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| **RISTM Ristocetin Cofactor Assay** |
| **Purpose** | This procedure provides instructions for RISTM RISTOCETIN COFACTOR ASSAY. |
| **Principle** | BC-VWF:RCo (Siemens Healthcare Diagnostics), reagent contains fixed, lyophilized platelets coated with GPIb on their surface, which bind active VWF present in the plasma through its GPIb receptor in the presence of ristocetin. VWF-platelet complexes are formed, followed by platelet agglutination, which is proportional to the capacity of plasma VWF to bind platelets and is determined by measuring the decrease of light transmission caused by this agglutination.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• SB ( Stir Bar ) Cuvettes SMN 10481761• SB ( Stir Bar ) Set Tool SMN 10481762• Teflon Mixing Bars ( for reagent ) SMN 10482200 | * BC von Willebrand Reagent, PN 10714565, 5 x 4 mL

Dilute with 1ml type I deionized water, shake vigorously before use.Stability: 48 hrs.* 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. | * Type I deionized water, available in canisters used to collect Type I water from the Millipore system.

Stability: 7 days.• Owrens Veronal Buffer  (OVB) PN 10445724, (10 x 15 ml).* CA Clean IPN 10445689,

(50ml)Stability: 5 days on board analyzer, 1 month 2-8°C.* CA Clean II PN 10708787, (45ml) or CA Clean II PN10445688 (500ml)

Stability: 5 days on board analyzer, 2 months 5-35°C. Ready to use. |
| **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), this is the minimum required when ordering as part of a von Willebrand Workup which may require Multimer Analysis.

- 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. Do not send through the tube system.
4. Check sample for clots with applicator sticks.
5. Centrifuge in Stat Spin for five minutes – or - 10 minutes at 3000 rpm at room temperature.
6. Remove plasma and place in a 4 mL plastic cup; centrifuge again. When ordered as part of a von Willebrand Workup divide remaining sample into two tubes with a minimum of 0.5ml in each tube and freeze immediately. The extra tube will be used if Multimer Analysis is indicated. Samples that have been frozen after thawing are not acceptable.
7. Test within:
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) hours1. Frozen plasmas are thawed at 37°C for three (3) minutes, test immediately.
2. 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. Specimen rejection: Notify unit or physician of unacceptable specimens, enter appropriate comment in computer.
2. Clotted specimens
3. Insufficiently filled tubes (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)
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| **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** | 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) |
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| **Procedure** | Follow the activities in the table below for RISTM PERFORMING ANALYTIC RISTOCETIN COFACTOR ASSAY. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load BC von Willebrand Reagent with a stir bar added to any mixing position on the reagent table. Mixing positions on the reagent table are indicated by a slightly heavier gray border around the circle. 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 [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | 2 | Load SB Cuvettes on the dispensing table.When the status of the Main Unit is displayed as [Ready] or [Int. Ready] on the IPU status bar, set SB Cuvettes using the SB Set tool. Determine how many SB Cuvettes are necessary, each sample requires a minimum of one SB Cuvette. | Instructions for loading SB Cuvettes using the SB Set Tool:[CS5100 Instructions for Use pages 123-131](https://starnet.childrenshc.org/References/labsop/coag/res/cs-5100-instructions-for-use.pdf) |
|  | 3 | 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](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. |  |
|  | 4 | Job analysis progress will be displayed on the Joblist; |  |
| **Interpretation/****Results/Alert Values** | See [Document E – Ristocetin Cofactor Reference Values.](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200673.pdf) |
| **Result Reporting** | 1. The ristocetin cofactor activity is expressed in % of normal.
2. Low values below the bottom of the curve are reported as less than <10%.
3. Sunquest reporting:

On-line mode (OEM):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 CS5100, 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>Manual Entry Mode (MEM):Function: MEM <CR>Worksheet: FAC <CR>Test-1: RISTM <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> |
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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 |