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| **Prothrombin Time in Plasma** |
| **Purpose** | This procedure provides instructions for MEASURING PROTHROMBIN TIME IN PLASMA ( PT ). |
| **Principle** | The Prothrombin Time is a rapid screening test used to detect single or combined deficiencies of the extrinsic coagulation system indicative of hereditary and acquired coagulation disorders, liver disease or Vitamin K deficiency. Innovin® initiates clotting of plasma from plasma specimens via the extrinsic and common pathways in a global screening test. After addition of thromboplastin and calcium (Innovin®)the obtained clotting time reflects the activity of the coagulation factors II, V, VII, X and fibrinogen. In addition, the PT is used to monitor anticoagulant therapy (coumadin).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.
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| **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 | * Siemens Innovin**:**

PN 10445706, 1. X 10ml).

 One year’s worth of  reagent is sequestered,re-order by using lot number.Reconstitute with 10mlType I deionized water.Swirl to mix allow to clear, let sit 15 minutes at room temperature before use. Stability: 72 hours (3 days) on board analyzer.  10 days when stored at  2-8°C, (reconstituted).* Ci-Trol Level 1**:**

PN 10445731, 20 x 1ml, for the control of coagulation and fibrinolysis in the normal range. • Ci-Trol Level 3:  PN 10445733, 20 x 1 ml,  for the control of  coagulation and fibrinolysis in the pathological range.Dilute with 1ml type I deionized water.Invert gently, let stand 15 minutes before use. Stability: 24 hours 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. |
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| **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 the computer.
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| **Quality Control** | 1. Control plasmas (Ci-Trol 1, and Ci-Trol 3) 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 Ci-Trol 3) 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 PT MEASURING PROTHROMBIN TIME IN PLASMA. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Innovin in any reagent rack.Load controls into the C-Rack using SLD Mini cups. | Training WorkbookPages 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 appropriate procedural 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 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 CS-5100 System Training 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;  |  |
|  |  |
| **Procedure Notes** | 1. All prothrombin times are reported with an INR.
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 29-37. [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-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.
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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 PT. [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. Critical Values: **All ages: PT >17.0 (INR) >1.7 WPT/INR <1.4 or >3.5**
2. Call results to the patient’s caregiver within 10 minutes
3. Extremely high results should be reported as >120.0 seconds, INR >9.0
4. Documentation:
	* In Sunquest, append all of the following
	* - RP
	* -;first and last name of caregiver and time called.
5. 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 80-90. [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)1. Sample decomposition (especially Factor V) occurs more rapidly in stored samples that are not refrigerated or frozen.
2. Hemolyzed specimens may have activated clotting factors that interfere with endpoint readings.
3. The PT is used both as a screening procedure and as a procedure to monitor oral anticoagulants.
4. The factor sensitivity of Innovin was investigated for factor V deficiency and factor VII deficiency on the CS-5100 analyzer according to the recommendations of CLSI guideline H47-A2. In this study using five different reagent lots of Innovin factor sensitivity levels ranged from 37-50% of Norm for factor V and from 42-54% of Norm for factor VII.
5. Minimal contamination with tissue thromboplastin may produce serious errors as can agitation of the blood sample, air bubbles and foaming.
6. The PT is abnormal in the following conditions:
7. Single factor deficiency ( I, II, V, VII, or X )
8. Active fibrinolysis
9. Liver disease
10. Presence of antithrombins ( high doses of heparin )
11. Presence of antithromboplastins
12. Presence of non-specific inhibitors ( lupus-like )
13. Specific factor inhibitors
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| **Reference Ranges** |

|  |  |  |
| --- | --- | --- |
| Age | Prothrombin Time | INR |
| 6 months and older | 8.5 to 12.4 seconds | 0.79 to 1.20 |
| Less than 6 months | 8.5 to 12.4 seconds | 0.79 to 1.20 |
| Transfused <6 months | 8.5 to 12.4 seconds | 0.79 to 1.20 |

INR Therapeutic Ranges:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Prophylactic Dose | Standard Dose | High Dose |
| INR | 1.4 to1.9 | 2.0 to 3.0 | 2.5 to 3.5 |

1. Results must be interpreted in conjunction with the physical condition of the child.
2. The CS-5100 automatically calculates the INR geometric mean of the patient population in the normal range.
3. INR can be calculated manually as follows:
4. INR = RISI
5. Where R = patient PT in seconds

 Mean of normal range1. ISI = International Sensitivity Index of the reagent/instrument combination found in the package insert of Innovin. The ISI changes from lot to lot of reagent.
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| **Result Reporting** | Sunquest:1. On-line mode (OEM):

Function: OEM <CR>Device: CS5M1/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 CS-5100, 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 CS5M1/CS5M2 for each(A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>PTA or PTW (Warfarin) Enter results (xxx.x) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
| **Maintenance** | 1. Night Shift performs daily maintenance:

[MAI 2.2 Performing CS-5100 Daily Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.2%20Performing%20CS-5100%20Daily%20Maintenance.docx)1. Day Shift performs weekly, monthly, and “as needed” maintenance:

 [MAI 2.3 Performing CS-5100 Weekly Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.3%20Performing%20CS-5100%20Weekly%20Maintenance.docx)  [MAI 2.4 Performing CS-5100 Monthly - As Needed Maintenance.docx](https://vcpsharepoint4.childrenshc.org/references/Documents/Lab%20SOP/Coag/CS5100/MAI%202.4%20Performing%20CS-5100%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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10. Massicotte, P., et al.: Home Monitoring of Warfarin Therapy in Children with a Whole Blood Prothrombin Time Monitor, Jour of Ped, 127:3, September 1995, pp. 389-94.
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12. One Stage Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) Test, NCCLS Document H47-A, Vol 16, No. 3, June 1996.
13. Sysmex CS-5100 System Application Sheet PT/INR Dade Innovin (V.06) RG\_39\_EN-U Rev. 2.11
14. Sysmex CS-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)  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS5100 application |