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| **Activated Partial Thromboplastin Time Assay** |
| **Purpose** | This procedure provides instructions for performing ACTIVATED PARTIAL THROMBOPLASTIN TIME ASSAY (PTT). |
| **Principle** | Incubation of plasma with the optimal quantity of phospholipids (platelet substitute) and a surface activator (micronized silica) leads to activation of factors of the intrinsic coagulation system, namely factors VIII, IX, XI, XII, Fletcher, Fitzgerald and Passavoy. The addition of calcium ions triggers the coagulation process; the time to formation of a fibrin clot is measured photometrically. The test can be used for screening factor deficiencies (hemophilia), for detecting inhibitors of coagulation, (lupus anticoagulant or specific factor inhibitors) and for monitoring replacement therapies. The test will not detect platelet disorders, qualitative or quantitative, isolated factor VII and factor XIII deficiencies or vascular disorders.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, the section supervisor, and section 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 | * Dade Actin® FSL PN 10445714 10x10 mL. Purified soy phosphatides and rabbit brain phosphatides in .0001M ellagic acid with added buffer, stabilizers and preservative. A reagent for the determination of the activated partial thromboplastin time in citrated plasma.
1. One year’s worth of reagent is sequestered, reorder by using lot number.
2. Ready for use; mix 5-8 times, place on instrument in either cooler rack with bar code facing left.
3. Stability:

• 72 hours (3 days) on  board analyzer.* Until date on label when stored at 2 to 8°C, unopened
* Seven (7) days when stored at 2-8°C, opened.
1. If the reagent is left to stand, a green deposit may form consisting of ellagic acid and lipids, before use mix by inverting.
 | * 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)Stabilty: 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. |
|  |  | * 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.  • Calcium Chloride 0.025 M:  PN 10446232. Ready for use. Stability :  • 72 hours (3 days) on  board analyzer. • Until date on label when  stored at 2-8°C, unopened. • 8 weeks when stored at 2-8°C  opened. |  |
| **Records/Forms****Documents Required** | 1. Periodically check the pending log (PL) for tests missed.
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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. Sample for testing:
6. For primary tube testing, leave plasma on cells

- OR -1. Remove plasma and place in a 4 mL plastic cup; allow for 100 μl of deadspace
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 (3) minutes, test immediately.
9. Delay in sample transport:
10. Notify supervisor or pathologist
11. 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 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. 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 inst 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 to perform aPTT ACTIVATED PARTIAL THROMBOPLASTIN TIME. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Actin FSL and CaCl 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, do the following depending on condition: | TrainingWorkbook,page 27.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)TrainingWorkbook,page 26.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
| **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.
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| 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. |
| 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;   |  |
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| **Procedure Notes** | 1. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis.
2. 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)
3. Repeat patient samples with an invalid or questionable result flag.
4. Repeat extremely high patient samples when encountered the first time unless the cause is known, i.e., heparin.
5. 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 PTT [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 Value: **All ages: 48 seconds**
2. Call results to the patient’s caregiver within 10 minutes
3. Extremely high results should be reported as >120.0 seconds
4. Documentation:
	* In Sunquest, append all of the following
	* - RP
	* -;first and last name of caregiver and time called.
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|  | 1. Evaluating Curves ( Trouble Shooting ):

 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. Increased contact activation may result in prolonged PTTs in plasma containing heparin. Studies have indicated great variability of the PTT in heparinized plasma with different lengths of activation time.
2. Report extremely elevated PTT results as >120.0 seconds.
3. Sample decomposition (especially FVIII) occurs more rapidly in stored samples that are not refrigerated or frozen.
4. Elevated PTT results are seen in patients receiving diphenylhydration, heparin, warfarin (coumadin), naloxone, and radiographic agents.
5. Hemolyzed specimens may have activated clotting factors and/or interfere with end point readings.
6. Factor deficiencies, which should produce prolonged PTTs (such as IX), may be compensated for or made to appear normal by elevated levels of one or more different clotting factors (such as VIII).
7. The factor sensitivity of Actin FSL was investigated for factor VIII deficiency and factor IX deficiency on the CS-5100 analyzer according to the recommendations of CLSI guideline H47-A2. In this study using five different reagent lots of Actin FSL factor sensitivity levels ranged from 47-57% of Norm for factor VIII and from 41-49% of Norm for factor IX.
8. Minimal contamination with tissue thromboplastin may produce serious errors as can agitation of the blood sample, air bubbles and foaming.
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| **Reference Intervals** | 1. Newborn to 3 mos 25.0 to 43.6 sec
2. 3 mos to 6 mos 23.2 to 40.1 sec
3. 6 months and older: 20.0 to 34.4 seconds
4. Transfused less than 6 months: 20.0 to 34.4 seconds
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| **Result Reporting** | Sunquest: MPLS.- See procedure [COA 1.3 Autoverification of Coagulation Results in Sunquest.doc](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Other/198716.pdf)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>PTTA: 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 Dade Behring Technical Services (TAC) 1-800-242-3233, be prepared to give the following:
* Serial number
* Functional location number
* What was happening at time of instrument malfunction
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| **References** | 1. Activated Partial Thromboplastin Time (APTT), NCCLS Document H29-T, Vol 12, No 23, December 1992.
2. Actin® FSL Dade Behring product insert , Dade Behring Marburg GMBH, edition June 2006.
3. 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.
4. Ci-Trol 1, Dade Behring product insert , Dade Behring Marburg GMBH, edition July 2004.
5. Ci-Trol 3, Dade Behring product insert, Dade Behring Marburg GMBH, edition July 2004.
6. Corriveau D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107.
7. Harmening D: Clinical Hematology and Fundamentals of Hemostasis, 2nd edition, FA Davis Company, Philadelphia, 1992, pp. 427-437.
8. Lusher J: Acquired Bleeding Disorders in Children, Vol 3, Masson Publishing, New York, pp. 13-25, 1981.
9. Sysmex CS-5100 System Application Sheet RG\_39\_EN-U Rev. 2.11
10. SysmexCS-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** |
|  | 8 | Al Quigley | 9/19/22 | Initial Version, CS-5100 application |