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| **Performing Protein C Testing** |
| **Purpose** | This procedure provides instructions for PERFORMING PROTEIN C TESTING. |
| **Clinical Significance/ Principle** | The first step in the process is the activation of Thrombomodulin by Thrombin. Protein C then combines with Thrombomodulin in order to produce activated Protein C. Activated Protein C then combines with Protein S on the surface of a Platelet.Protein C along with Protein S (which acts as a cofactor) is a vitamin K dependent inhibitor which regulates the activity of Factor V and Factor VIII. Congenital heterozygous deficiency leads to a high age-dependent incidence of venous thrombosis. Homozygous deficiency in neonates is associated with very severe thrombotic manifestations. An acquired deficiency may be due to vitamin K deficiency, i.e. as a result of absorption disturbances or oral anticoagulant therapy. In vitamin K deficiency, other vitamin K dependent coagulation factors are also diminished in activity, and therefore the risk of thrombosis under these conditions is small. Due to the short half-life of Protein C, the induction of oral anticoagulant therapy may lead to very low levels of Protein C activity with the risk of coumadin necrosis. Berichrom® Protein C detects the amidolytically active portion of the activated Protein C, including the non-carboxilated molecules synthesized in vitamin K deficiency. Thus, in conditions of vitamin K deficiency, a higher Protein C activity is found with Berichrom® Protein C than when using the coagulometric method. To obtain a complete picture of a Protein C deficiency, it is therefore advisable to also use the coagulometric method of the antigenic determination technique.[Document M - Protein C Mechanism of Activation](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200762.pdf)Protein C in the patient sample is activated by a specific snake venom activator. The resulting Protein C(a) is assayed in a kinetic test by measuring the increase in absorbance at 405nm. The assay is based on the following reactions. Protein C (sample) Protein C Activator > Protein C (a)p-Glu-Pro-Arg-MNA Protein C(a) > p- Glu-Pro-Arg-OH + MNA |
| **Test Code** | PRC |
| **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
 | Berichrom® Protein C kit, PN 10446499Containing:● Protein C Activator Reagent:Extracted from venom ofAgkistrodon contorix, stabilized and lyophilized.Dissolve contents of the vial with the volume of Activator Diluent indicated on the label.**Stability:****Onboard Analyzer 96 hrs****2-8°C 2 Weeks****-20°C 4 Weeks** ● Activator Diluent (Hepes Buffer Solution): Hepes (25 mmol/L) polyethylene glycol (2.5g/L) calcium chloride (5g/L, pH 8.25;Preservative; sodium azide (<1g/L).Reagents containing sodium azide should be handled with caution, refer to package insert. **Stability:****6 Months once opened and stored at 2-8°C.**● Substrate Reagent:Pyro-glutmic acid-proline arginine-methoxy-nitroanilde (p-glu-pro-arg-MNA), lyophilized; concentration in the working solution: 4mmol/L.Dissolve the contents of the vial with the quantity of distilled water indicated on the label.**Stability:****Onboard Analyzer 96hrs****2-8°C 6 Weeks****-20°C 6 Months.*** 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 hrs. on board analyzer* Control Plasma P (BEP): PN10446472,

(10 x 1 mL)Dilute with 1ml type I deionized water. Invert gently, let stand 15 minutes before use.Stability: 16 hrs. 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. | * TypeI deionized water, available in canisters used to collect Type I water from the Millipore system. Stable seven (7) days
* Owrens Veronal Buffer (OVB) PN10445724, (10 x mL )

Stability: 4 days on board analyzer, 8 weeks 2-8°C* CA System Buffer PN 10873440 ( 8 x 250 mL)

Stability: 4 days on board analyzer, 8 weeks 2-8°C* 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).

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.
 |
| **Calibration** | 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)  |
| **Quality Control****Procedure** | 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.
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)Follow the activities in the table below for PERFORMING PROTEIN C TESTING. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load the Protein C Activator Reagent and Substrate Reagent in any reagent rack.Load controls into a C-Rack using SLD Mini cups.Load the Owren’s 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**.

After the barcodes have been read, confirm the sample order status and progress on the Joblist screen. | TrainingWorkbook,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. |  |
| **Interpretation/****Results/Alert Values** | 3 | Job analysis progress will be displayed on the Joblist;1. Protein C may be reduced during an acute event (thrombotic, surgical, etc.) therefore it is preferable not to test for it during this time. However a normal value at the time of an acute event excludes a congenital deficiency.
2. Elective testing for Protein C deficiency is best done at least 30 days after cessation of coumadin therapy.
3. If other factor deficiencies, such as decreased Antithrombin, or if inherited conditions such as Factor V Leiden or Prothrombin20210 Mutation are also present the effects of a Protein C or Protein S deficiency can be exacerbated.
4. Fresh frozen plasma contains both Protein C and Protein S, and it can be used as a short term preventative when a patient is having a surgical procedure.
5. A Protein C concentrate was recently approved by the FDA for use in patients with Protein C deficiency.
6. The advantage of this assay is that it is not affected by Lupus anticoagulants, Factor VIII levels, Factor V Leiden, or other coagulation abnormalities that can interfere with clot-based assays.
7. Protein C may be reduced during an acute event (thrombotic, surgical, etc.) therefore it is preferable not to test for it during this time. However a normal value at the time of an acute event excludes a congenital deficiency.
8. Protein C deficiencies are either quantitative (type I) or qualitative (type II). In type I deficiencies normal Protein C molecules are made, but reduced in quantity. In type II deficiencies normal amounts of Protein C are made, but the Protein C is defective. Functional assays measure Protein C function (activity). Antigenic assays measure the quantity of Protein C regardless of the quality of its function. Accordingly, type I deficiencies have decreased Protein C in both functional and antigenic assays. Type II deficiencies have normal antigenic Protein C levels, with decreased functional Protein C. Thus, a functional assay should be used as the initial screening assay. If the result is decreased, an antigenic assay may be performed to determine if the deficiency is type I or type II.
9. This assay will not detect rare mutations affecting Protein C’s ability to interact with thrombin, endothelial cell Protein C receptor, phospholipid, or Protein S. This assay also does not directly measure Protein C’s ability to inactivate Factors V and VIII. Some cases of type II Protein C deficiency have been reported with a normal chromogenic (functional) and a normal antigenic result but an abnormal clot-based assay result.
10. Various anticoagulants may affect the Protein C assay

 [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** | Reference range is 70-140%.At birth Protein C levels are only 35% of adult normal values. Mean Protein C levels rise to above 50% of adult normal values by age 6 months, but may remain below normal adult range until the age of 10-16 years. See table below:

|  |  |
| --- | --- |
| Newborn | 14-42% |
| 1-4 day | 26-44% |
| 5-29 day | 31-53% |
| 30-89 day | 32-54% |
| 90-179 day | 41-67% |
| 180-364 day | 48-70% |
| 1-5 year | 40-92% |
| 6-10 year | 45-93% |
| 11-16 year  | 55-111% |
| 17 yr and older | 70-140% |

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| **Method Performance Specifications** | Berichrom® Protein C detects the amidolytically active portion of the activated Protein C, including the non-carboxilated molecules synthesized in vitamin K deficiency. Thus, in conditions of vitamin K deficiency, a higher Protein C activity is found with Berichrom® Protein C than when using the coagulometric method.The measuring range extends from 0-140%. |
| **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)PRC: CS5M1 or CS5M2<CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>PRC: Enter resultAccept (A), Modify (M), or Reject (R): A <CR> |
| **References** | * 1. Siemens Berichrom Protein C package insert OUVV G15 E0501 (699), Siemens Healthcare DiagnosticsInc.,Newark, DE, May 2008.
	2. Control Plasma N package insert, Siemens Healthcare Diagnostics, Newark, DE, December 2007.
	3. Control Plasma P package insert, Siemens Healthcare Diagnostics, Newark, DE, December 2007.
	4. Application Sheets for Protein C with Berichrom Protein C on BCS and BCS XP.
	5. BCS System Instruction Manual
	6. BCS XP System Instruction Manual
	7. Thrombophilia Powerpoint presentation Kandice Kottke-Marchant M.D. PhD. <http://aniaracorp.s3.amazonaws.com/PhyFiles/Thrombophilia2/Marchant_medium.wmv>
	8. An Algorithmic Approach to Hemostasis Testing Kottke-Marchant ,CAP Press,Copyright 2008.
	9. Andrew M, Paes B, Milner R, et al, “ Development of the Human Coagulation System in the Full-Term Infant,” Blood, 1987,70(1) : 165-72.
	10. Andrew M, Vegh P, Johnston M, et al, “Maturation of the Hemostatic System During Childhood,” Blood,1992, 80(8) : 1998-2005.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-5100 application |