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|  **Fibrinogen Assay** |
| **Purpose** | This procedure provides instructions for PERFORMING FIBRINOGEN ASSAY. |
| **Principle** | The thrombin clotting time of dilute plasma is inversely proportional to the fibrinogen concentration of the plasma. At high thrombin concentrations ( 100 NIH units/mL) and low fibrinogen concentrations (35 to 80 mg/dL), the reaction rate is determined by the fibrinogen concentration. Using this principle, Clauss developed a simple quantitative assay for fibrinogen by measuring the clotting time of dilute plasma when a large excess of thrombin is added. The clotting time is then compared with that of a standardized fibrinogen curve to yield mg/dL of fibrinogen. |
| **Policy Statements** | * This procedure applies to all laboratory technologists who perform 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 | • Dade Thrombin: PN10445721, 10 x 5ml,  Dilute with 5ml type I  deionized water.Invert gently, let stand, allow to clear before use. Stability: 72 hours on  board analyzer.* Ci-Trol Level 1**:** PN10445731, 20 x 1ml, for the control of coagulation and fibrinolysis in the normal range.

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

Stability: 7 days.* Owrens Veronal Buffer (OVB) PN10445724, (10 x mL )

Stability: 24 hours on board analyzer, 8 weeks 2-8°C* 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. |
| **Sample****Calibration****Quality Control** | 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).

e) Plasma must be frozen if testing cannot be completed within four (4) hours.1. Frozen plasmas are thawed at 37°C for three (3) minutes, test immediately.
2. 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:
* Mpls “-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.

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 selct 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)1. Control plasmas (Ci-Trol Level 1, and Control Plasma P (BEP) should have their ranges established by each laboratory when there is a change in lot number of reagent or control material.
2. Control Plasma Ci-Trol Level 1 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.
3. Patient results cannot be reported unless control values are within expected tolerance limits.
4. If values do not fall within the expected range, test new controls then new reagents.
5. If QC is still out of range, notify the supervisor.
6. Control values are recorded daily.
7. 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.
8. 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.

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 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 FIBRINOGEN ASSAY. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-2500. Load Dade Thrombin in any reagent rack.Place controls and into a C-Rack using SLD Mini cups.Load the Owrens Veronal Buffer (OVB) or CA System Buffer on the Buffer Table. | Training WorkbookPages 15 - 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.
 | TrainingWorkbook,page 28.[Sysmex](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)[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.
 | TrainingWorkbook,page 27.[Sysmex](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)[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** | 1. Results with flags or markings are to be examined in more detail. Refer to the System Training Workbook, Sample Processing Section pages 29-35. [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)
2. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis.
3. Repeat patient samples with an invalid or questionable result flag.
4. Repeat extremely abnormal patient samples when encountered the first time unless the cause is known, i.e., DIC.
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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| **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
 |
| **Result Reporting** | 1. Online 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. Mpls (Sunquest):

Function: MEM <CR>Worksheet: C1 <CR>Test-1: FIBA <CR>Test-2: <CR>CAP Method: Accept (A)Workload data for - <CR>Acc. No.: Enter ##### <CR>FIBA: Enter results (xxx) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
| **References** | 1. 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.
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4. Corriveau, D.M., et al: Hemostasis and Thrombosis in the Clinical Laboratory, JB Lippincott Company, Philadelphia, 1988, pp. 104-107.
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6. Harmening, D.: Clinical Hematology and Fundamentals of Hemostasis, 2nd edition, FA Davis Company, Philadelphia, 1992, pp. 427-437.
7. Lusher, J.: Acquired Bleeding Disorders in Children, Vol 3, Masson Publishing, New York, pp. 13-25, 1981.
8. National Institute for Biological Standards and Control Website, <http://www.nibsc.ac.uk>
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10. Procedure for the Determination of Fibrinogen in Plasma, NCCLS Document H30-A, Vol 14, No 2, February, 1994.
11. Sysmex CS-2500 System Application Sheet RG\_36\_EN-U Rev. 2.11
12. 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** |
|  | 8 | Al Quigley | 9/19/22 | Initial Version, CS-2500 application |