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|  **Anti-Thrombin III Testing** |
| **Purpose** | This procedure provides instructions for PERFORMING ANTI-THROMBIN III TESTING ( ATIII ). |
| **Principle** | Antithrombin III is the plasmatic inhibitor of thrombin and activated Factor X and forms an irreversible inactive complex with these enzymes. The inactivation of the activated coagulation factors is greatly accelerated by heparin. Berichrom Antithrombin III (A) reagent is used for the rapid determination of the physiologically active antithrombin III and permits the diagnosis of congenital and acquired antithrombin III deficiency, a condition associated with an increased risk of thrombosis. Acquired antithrombin III deficiencies frequently occur due to consumption following major operations or due to disseminated intravascular coagulation (DIC) in cases of septicemia, nephrosis, liver parenchymal damage (hepatitis, drug intoxication, alcoholism) and estrogen-containing contraceptives. The test permits early detection of patients at increased risk for thrombosis.The antithrombin III in the sample is converted into an immediate inhibitor by heparin and inactivates thrombin present. The residual thrombin content is determined in a kinetic test by measuring the increase in absorbance at 405 nm, based on the following reaction:ATIIIsample + thrombinexcess heparin [ATIII-thrombin] + thrombinresidueTos-gly-pro-arg-ANBA-IPA thrombin residue tos-gly-pro-arg-OH + ANBA-IPAThe absorbance decreases linearly with the amount of antithrombin III present in the patient sample. Each analyzer run is to be calibrated using Standard Human Plasma. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist.
 |
| **Materials** | **Equipment** | **Reagents** | **Supplies** |
|  | * Sysmex CS-2500 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 Antithrombin III (A) kit:

Substrate – dilute with 3ml water. Let sit for 30 minutes at room temp. before use.Stability: 5 days on board analyzer, 6 weeks 2-8°CThrombin – dilute with 5ml buffer. Let sit for 30 minutes at room temp. before use. Stability: 5 days on board analyzer,2 weeks 2-8°C* 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 -
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%. 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. Sample for testing:
10. For primary tube testing, leave plasma on cells

- OR -1. Remove plasma and place in a 4 mL plastic cup; allow for 150 μl of dead space.
2. Specimen Stability:
3. Four (4) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C.
4. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C.
5. Two (2) weeks when stored -20°C.
6. Six (6) months when stored -70°C (rapidly frozen).
7. Plasma must be frozen if testing cannot be completed within four (4) hours.
8. Frozen plasmas are thawed at 37°C for three minutes, test immediately.
9. Delay in sample transport:
10. Notify supervisor or pathologist
11. If approval is given to run test, append 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:**“-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.
2. Do not use Heparin inactivated plasma (HEPN/ Hepzymed) specimen for ATIII 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 15-23 for more details on steps 2-6.  [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-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 select 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 43-48 for more details on steps 7-11.  [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-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) |
| **Procedure** | Follow the activities in the table below for PERFORMING ATIII ANTI-THROMBIN III TESTING. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-2500. Load the Thrombin Reagent and the 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 Pages15-23[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-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* 28.[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-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 27.[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-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** | Please Note:Results with flags or markings are to be examined in more detail. Refer to the System Training Workbook, Sample Processing Section pages 30 - 38. [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)1. Patients with high ATIII levels will be reflexively run on dilution.
2. Grossly prolonged results can be encountered with reagents and samples that contain air bubbles at the surface; remove all bubbles in reagents and samples.
3. Report results as they appear across the interface.
4. Results above or below reportable range must be changed after they cross the interface to reflect this in Sunquest.
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| **Reference Intervals** | Reference Ranges:0-5 days: 51-75%5 days to 1 month: 54-80%1 to 3 months: 63-93%3 to 6 months: 85-109%>6 months: 92-118%1. Limitations: The measurement range extends from 0 to 140% of the norm.
2. Specificity:
3. Aprotinin in the Thrombin Reagent blocks the activity of plasmin if present in the patient sample.
4. Interferences due to heparin cofactor II are negligible as bovine thrombin is used in the test.
5. Berichrom Antithrombin III reagent is used for the rapid determination of the physiologically active Antithrombin III, and permits the diagnosis of congenital and acquired Antithrombin III deficiency, a condition associated with an increased risk of thrombosis.
6. Acquired Antithrombin III deficiencies frequently occur because of consumption following major operations or because of disseminated intravascular coagulation (DIC), in cases of septicemia, nephrosis, liver parenchymal damage (hepatitis, drug intoxication, alcoholism) and estrogen containing contraceptives.
7. Normally, ATIII is a relatively weak inhibitor of the serine protease factors that can be activated by various glycoaminoglycans in particular heparin and heparin sulfates:
8. Heparin causes a conformational change in the ATIII molecule, making its active site more available to thrombin and other serine proteases.
9. A complex is formed between ATIII and the serine proteases that possess no enzyme or inhibitor activity.
10. As the complex forms, the heparin molecule falls off and is ready to react on another ATIII molecule.
11. Thus, heparin administered even in small doses converts ATIII from a slow, relatively ineffective inhibitor to a fast effective one.

6. Various anticoagulants may affect the result of the ATIII 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) |
| **Result Reporting** | 1. Mpls (Sunquest): MPLS- see procedure “Autoverification in Coagulation”

Function: MEM <CR>Worksheet: C1<CR>Test-1: ATIII<CR>Test-2: <CR>CAP Method: Modify (M)ATIII: CS2S1 or CS2S2<CR>Workload data for - A <CR>Acc. No.: Enter ##### <CR>ATIII: Enter resultAccept (A), Modify (M), or Reject (R): A <CR> |
| **References** | 1. Andrew M, Paes B, Johnston M: Development of the hemostatic system in the neonate and young infant. Am J Pediatric Hematol Oncol 1990; 12:95.
2. Andrew, M., Vegh, P., Johnston, M., Bowker, J., Ofosu, F., and Mitchell, L.: Maturation of the Hemostatic System During Childhood. Blood, Vol 80, No 8 (October 15), 1992: pp 1998-2005.
3. Andrew, M., Paes, B., Milner, R., Johnston, M., Mitchell, L., Tollefson, Douglas M., and Powers, P.: Development of the Human Coagulation System in the Full-Term Infant. Blood, Vol 70, No 1 (July), 1987: pp 165-172.
4. Corriveau, D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107.
5. Harmening, D.: Clinical Hematology and Fundamentals of Hemostasis, 2nd edition, FA Davis Company, Philadelphia, 1992, pp. 427-437.
6. Dade Behring – Berichrom Antithrombin III (A) Reagents for the chromogenic determination of antithrombin III Autoanalyzer method for undiluted sample. Package Insert. OWWR G17 E0533 (834) H 1, Dade Behring Marburg GmbH, Marburg, Germany. Edition
7. Sysmex CS-2500 System Application Sheet RG\_36\_EN-U Rev. 2.11
8. SysmexCS-2500Training Workbook, Effective Date: 14-Jan-2021 | HOOD05162003158939

[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf) |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-2500 application |