|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Document Title:** D-Dimer Assay HS 500 - ACL TOP 350 | | | | | | |
|  | | | | | | |
|  | | | | | | |
| **Author** | **Effective Date:**  *Note: The Effective Date is assigned after all approval signatures are obtained* | | | | **Supersedes Procedure #** | |
| Sue Baker |  | | | | New | |
|  | | | | | | |
| **Revised by:** | **Date Revised:** | | | | **Effective Date:**  *Note: The Effective Date is assigned after all approval signatures are obtained* | |
|  |  | | | |  | |
|  | | | | | | |
| **Approval Signature** | | | | **Approval Date** | | |
|  | | | |  | | |
|  | | | |  | | |
| Xiaolan Ou MD, Medical Director | | | |  | | |
| . | | | |  | | |
| Mary Johnson, Chief Supervisor | | | |  | | |
|  | | | |  | | |
|  | | | |  | | |
|  | | | |  | | |
|  | | | | | | |
| **Distributed to** | | **# of Copies** | **Distributed to** | | | **# of Copies** |
| Bench copy | | 1 |  | | |  |
| Office | | 1 |  | | |  |
| SharePoint | | 1 |  | | |  |
|  | |  |  | | |  |
|  | |  |  | | |  |

# REVISION HISTORY

|  |  |  |
| --- | --- | --- |
| **Procedure #** | **Revision Date** | **Reason for Revision** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

# TITLE: Prothrombin Time (PT/INR) – ACL TOP 350

1. Purpose
   1. The determination of D-Dimer is becoming a widespread tool for diagnosing thrombosis and monitoring thrombolytic therapy. This procedure provides instructions for the analysis of D-Dimer using HemosIL D-Dimer HS 500 on the ACL TOP 350.
2. PRINCIPLE
   1. D-Dimer is contained in the soluble derivatives formed upon plasmin degradation of Factor XIIIa cross-linked fibrin (XDP). These soluble fibrin degradation products contain a neoantigen (D-Dimer domain), which is not present on the original fibrinogen molecule, its degradation products or on soluble fibrin.
   2. The Latex Reagent is a suspension of polystyrene latex particles of uniform size coated with the F(ab’)2 fragment of a monoclonal antibody highly specific for the D-Dimer domain included in fibrin soluble derivatives. The use of the F(ab’)2 fragment allows a more specific D-Dimer detection avoiding the interference of some endogenous factors like the Rheumatoid Factor.
   3. When plasma, which contains D-Dimer, is mixed with the Latex Reagent and the Reaction Buffer included in the D-Dimer HS500 kit, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of D-Dimer in the sample and is determined by measuring the decrease of the transmitted light caused by the aggregates (turbidometric immunoassay.)
3. SCOPE
   1. To provide UR laboratory personnel with a guide to accurately and precisely measure D-Dimer levels on the ACL TOP 350 (TOP 350) instruments at the Strong West Laboratory, 156 West Ave., Brockport, NY 14420.
4. RESPONSIBILITIES

| **Group/Person** | **Responsibility** |
| --- | --- |
| Quality Assurance | * Supports the development of this document. |
| Medical Director | * Ensures that the procedure is followed. * Review and approval of this document. |
| Supervisor/Manager | * Ensures that the procedure is followed. * Review and approval of this document. |
| Technical Staff | * Follows the procedure. |

1. ACRONYMS/DEFINITIONS

|  |  |
| --- | --- |
| URMC | University of Rochester Medical Center |
| SW | Strong West Laboratory |
| BR | Bailey Road Laboratory |
| SMH | Strong Memorial Hospital |
| CLSI | Clinical and Laboratory Standards Institute |
| PTT | Partial Prothrombin Time |
| PT | Prothrombin Time |
| INR | International Normalized Ratio |
| ISI | International Sensitivity Index |
| DD | D-Dimer |
| QC | Quality Control |
| LIS | Laboratory Information System |

1. SPECIMENS
   1. Nine parts of freshly drawn venous blood are collected into one part 3.2% trisodium citrate. Refer to the most recent Clinical and Laboratory Standards Institute (CLSI) Document H21-A5 for further instructions on specimen collection, handling and storage. No other anticoagulant is acceptable.
   2. Frozen samples: Thaw frozen specimens rapidly at 37°C and centrifuge plasma before testing. After thawing the assay must be performed within 2 hours.
   3. Samples for DD testing are processed by centrifuging specimens for r 3 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge.)
   4. Plasma Storage
      1. 8 hours at 20 ± 5º C
      2. 12 hours at 2-8º C
   5. Sample volume is CRITICAL to obtain accurate coagulation results. A 90% draw is the minimum volume acceptable for accurate testing. Do not run or report these samples. Place “.ND” in the result field with the canned text comment “Sample volume inadequate, unable to perform required testing.” In Order Entry add the SPROB test by following procedure. See Problem test in the Laboratory Information System SH.CP.SM.loe.0180. Refer to the minimum volume indicator on the tube, see the BD Tube draw volume guide.

F. Clotted samples: Each specimen is checked visually for the presence of clots prior to analysis. If a clot is suspected, the tube is uncapped, and checked with a pair of applicator sticks. *ANY* clot present in the specimen makes it inadequate for ALL coagulation testing. Clotted specimens are rejected and a new specimen should be requested.

H. Plasma Coloration Interference (hemolysis, lipemia, and icterus): Refer to TOP 350 SOP # section\_\_\_\_\_ for instructions on how to handle specimens with plasma coloration interference.

1. **QUALITY CONTROL**
   1. Normal and abnormal controls are recommended for a complete quality control program.
   2. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing.
   3. Controls should be analyzed at least once every 8 hour shift of patient testing and with each reagent change in accordance with good laboratory practice.
   4. Refer to the instrument’s On-Line Help for additional information.
   5. Refer to Westgard et al for identification and resolution of out-of-control situations.
   6. QC data is transmitted to HemoHub. Remedial action for out of range values is documented in HemoHub.
      1. When a value is out of range, the operator should:
         1. Repeat the control using the same control material. If acceptable, document in HemoHub and continue running patient samples.
         2. If still not acceptable, reconstitute a new control and rerun. If acceptable document in HemoHub and continue running patient samples.
         3. If still not acceptable, reconstitute new reagent for the out-of-range test and rerun both levels of control. If acceptable, document in HemoHub and continue running patient samples.
         4. If still not acceptable notify Hematology Supervisory personnel to begin maintenance and/or troubleshooting procedures as necessary.
         5. If supervisory personnel are not immediately available, set up backup analyzer for STAT/routine testing until issue is resolved.
         6. Refer to QC procedures SW.CP.GL.adm.0002
         7. **FOR SPECIMENS RUN PRIOR TO THE OUT OF RANGE VALUE:**
            1. Rerun the five most recent specimens on the backup analyzer.

If no clinically significant difference is noted then no further action is necessary.

If clinically significant differences are noted, then continue to perform result look back until comparison is within acceptable bias, usually 10%. Correct all results not within acceptable bias limit.

1. SPECIAL SAFETY PRECAUTIONS
   1. All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC (Center for Disease Control) recommendations and in compliance with the Federal OSHA (Occupational Safety and Health Administration) Blood-borne Pathogen Standard, 29 CFR (Code of Federal Regulations) part 1910.1030. All animal products should be treated as potentially infectious. Avoid contact with skin and eyes. Do not empty into drains. Wear suitable protective clothing. Follow specimen handling as outlined by Laboratory Safety Policy (SW.CP.GL.adm.0005).
   2. Wherever there are moving parts use caution with correcting malfunctions and when operating system.
   3. The reaction buffer contains less than 0.1% sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures.
   4. For in vitro diagnostic use only.
   5. Disposal of all waste material should be in accordance with local guidelines.
2. MATERIALS
   1. Equipment
      1. ACL TOP 350 LAS Coagulation Analyzer (hereafter referred to as “TOP 350”)
      2. Centrifuge
   2. Supplies
      1. Pipettes and pipette tips
      2. CLSI CLRW Type (or equivalent) water
   3. Reagents
      1. HemosIL D-Dimer HS500 kit (PN 0020500100) consists of:
         1. Latex Reagent: 3 x 4 mL vials of a suspension of polystyrene latex particles coated with F(ab’)2 fragment of a mouse antibody (MA-8D3) directed against DD. The reagent contains bovine serum albumin, buffer, stabilizers and preservative.
         2. Reaction Buffer: 3 x 6 mL vials of HEPES buffer containing bovine serum albumin, stabilizers, and preservatives
         3. D-Dimer Calibrator: 2 x 1 mL vials of a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin. It contains bovine serum albumin, buffer, stabilizers, and preservative.
      2. The following are not supplied with the kit and those required may be purchased separately:
         1. HemosIL D-Dimer HS500 Controls (PN 20500200)
         2. HemosIL Factor Diluent (PN 9757600)
         3. HemosIL Cleaning Agent (PN 9832700)
         4. HemosIL Cleaning Solution (PN 9831704)
         5. HemosIL ACL TOP Rinse Solution (PN 20302400)
   4. Controls
      1. HemosIL D-Dimer HS500 Controls (PN 20500200) consists of:
         1. Low DD HS500 control: 5 x 1 mL vials of a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin. The control contains bovine serum albumin, buffers, stabilizers, and preservative.
         2. High DD HS500 control: 5 x 1 mL vials of a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin. The control contains bovine serum albumin, buffer, stabilizers and preservative.
3. PROCEDURE – (STEP/ACTION)

**Note**: Please refer to the TOP 350 onboard help manual for additional information on the procedures below.

**Note**: Make sure all maintenance has been done and is up to date.

* 1. Reagent/Control Preparation and Stability:
     1. Unopened reagent is stable until the expiration date shown on the vial when stored at 2-8°C. For optimal stability, remove reagents from the system and store them at 2-8°C in the original vial.

**NOTE:** Avoid foam formation when homogenizing reconstituted calibrators or controls. Bubbles on top of the liquids may interfere with the instrument liquid sensors.

* + 1. Stability after reconstitution
       1. 30 days at 2-8˚C in the closed original vial.
       2. 7 days at 15°C on the ACL TOP® Family in the original vial with no stirring.

**NOTE:** the Latex Reagent and the Reaction Buffer have the same stability claims and should be used as a pair on the instrument. If one vial is replaced the other component should be replaced at the same time regardless of the residual contents in the vial.

* + 1. D-Dimer Calibrator: Dissolve the contents of each vial with 1 mL of CLSI CLR water or equivalent. Replace the stopper and swirl gently. Ensure the complete reconstitution of the product. Keep the calibrator at 15-25°C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.
       1. D-Dimer calibrator is stable after reconstitution for 3 days at 15-25°C, 1 month at 2-8°C, or 2 months at -20°C in the original vial. Frozen calibrator may be thawed at 37° and gently mixed before use. Do not refreeze.
    2. D-Dimer Controls: Dissolve the contents of each vial with 1 mL of CLSI CLR water or equivalent. Replace the stopper and swirl gently. Ensure the complete reconstitution of the product. Keep the control at 15-25°C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.
       1. D-Dimer controls are stable for reconstitution 1 month at 2-8°C, 8 hours at 15-25°C onboard the TOPS 350, or 2 months at -20°C in the original vial. Frozen controls may be thawed at 37°C and gently mixed before use. Do not refreeze.
    3. Cleaning Agent (Clean B Diluted): Diluted Cleaning agent 1:8 with CLSI CLRW Type water or equivalent. Reconstituted Clean B is stable onboard for 24 hours
  1. Calibration
     1. Calibration and storage of a valid DD calibration curve is required to obtain DD results
     2. Calibration will be performed:
        1. Every 6 months
        2. With a change of reagent lot number
        3. After major parts replacement
        4. To satisfy local regulatory requirements
        5. At the laboratory’s discretion
     3. Method for calibration:
        1. Define Materials if necessary (**Setup, Material List**). Select the appropriate materials from the Material List.

b. Add D-Dimer HS 500 Latex, D-Dimer HS 500 Buffer, D-Dimer HS 500 Calibrator and Factor Diluent to the Materials Programming Window if necessary (**Setup, Display, Materials Programming Window**).

c. Define Results Units and Rerun Rules in the DD HS 500 Test Definition if necessary (**Setup, Test List, Test Code, Result Units and Rerun Rules**).

d. Choose **Setup, Materials List**.

e. Double-click on the appropriate calibrator to open the **Materials Definition** screen.

f. Choose the **Lot Specific Information** tab and enter the Calibration Plasma lot number and Expiration Date

g. Enable **Lot Management** from the Lot Specific Information tab.

h. Select the **Save** icon to store the lot number. Once the lot number is saved, the **Assign Values** icon becomes available.

i. Select the **Assign Values** icon.

j. Enter the calibration value from the D-Dimer HS 500 package insert. Press **OK**.

k. Choose the **Previous Screen** icon to exit.

l. Load the D-Dimer HS 500 Latex, D-Dimer HS 500 Buffer, D-Dimer HS 500 Calibrator and Factor Diluent onto the ACL TOP® Family instrument.

m. Select **Calibration, Status List**.

n. Double-click on the DD HS 500 test to open the **Calibration Details** screen.

o. Choose the **Run** icon.

p. Select **OK** at the “Do you confirm the operation?” prompt.

q. Choose the **Previous Screen** icon to exit.

r. Verify the Job Status for the DD HS 500 test code says **Active**.

s. Once the calibration is complete, review calibration results. This calibration uses a spline curve fit, therefore there are no slope, intercept or r2 values displayed. If the calibration is acceptable, choose the **Validate** icon to validate the calibration curve.

4. For more details on performing calibration, see ACL TOP 350 Standard Operating Procedure (SW. CP. GL.lab.0019) or see the ACL TOP 50 series On-line help manual.

C. Procedure

1. Make sure all maintenance has been done and is up to date.

2. Load D-Dimer HS500 reagent and Clean B Diluted onto the TOP 350 analyzer using a reagent rack ® (Clean B Diluted should be loaded on the same rack as any thrombin-containing reagents)

3. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the TOP 350 analyzer. (If running QC from the sample rack, refer to **Quality Control, Performing a QC Test** in the On-line Help manual).

4. Choose **QC** from the Main Menu and select **Test Status List**

5. Double click on the test code to reveal the **Test Materials** Definition tree, making sure that the tree is organized by **Material/Tests**.

6. Select the box in from of the DD QC control and choose the **Program QC** icon. This will run all QC levels for that test.

7. Verify that all QC is in before running patient samples by viewing the QC **Test Status** list as well as verifying the results in HemoHub.

8. If QC is out of range, the analyzer will alert the operator by posting a “FAILED” result and with an audible beeping sound and flashing red exclamation point in the **Alarm Status** bar. Investigate and repeat QC according to laboratory protocol. (See section VII)

9. Place sample tubes in a sample rack with barcodes facing outwards.

10. Select an available sample track and load the sample rack when the barcode reader is in position.

11. Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually using the **Rack Details** screen (refer to **Samples Analysis, Managing Patient Samples, Programming Bar Coded Samples, and Programming Non-Barcoded Samples** in the SCL TOP 350 on-line help manual)

12. Choose the **Run** icon in the TOP 350 analyzer is not currently running to start processing the sample.

13. If the test has completed successfully, the mean result for each sample is displayed. IF the test completed, but the result failed, the work “FAILED” is displayed. If the test failed, the operator will need to investigate and rerun or load reagents, if necessary.

14. If the result is:

**Purple and Bold** – result is outside the Test Range

**Red and Bold** – results is outside of Linear Range but within Test Range

**Orange and Bold** – result is out of Therapeutic Range

Blue and not bold – result is out of Normal Range, but within Linear Range

Black and not bold – result is within Normal Range

15. The results will auto-validate (indicated by a green V) if they are within the test range for DD and there are no error codes associated with the result.

16. If the results do not auto-validate, the operator will need to investigate and address whatever issues are preventing the result from validating. If any flags or alarms are present, refer to Online Help for details. If the sample reruns and is still out, the operator may need to validate the result on the TOP 350 as well as on the LIS.

18. If the DD result is outside the Linear or Test Range (the result will be **PURPLE** or **RED**), the TOPS 350 is set up to automatically rerun the sample at a higher alternative dilution, thereby expanding the test range from 0.215-7.65 µg/mL to 0.215-128.0 µg/mL.

1. LIMITATIONS
   1. Prothrombin Time (PT) results on the ACL TOP® Family are not affected by these substances up to:

|  |  |  |
| --- | --- | --- |
| **Hemoglobin** | **Triglycerides** | **Bilirubin** |
| 500 mg/dL | 1327 mg/dL | 18 mg/dL |

* 1. The monoclonal antibody (MA-8D3) used in the Latex Reagent has major specificity for the D-Dimer domain of cross-linked Fibrin Degradation products. A low cross-reactivity to Fibrinogen Degradation Products was seen with plasma samples spiked with purified Fragments D and E above 10 µg/mL.
  2. Specimens from patients who have received a preparation of mouse monoclonal antibody for diagnosis or therapy may contain human anti-mouse antibody (HAMA). The presence of HAMA may cause an over-estimation of results in immunoassays that utilize mouse monoclonal antibodies. The Reaction Buffer contains a blocking agent against HAMA to minimize this interference on the assay results

1. CALCULATIONS
   1. NA
2. INTERPRETATION
   1. Elevated levels of DD are found in clinical conditions such as dep vein thrombosis (DVT, pulmonary embolism (PE), and disseminated intravascular coagulation (DIC).
   2. DD levels also rise during the normal pregnancy but very high levels are associated with complications.
   3. A negative DD result when combined with a clinical assessment of low pretest probability has been shown to have a high negative predictive value for DVT and PE.
3. RESULT REPORTING
   1. Reportable range: DD = 0.215 – 128.0 µg/mL FEU (Fibrinogen Equivalent Units (also known as Linear Range)

**NOTE**: For reporting purposes within the URMC laboratory network, all DD results that are greater than 128 µg/mL FEU will be reported out by the LIS as “>128.0 µg/mL FEU”

* 1. Normal Reference Range: PT = 0.0 – 0.50 µg/mL FEU

1. PROFICIENCY TESTING
   1. Proficiency testing is performed on:
      1. College of American Pathologists (CAP) (5 specimens) 3 times per year
      2. Other proficiency testing as applicable
2. LOT CONVERSION/INR CALCULATION:
   1. Lot conversion data is on file in the laboratory.
3. TRAINING
   1. Staff is initially trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

|  |  |
| --- | --- |
| **Role** | **Training Needed** |
| Management | Read the procedure. |
| Technical Staff | Read the procedure. |

1. REFERENCES
   1. HemosIL D-Dimer HS500 (PN 0020500100) package insert.
   2. ACL TOP® Family 50 series On-Line Help Manual.
   3. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition, CLSI Document H21-A5; Vol. 28, No. 5.
   4. ACL TOP 350 General Operations SOP, UR.CP.GL.lab.0019
   5. Laboratory Safety Policy (SW.CP.GL.adm.0005)

**Training or Read/Review Signature Log**

|  |  |  |  |
| --- | --- | --- | --- |
| **Document Title:** | D-Dimer HS 500 - ACL TOP 350 | | |
| **Document Number:** | SW.CP.CL.lab.0020.0001 | | |
|  |  | | |
| **Document Type:** | X SOP | □ Policy | □ Other \_\_\_\_\_\_\_\_\_  (specify: Article, Job Aid, Form, MSDS revision) |

|  |
| --- |
| Brief Description: (i.e. Revised) |
| Trainer(s):  (if applicable, or NA) |

***Your signature below indicates that you have read/been trained and understood the information.***

|  |  |  |
| --- | --- | --- |
| **Name (print)** | **Signature** | **Date** (mmm/dd/yyyy) |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |