|  |
| --- |
| **DDI D-Dimer Testing in Plasma** |
| **Purpose** | This procedure provides instructions for performing Quantitative D-Dimer Testing in plasma. |
| **Clinical Significance/****Principle** | Coagulation activation results in the cleavage of Fibrinogen to fibrin monomer. The fibrin monomers spontaneously aggregate to fibrin and are cross-linked by Factor XIII, producing a fibrin clot. In response to the coagulation process the fibrinolytic system is activated resulting in the conversion of plasminogen to plasmin, which cleaves fibrin (and fibrinogen) into the fragments D and E. Due to the cross-linkages between the D-domains in the fibrin clot, the action of plasmin releases fibrin degradation products with cross-linked D-domains. The smallest unit is the D-Dimer. Detection of D-Dimers, which specifies cross-linked fibrin degradation products generated by reactive fibrinolysis is an indicator of coagulation activity.Elevated D-Dimer levels are observed in all diseases and conditions with coagulation activation, e.g. thromboembolic disease, disseminated intravascular coagulation (DIC), Acute aortic dissection, myocardial infarction, malignant diseases, obstetrical complications, third trimester of pregnancy, surgery or poly trauma.The relevance of the D-Dimer assay is as an aid in the diagnosis of thromboembolic events. Elevated concentrations of D-Dimer are indicative of the presence of a clot and have been reported in deep vein thrombosis, pulmonary embolism, and disseminated intravascular coagulation (DIC).Polystyrene particles covalently coated with monoclonal antibody are aggregated when mixed with samples containing D-Dimer. D-Dimer cross-linkage region has a stereo symmetrical structure, i.e. the epitope for the monoclonal antibody occurs twice. Consequently, one antibody suffices in order to trigger an aggregation reaction, which is then detected turbidametrically via an increase in turbibity.[Document U - Diagram of D-Dimer Formation](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/202640.pdf) |
| **Test Code** |  DDI |
| **Policy Statements** | * This procedure applies to all clinical laboratory scientists performing coagulation tests, section supervisor and section pathologist.
 |
|  |
| **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 | • Innovance D-Dimer Kit, PN 10445981 Containing;● Innovance D-Dimer Reagent: Dissolve with 4.0 ml water. Invert 3 Times. Let sit for 15 minutes at room temperature. Stability:  On board analyzer 48 hrs  2-8°C 4 weeks </= -18°C 4 weeks ● Innovance D-Dimer Buffer/ Supplement/Diluent: Ready to use.  Stability:  On board analyzer 48 hrs 2-8°C 4 weeks </= -18°C 4 weeks● Innovance D-Dimer Controls  PN 10446006 / Calibrator  (included in kit) Dissolve with 1.0ml water. Mix  carefully without foam formation. Let sit for 15 minutes at room  temperature. Stability:  Calibrator: 4 hours room  Temperature. Controls: On board analyzer 24 hrs 2-8°C 4 weeks </= -18°C 4 weeks Reagents that have been aliquoted and frozen must be thawed at 37°C within a maximum of 10 minutes, and must be used within 4 hours of thawing.Reagents that are frozen should be in their original containers**.** Do not refreeze after thawing. | * 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. |
| **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 3,000 rpm at room temperature.
5. Remove plasma, place in 4 mL plastic cup, centrifuge again.
6. Sample for testing: Remove plasma and place in a 4 mL plastic cup; allow for 100 μl of dead space.
7. Specimen Stability:
8. Four (4) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C.
9. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C.
10. Two (2) weeks when stored -20°C.
11. Six (6) months when stored -70°C (rapidly frozen).
12. Plasma must be frozen if testing cannot be completed within four (4) hours.
13. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
14. If there is a delay in sample transport:
15. Notify supervisor or pathologist
16. 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. Tube insufficiently filled (tubes may vary by no more than 10%, see comparison tubes by centrifuge).
4. Incorrect ratio of anticoagulant to blood.
5. Reject 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.
 |
| **Quality Control** | Innovance D-Dimer Control 1 and Innovance D-Dimer Control 2 are assayed normal and pathological level, intra laboratory quality controls for assessment of precision and analytical bias in the quantitative determination of D-Dimer on Siemens Systems.1. D-Dimer Control 1 (DDIL) and D-Dimer Control 2 (DDIH) are run:
2. Each time a patient sample is run up to once per eight hour shift.
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 each day they are performed.
8. All control values must be entered into Sunquest (method code CS5M1 or CS5M2) whether in or out of control range.
* Out of control values must have an appropriate modifier appended.
1. 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 in Sunquest from

 [Table P - Exclusion Codes](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/200727.pdf) |
| **Calibration** | Calibration is done using D-Dimer calibrator included in test kit, 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 14-22 for more details on steps 2-6.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-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 42-46 for more details on steps 7-11.  [Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)  |
|  | Follow the activities in the table below for PERFORMING DDI TESTING. |
| **Procedure** | **Step** | **Action** | **Related Document** |
|  | 1 | Load reagent vials on CS-5100. Load Innovance D-Dimer Reagent, Innovance D-Dimer Buffer/ Supplement in any reagent rack. Load D-Dimer Diluent on the Buffer Table.Place controls and into a C-Rack using SLD Mini cups. |  |
|  | 2 | To load patients, follow the procedural step below that matches 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.
 | Training Workbook, 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**.
4. After the barcodes have been read, confirm the sample order status and progress on the Joblist screen.
 | TrainingWorkbook,page 26.[Sysmex](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[CS5100](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[System](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[Training](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[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; |  |
| **Interpretation/****Results/Alert Values** | * 1. The D-Dimer concentration in mg/L is calculated automatically by the analyzer based on the reference curve. The D-Dimer level is expressed as fibrinogen equivalent unit (FEU). An FEU is the quantity of fibrinogen initially present that leads to the observed D-Dimer level. Increases in D-Dimer concentration observed with thromboembolic events can be variable due to localization, extension and age of the thrombus. Therefore, a thromboembolic event cannot be excluded with certainty solely on the basis of a D-Dimer concentration being within the reference range of ostensibly healthy persons.
	2. Results of the D-Dimer test should always be interpreted in conjunction with the patient’s medical history, clinical presentation, and other findings. Clinical diagnosis should not be based on the results of Innovance D-Dimer alone.

All D-Dimer results will be appended with the comment –DDIC in MIQ which states the following; “A negative D-Dimer does not completely rule out Deep Vein Thrombosis or Pulmonary Embolism. Results of the D-Dimer test should always be interpreted in conjunction with the patient’s medical history, clinical presentation, and other findings”.* 1. Patients with distal (in one of the three major veins in the calf) DVT may have a normal Innovance D-Dimer result.
	2. The following patients should not have D-Dimer testing used as an aid in the diagnosis of DVT and PE:
		1. Patients with therapeutic dose of anticoagulant therapy within the last 24

Hours.* + 1. Fibrinolytic therapy within the previous 7 days.
		2. Trauma or surgery within previous 4 weeks.
		3. Disseminated malignancies.
		4. Aortic aneurysm.
		5. Sepsis, severe infections, pneumonia, severe skin infections.
		6. Liver cirrhosis.
		7. Pregnancy

 [Document V - Wells Clinical Score for DVT and PE](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/202674.pdf) |
| **Reference Intervals** | Reference range: Clinical cutoff of 0.5 mg/L FEU when used along with a low clinical pretest probability assessment model to exclude DVT / PE. In a multi-center study using Innovance D-Dimer at a clinical cut-off of 0.5 mg/L FEU, the negative predictive value of 98% was established for ED patients using the Wells Pretest Probability Model and diagnosed by standard objective tests. At this cut-off the Sensitivity and Specificity for this method were found to be 97% and 42% respectively.Innovance D-Dimer assay has a new FDA-cleared clinical indication; the Innovance D-Dimer assay may now be used in conjunction with a low clinical pretest probability (PTP) assessment model to exclude deep-vein thrombosis (DVT) in addition to the FDA-cleared exclusion for pulmonary embolism (PE). [Document V - Wells Clinical Score for DVT and PE](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/202674.pdf) |
| **Method Performance Specifications** | * 1. Turbidity and particles in plasma may interfere with the determination. Plasmas containing particles should be ultrafuged prior to testing. Higher levels of lipids or turbid samples can lead to falsely elevated or decreased results.
	2. Patient samples may contain heterophilic antibodies ( i.e. human anti-mouse antibodies (HAMA) and rheumatoid factors) that could react with immunoassays to give a falsely elevated or depressed result. This assay has been designed to minimize interference from heterophilic antibodies, but complete elimination from this interference cannot be guaranteed.

The measuring range extends from approximately 0.2 mg/L FEU (lowest point on the calibration curve) to approximately 4.4 mg/L FEU or the highest point on the calibration curve.Samples initially outside the measuring range are reflexively ordered on the CS-5100 as DDi Hi which performs a sample dilution of 1:8 resulting in a measurement range up to 35.2 mg/L FEU in this example (4.4 x 8). Samples with values reported with a greater than sign (>) should be reported out as greater than (>) the number displayed on the analyzer (in this example >35.2). |
| **Result Reporting** | 1. Mpls (Sunquest): MPLS- see procedure “Autoverification in Coagulation”

Function: MEM <CR>Worksheet: C1<CR>Test-1: <CR>Test-2: <CR>CAP Method: Modify (M)DDI: CS5M1 or CS5M2 <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>DDI: Enter resultAccept (A), Modify (M), or Reject (R): A <CR> |
| **References** | 1. Innovance D-Dimer package insert. Siemens Healthcare Diagnostics Marburg, Germany, November 2008.
2. Innovance D-Dimer Control package insert. Dade Behring. Marburg, November, April 2008.
3. Clinical Laboratory Standards Institute. Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays: Approved Guideline-Fifth Edition.

CLSI Publication H21-A5. Wayne,PA, January,20081. Dade Behring BCS Operators Manual
2. Dade Behring BCS-XP Operators Manual
3. Application Sheet for Innovance D-Dimer on BCS System
4. Application Sheet for Innovance D-Dimer on BCS-XP System
5. An Algorithmic Approach to Hemostasis Testing Kottke-Marchant ,CAP Press,Copyright 2008.
6. Siemens Application Sheets RG\_39\_EN-U Rev. 2.11 D-Dimer / INNOVANCE V.07
7. Sysmex CS-5100 Instructions for Use, March 2020
 |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-5100 application |