C. 9. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C. 10. Two (2) weeks when stored -20°C. 11. Six (6) months when stored -70°C (rapidly frozen).   e. Plasma must be frozen if testing cannot be completed within four (4) hours.   1. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately. 2. If there is a delay in sample transport: 3. Notify supervisor or pathologist 4. 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. Tube insufficiently filled (tubes may vary by no more than -10%, see comparison tubes by centrifuge). 4. Incorrect ratio of anticoagulant to blood. 5. Reject grossly hemolyzed specimens 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 computer. | | | | | | | | |
| **Quality Control** | Control Plasma N (BEN) and ProC Control Plasma (AFVL), expected values for APCRA (APCR Activated), APCRN (APCR Normal) and APCRR (APCR Ratio) are determined for each new lot of reagent.   1. Control Plasma N (BEN) and ProC Control Plasma (AFVL) 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](file:///G:/LAB/Hematology/Heme%20Section%20Procedures/Heme%20Coag%20Procedures%20under%20Construction/CS5100%20Proceudres/COA%201.1%20Anti-Thrombin%20III%20Testing.docx)   1. 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. | | | | | | | | |
| **Procedure**  **(Computer)** | Results are transmitted on line in function OEM, device code XP1. If it is necessary to enter results manually use function MEM, worksheet FAC, test APCRB. For each control (C-BEN, C-AFVL) enter results for APCRA, APCRN. APCRR will be calculated in Sunquest. | | | | | | | | |
| **Procedure** | Follow the activities in the table below for PERFORMING FACTOR V LEIDEN (APCR) TESTING. | | | | | | | | |
|  | **Step** | **Action** | | | | | | | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Actin Activator Reagent and PR3V Reagent 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 step below that matches 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](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  [CS-5100](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  [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 | Job analysis progress will be displayed on the Joblist; | | | | | | |  |
| **Calculations** | The APCR Ratio is equal to: Clotting time PR3V with Activator / Clotting time PR3V with Saline. | | | | | | | | |
| **Interpretation/**  **Results/Alert Values** | This clotting time assay identifies individuals who have resistance to APC. The DNA test identifies Factor V Leiden as the cause of the APC resistance. Activated Protein C resistance is the most prevalent hereditary predisposition to venous thrombosis. It is present in 5% of the general Caucasian population and is less common or rare in other ethnic groups. It accounts for 20% of unselected patients with a first deep vein thrombosis and 50% of familial cases of thrombosis. The vast majority of cases are due to the Factor V Leiden mutation, which renders Factor V resistant to the degradation by activated Protein C, resulting in an increased risk for venous thrombosis.  If APCR is positive or borderline, results should be confirmed by genetic analysis, as Factor V Leiden is a genetic mutation.  Various anticoagulants may affect 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** | APC Ratios less than 1.5 suggest Factor V Leiden is present. The text code (**APCA**) ; “Abnormal ratio, patient has resistance to activated Protein C. Confirm with molecular test for Factor V Leiden” will be appended to the result.  APC Ratio greater than 2.0 – Negative for the presence of Factor V Leiden. The text code (**APCN**); “Normal ratio, patient does NOT have activated Protein C resistance. A molecular test for Factor V Leiden is not indicated” will be appended to the result.  APC Ratios between 1.5 and 2.0 may suggest low levels of Protein C and should be investigated further. The text code (**APCB**); “Borderline ratio, may be suggestive of activated Protein C resistance. Confirm with molecular test for Factor V Leiden” will be appended to the result.  Samples with positive or borderline results should be confirmed by genetic analysis as Factor V Leiden is a genetic mutation.  Some laboratories report the result as a normalized ratio, which is the result of the APC resistance assay of the patient, divided by the result for normal pooled plasma. Using the normalized ratio reduces intra and inter-laboratory variability in the assay. However, it has not improved the ability of the assay to distinguish APC resistance from normal. | | | | | | | | |
| **Method Performance Specifications** | Tests with the Siemens Factor V Leiden Assay have been performed on patients on stabilized oral anticoagulant, heparin and low molecular weight heparin, and patients with Lupus Anticoagulant. Samples from these patients may give substantially longer clotting times from normal individuals. However, as saline times are also prolonged the ratios of activator/saline are reliable and remain diagnostic.  This test should not be performed on patients that are on argatroban or other direct thrombin inhibitors. These samples will not clot when endogenous Protein C is activated by incubation of plasma with Agkistrodon contorix (Southern Copperhead) venom or they will be markedly elevated which could result in a false negative being reported. Cancel these tests with the comment UNCA (Unable to calculate).  Activated Protein C resistance may also be caused by other mutations in the Factor V gene (e.g. Factor V (Cambridge) and Factor V (Hong Kong)). | | | | | | | | |
| **Result Reporting** | 1. Mpls (Sunquest): MPLS- see procedure “Autoverification in Coagulation”   Function: MEM <CR>  Worksheet: FAC<CR>  Test-1: <CR>  Test-2: <CR>  CAP Method: Modify (M)  APCRB: CS5M1 or CS5M2 <CR>  Workload data for - <CR>  Acc. No.: Enter ##### <CR>  APCRB: Enter result  Accept (A), Modify (M), or Reject (R): A <CR> | | | | | | | | |
| **References** | 1. Factor V Leiden Assay, Siemens Package Insert, Siemens Healthcare Diagnostics,Marburg Germany, May 2005. 2. Bertina RM, Koeleman BP,Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-67. 3. Dahlback B. Physiological anticoagulation. Resistance to activated protein C and venous thrombo-embolism. J Clin Invest 1994; 94 923-7. 4. Thrombophilia Powerpoint presentation Kandice Kottke-Marchant M.D. PhD. <http://aniaracorp.s3.amazonaws.com/PhyFiles/Thrombophilia2/Marchant_medium.wmv> 5. An Algorithmic Approach to Hemostasis Testing Kottke-Marchant ,CAP Press, Copyright 2008. 6. ProC Control Plasma package insert, Siemens Healthcare Daignostics, Marburg Germany, May 2008 7. Application Sheet for Factor V Leiden Assay on the BCA and BCS XP System. 8. An Algorithmic Approach to Hemostasis Testing Kottke-Marchant ,CAP Press,Copyright 2008. 9. Control Plasma N package insert, Siemens Healthcare Diagnostics, Newark, DE, December 2007. 10. 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. Sysmex CS-5100 System Application Sheet RG\_39\_EN-U Rev. 2.11 | | | | | | | | |
|  |  | | | | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | | | **Effective Date:** | **Summary of Revisions** | | |
| 1 | | Al Quigley | | | 9/19/22 | Initial Version, CS-5100 application | | |