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|  **Heparin Assays of Plasma** |
| **Purpose** | This procedure provides instructions for PERFORMING HEPARIN ASSAYS OF PLASMA,Unfractionated Heparin (HEPU), Low Molecular Weight Heparin (HLMW). |
| **Principle** | The INNOVANCE HEPARINassay is a one stage chromogenic assay. The reagent kit consists oftwo components. One component (Reagent) contains Xa, the other (Substrate) a chromogenicsubstrate specific for Xa. Upon mixing Reagent Xa and Substrate the chromogenic substrate is converted into two products, one of them is paranitroaniline. The formation of paranitroanilinecan be quantified by the coagulation analyzer employing light absorption at a specificwavelength (405 nm).In the presence of a sample containing heparin the formation of paranitroaniline will bereduced in a time dependent manner. This is due to inhibition of Xa by the heparin/ATcomplex. This complex is formed in the patient's plasma and competes with the substrateconversion by Xa. The concentration of the complex is not only dependent on theconcentration of heparin but also on the availability of the patient’s endogenous antithrombin.By comparison to a reference curve the heparin activity of the sample can be quantified.To reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextransulfate is included in the reaction mixture.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 testing, 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 | * **INNOVANCE® Heparin**

PN 10873535Heparin Xa Reagent: 5 x 3.2 ml Substrate Reagent: chromogenic substrate 5 x 4 ml Stability: 72 hours on board analyzer, 8 weeks in original capped vial at 2-8°C.* **INNOVANCE® Heparin Calibrator**

PN 10873530Five levels, 5 x 1mlDilute each vial with 1ml water.Allow to stand at least 15 minutes before use.Stability after reconstitution:8 hours on board analyzer.48 hours at 2-8°C.**HEPU Controls:*** **INNOVANCE UF Control 1**

PN 10873531 5 x 1ml Dilute with 1 ml water.Allow to stand at least 15 minutes before use.Stability after reconstitution:8 hours on board analyzer.48 hours at 2-8°C.* **INNOVANCE UF Control 2**

PN 10873532 5 x 1ml Dilute with 1 ml water.Allow to stand at least 15 minutes before use.Stability after reconstitution:8 hours on board analyzer.48 hours at 2-8°C.**HLMW Controls:*** **INNOVANCE LMW Control 1**

PN 10873534 5 x 1mlDilute with 1 ml water.Allow to stand at least 15 minutes before use.Stability after reconstitution:8 hours on board analyzer.48 hours at 2-8°C.* **INNOVANCE LMW Control 2**

PN 10873533 5 x 1mlDilute with 1 ml water.Allow to stand at least 15 minutes before use.Stability after reconstitution:8 hours on board analyzer.48 hours at 2-8°C. | * 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. |
| **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: Remove plasma from RBCs (Procedure Notes #4) and place in a 4 mL plastic cup; allow for 100 μl of dead space.
6. Specimen Stability:
7. Plasma two (2) weeks when stored -20°C.
8. Plasma six (6) months when stored -70°C (rapidly frozen).
9. Plasma must be frozen if testing cannot be completed within two (2) hours.

f. Samples should be centrifuged within one hour from the time of specimen collection.1. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
2. If there is a 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. 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, 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. Lipemic samples: Grossly lipemic samples should be ultra-centrifuged before testing.
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| **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. The heparin calibrators come as a set of five vials at different concentrations.
3. Load reagents. Slowly dispense the entire volume of the calibrator(s) 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 - 24 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** | 1. Level 1 and Level 2 Heparin Controls are run:
2. At the beginning of each shift or once every eight (8) hours as needed.
3. Each time a reagent is changed.
4. Patient results cannot be reported unless control values are within expected tolerance limits.
5. If values do not fall within the expected range, test new controls then new reagents.
6. If QC is still out of range, notify the supervisor.
7. Control values are recorded daily.
8. 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.
9. 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.
10. To enter corrective action in Sunquest; after the standard deviation is displayed, the prompt ENTER QC MODIFIER is displayed, use the QC modifier that best describes the action taken 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 HEPU and HLMW HEPARIN ASSAYS OF PLASMA. |
|  | **Step** | **Action** |
|  | 1 | Load reagent vials on CS-2500. Load the Heparin Xa 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 Pages 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 patients, follow the procedural steps below that match the situation: |
|  | 3 | **To load patients using Manual Order Processin**g: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**.
4. After the barcodes have been read, confirm the sample order status and progress on the Joblist screen.

Training Workbook, 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. |
|  | 4 | Job analysis progress will be displayed on the Joblist; |
|  | 5 | **Result Reporting;**Sunquest:1. On-line mode (OEM):

Function: OEM <CR>Device: CS2S1 or 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 or CS2S2 (A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>HEPU or HLMW: Enter results (xxx.x) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
|  |  | Additional Notes:1. Linearity: 0.01 – top point of curve, generally around 1.5.
2. Samples should **not** be collected from a heparinized line.
3. Samples should not be left on the RBCs, as heparin will be absorbed onto the cell surface or neutralized by the release of platelet factor 4. Dextran sulfate has been added to the INNOVANCE ® Heparin reagent to reduce the influence from heparin antagonists such as platelet factor 4.
4. High values should be reported as “greater than” the highest measurable point on the calibration curve. **Do not dilute samples.**

The antithrombin activity in samples with pathological low antithrombin levels will be compensated by diluting with products such as Standard Human Plasma, which may lead to an overestimation of the anticoagulation effect in these patients.1. If the values are <0.10 IU/mL and the patient is receiving heparin, call the unit to obtain a new sample.
2. The correct test must be selected to determine the heparin level.
3. Generally, in-house patients are treated with unfractionated heparin.
4. Generally, outpatients are treated with low molecular weight heparin.
 |
|  |  | 1. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis.
2. There has been evidence to suggest that dextran sulfate dissociates protamine/heparin complexes which may lead to inadequate management of heparin reversal.
3. If a value of <0.00 is obtained, verify the result by performing testing on the other

 analyzer before reporting. Abnormal results can be encountered with reagents and  samples that contain air bubbles at the surface; remove all bubbles in reagents and  samples. If results match, a value of <0.01 should be reported. [Table T - Comparative properties of Unfractionated vs Low Molecular Weight Heparin](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200687.pdf)[Table U - Trade names of LMW heparin in the United States](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200688.pdf) |
| **Interpretation/****Results/Alert Values** | 1.Critical Value: All ages:  **1.00 IU/mL**1. Call results to the patient’s caregiver within 10 minutes
2. Documentation:
* In Sunquest, append all of the following
* - RP
* -;first and last name of caregiver and time called.+

2. Various anticoagulants may affect results for heparin assays [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)3. A class of anticoagulants referred to as Direct Thrombin Inhibitors such as Hirudin (Refludan) and argatroban (Novastan®) may cause a falsely elevated heparin level. |
| **Reference Intervals** | See [Table V – Heparin Assay of Plasma – Reference Ranges](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200689.pdf) |
| **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 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
 |
| **References** | 1. Andrew, M., et al. Clinical Problems in Anticoagulation Therapy. HEMATOLOGY – 1997. Education Program American Society of Hematology, pp. 8-28, 12/97.
2. Behring Coagulation System Instruction Manual, Dade Behring 1 000 074.0698, Dade Behring Marburg GMBH, Version 2.0, June 1998.
3. Behring Coagulation System Customer Training Guidebook, Document #CT26, Dade Behring, Newark, DE, 04/10/00.
4. Bick, R.L., Heparin Therapy and Monitoring: Guidelines and Practice Parameters for Clinical and Laboratory Approaches. Clin Appl Thrombosis HEMOSTASIS. 2(Suppl 1) 512-520, 1996.
5. Check, W. In Coagulation, a Cascade of Questions, CAP TODAY, 1/98, Vol 12, No 1.
6. Chromogenix AB. Heparin, Taljegardsgatan 3, S-421 53 Molndal, Sweden, version 1.1.
7. Cleaner SCS, Dade Behring product insert OQUB G19 C0530 (1785) W, Dade Behring Marburg GMBH, edition July 1998.
8. 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.
9. Corriveau, D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107.
10. Enoxaparin Guidelines, Children’s Thrombophilia Network, 4/22/96, pp. 1-6.
11. Heparin, Elkins-Sinn Product Insert J-1432K. Elkins-Sinn, Inc. Cherry Hill, NJ. 12/92 edition.
12. Hirsh, J., et al; “Low molecular weight heparin”. Blood, 79.1, 1-17, 1992.
13. Kovacs, M.J., et al. A Comparison of Three Methods of Measuring Low Molecular Weight Heparin after Total Knee or Hip Arthroplasty, Laboratory Hematology, Vol 2, pp. 111-114.
14. Lovenox® Package Insert IN-1107M, Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, PA, Rev 3/97.
15. Massicotte, P., et al.: Low-molecular-weight-heparin in pediatric patients with thrombotic disease: A dose finding study. J Pediat. 128:313, 1996.
16. Rosborough, T.K., Comparison of Anti-Factor Xa Heparin Activity and Activated Partial Thromboplastin Time in 2773 Plasma Samples from Unfractionated Heparin-Treated Patients. AJCP 108:6, 662-668.
17. Simonneau, G., et al.: Subcutaneous Low-Molecular Weight Heparin Compared with Continuous Intravenous Unfractionated Heparin in the Treatment of Proximal Deep Vein Thrombosis. Archives of Int Med, 7/93.
18. Triplett, D.A.:Laboratory Monitoring of Heparin Therapy, Hemoliance Times, 7/97, Vol 7 No 7.
19. Heparin / INNOVANCE® Heparin Application Sheet (V.O1)
20. INNOVANCE® Heparin Calibrator IFU 10873530GU11 Rev. 01 – en 2017-01
21. INNOVANCE® Heparin QC IFU 10873534GU11 Rev. 01 – en 2017-01
22. INNOVANCE® Heparin Reagent IFU 10873535GU11 Rev. 01 – en 2017-01
23. Siemens INNOVANCE® White Paper A91LD-HHS-171634-P1-4A00 · Printed in USA · 10-2017 · © Siemens Healthcare Diagnostics Inc., 2017
24. Dextran Sulfate included in factor Xa assay reagent overestimates heparin activity in patients after heparin reversal with protamine. Mouton C., Calderon J., Janier G., Vergnes MC., PMID: 14693175 [PubMed – Indexed for MEDLINE]
25. Sysmex CS-2500 System Application Sheet RG\_36\_EN-U Rev. 2.11
26. 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** |
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