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| **Thrombin Time in Plasma** |
| **Purpose** | This procedure provides instructions for THROMBIN TIME IN PLASMA ( TT ). |
| **Principle** | The thrombin time measures the rate of conversion of fibrinogen to fibrin by a low concentration of thrombin. Citrated plasma is clotted by the addition of human thrombin, which has been adjusted to give a clotting time of less than 20 seconds with normal plasma.The thrombin time is prolonged in the presence of even a low concentration of heparin, presence of fibrinogen degradation products, presence of abnormal fibrinogens (such as in the newborn where fetal fibrinogen is present in the presence of pathological antithrombins) and in severe hypofibrinogenemia. The thrombin time is shortened by higher molecular weight dextrans and in some cases of marked hyperfibrinogenemia.The Sysmex CS-2500 is a fully automated coagulation analyzer. The CS-2500 can analyze samples using clotting, chromogenic and immunoassay methods. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology, section supervisor, and pathologist.
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| **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 | * **Test Thrombin Reagent:**

PN 10446598 10 x 5 ml.Reconstitute with 5ml reagent diluent. Invert gently, let stand 15 minutes before use. Stability:72 hours (3 days) on board analyzer.  7 days when stored at  2-8°C, (reconstituted).* **Ci-Trol Level 1:**

PN 10445731, 20 x 1ml. Dilute with 1ml type I deionized water. Invert gently, let stand 15 minutes before use.Stability: 24 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 | * Type I deionized water, available in canisters used to collect Type I water from the Millipore system.

Stability: 7 days.* 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)

- 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 3000 rpm at room temperature.
5. Specimen Stability:
6. Twenty Four (24) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C.
7. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C.
8. Two (2) weeks when stored -20°C.
9. Six (6) months when stored -70°C (rapidly frozen).
10. Plasma must be frozen if testing cannot be completed within four (4) hours.
11. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
12. If there is a delay in sample transport:
13. Notify supervisor or pathologist.
14. 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:*** + “-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.
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| **Quality Control** | 1. Control plasmas ( Ci-Trol 1, and BEP ) should have their ranges established by each laboratory when there is a change in lot number of reagent or control material.
2. Control Plasmas ( Ci-Trol 1 and BEP ) are run:
3. At the beginning of each shift or once every eight (8) hours
4. Each time a reagent is changed.
5. Patient results cannot be reported unless control values are within expected tolerance limits.
6. If values do not fall within the expected range, test new controls then new reagents.
7. If QC is still out of range, notify the supervisor.
8. Control values are recorded daily.
9. All control values must be entered into Sunquest whether in or out of control range. Out of control values must have an appropriate modifier appended.
10. Patient results cannot be reported unless control values are within expected tolerance limits.
11. If values do not fall within the expected range, test new controls then new reagents.
12. If QC is still out of range, notify the supervisor.
13. Control values are recorded daily.
14. 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.
* To enter corrective action in Sunquest; after the standard deviation is displayed, the prompt ENTER QC MODIFIER is displayed, use the QC modifier which best describes the action taken from the following list:

IHM in-house maintenance; see instrument logINSR instrument recalibratedMN mean changed, entered by Supervisor on reviewO2I3 this control out 2 SD but in 3 SD, other controls in 2 SDOK result ok’d by supervisor/chief techRND repeated/new dilutionRNRG repeated/new reagentsRNV repeated/new vial of controlRSD repeated/same dilutionRSVC repeated/same vial of controlSH short samplesSUP excluded on supervisory reviewVENM vendor maintenance; see inst logWRSN - Westgard rule failure, supervisor notified<CR |
| **Procedure** | Follow the activities in the table below for TT THROMBIN TIME IN PLASMA. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load BC Thrombin in any reagent rack.Load controls into the C-Rack using SLD Mini cups. | Training WorkbookPages 21-23.[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf) |
|  | 2 | To load patient samples, follow the appropriate sub-step below: |  |
|  | **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**. 1. Press **Start**.
2. Confirm the sample order status on the Joblist screen.
 | TrainingWorkbook,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](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf) [CS-2500](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)[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** | 1. Be sure to make the thrombin reagent up with the accompanying reagent buffer; short control values are obtained when reconstituted with deionized water.
2. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis.
3. 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)
4. Repeat patient samples with an invalid or questionable result flag.
5. Repeat extremely high patient samples when encountered the first time unless the cause is known, i.e., heparin.
6. Greatly prolonged results can be encountered with reagents and samples that contain air bubbles at the surface; remove all bubbles in reagents and samples.
7. Be sure to make the thrombin reagent up with the accompanying reagent buffer; short control values are obtained when reconstituted with deionized water.
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| **Interpretation/****Results/Alert Values** | 1. The results must be interpreted in conjunction with the physical condition of the child.

 Various anticoagulants may affect the TT;  [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)1. Reference Range: All Ages <20.0 seconds
2. Critical Value: All Ages >/= 25.0 seconds
3. Call results to the patients care provider within 10 minutes.
4. Extremely high results are to be reported as >120.0 seconds.
5. Document in Sunquest with the first and last name of the care provider ad time called.
6. Evaluating Curves:

 Determine the probable cause of the questionable/invalid result and curve and attempt to correct It. For examples and how to correct them, refer to the System Training Workbook,  Trouble Shooting Section pages 78-88. [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf) |
| **Result Reporting** | Sunquest:1. On-line mode (OEM):

Function: OEM <CR>Device: CS2S1/CS2S2<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 CS-2500, 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. Manual entry mode (MEM):

Function: MEM <CR>Worksheet: C1 <CR>Test-1: <CR>Test-2: <CR>CAP Method: M <CR>Lots of tests appear one at a time Enter CS2S1/CS2S2 for each(A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>TTA Enter results (xxx.x) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
| **Maintenance** | 1. Night Shift performs daily maintenance:

[MAI 2.5 Performing CS-2500 Daily Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS2500/MAI%202.5%20Performing%20CS-2500%20Daily%20Maintenance.docx)1. Day Shift performs weekly, monthly, and “as needed” maintenance:

 [MAI 2.6 Performing CS-2500 Weekly Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS2500/MAI%202.6%20Performing%20CS-2500%20Weekly%20Maintenance.docx)  [MAI 2.7 Performing CS-2500 Monthly - As Needed Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS2500/MAI%202.7%20Performing%20CS-2500%20Monthly%20-%20As%20Needed%20Maintenance.docx) |
| **Troubleshooting** | 1. Call Siemens Technical Services (TAC) 1-800-242-3233. Be prepared to give the following:
* Serial number
* Functional location number
* What was going on at time of instrument malfunction
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| **References** | 1. BC Thrombin Reagent, package insert, ORKE G37 U01 W, Siemens Marburg GMBH, 1/97.
2. 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.
3. Ci-Trol 1, Siemens product insert , Siemens Diagnostics Inc. Marburg GMBH, edition July 2004.
4. Control Plasma P, Siemens product insert OUPZ G13 E0532 (0019) W 1, Siemens Diagnostics Inc. Marburg GMBH, edition April 1999.
5. Corriveau D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107.
6. Edson, JR, et al., Low Platelet Adhesiveness and Other Hemostatic Abnormalities in Hypothyroidism, Annals of Internal Medicine, 82:342-46, 1975.
7. Harmening D: Clinical Hematology and Fundamentals of Hemostasis, 2nd edition, FA Davis Company, Philadelphia, 1992, pp. 427-437.
8. Henry, JB, Todd-Sanford-Davidsohn, Clinical Diagnosis and Management by Laboratory Methods, WB Saunders Co., Philadelphia, 1979, pp. 1157-58.
9. Lusher J: Acquired Bleeding Disorders in Children, Vol 3, Masson Publishing, New York, pp. 13-25, 1981.
10. Sysmex CS-2500 Training 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 |