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| **D-dimer HS500****on the ACL TOP 500** | *Procedure #:* | ***HCO# 240*** |
| *Version #:* | ***1.0*** |

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| **Purpose** | This procedure provides instruction for the analysis of D-Dimer using HemosIL D-Dimer HS 500 on the ACL TOP Family analyzers. |
| **Principle/ Clinical Significance** | The HemosIL D-Dimer HS 500 is an automated latex enhanced immunoassay for the quantitative determination of D-Dimer in human citrated plasma for use, in conjunction with a clinical pretest probability (PTP) assessment model, to exclude venous thromboembolism (VTE) in outpatients suspected of deep venous thrombosis (DVT) and pulmonary embolism (PE). When plasma, which contains D-Dimer, is mixed with the Latex Reagent and the Reaction Buffer included in the D-Dimer HS 500 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 aggregated (turbidimetric immunoassay).Elevated levels of D-Dimer are found in clinical conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC). D-dimer levels also rise during normal pregnancy but very high levels are associated with complications. A negative D-Dimer result when combined with a clinical assessment of low pretest probability has been shown to have a high negative predictive value for DVT or PE. |
| **Scope** | This standard operating procedure applies to all laboratory technicians, technologists and supervisory personnel of the Baltimore VA Medical Center Pathology & Laboratory Medicine Service. |
| **Responsibilities** |

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| **Responsible Party** | **Responsibilities** |
| Hematology Supervisor | * review this procedure biennially and make any necessary revisions in a timely manner
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| Medical Director | * review all new or substantially revised procedures, before implementation
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| Staff | * read this procedure in its entirety and ask any questions before implementation
* govern yourself according to the contents of this procedure after implementation
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| **Safety Precautions** | Standard Precautions:* Gloves
* Fluid resistant laboratory coat
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| **Sample Requirements** | SAMPLE COLLECTION: 1. Collect 9 parts fresh venous whole blood to 1 part **3.2% Sodium Citrate anticoagulant**.
2. Centrifuge sample ONLY in a designated Coagulation centrifuge at the indicated speed and time which is known to produce **platelet poor plasma**.
3. When collected in the manner described above, 3.2% Sodium Citrated plasma may be aliquoted and **frozen** as described below.

SAMPLE STABILITY:1. 3.2% Sodium Citrated plasma stored at ROOM TEMP (18-25°C) or REFRIGERATED (2-8°C) may be analyzed within **24 hours of collection.**
2. 3.2% Sodium Citrated plasma may be stored FROZEN (-20°C) for up to 2 weeks.
3. 3.2% Sodium Citrated plasma may be stored FROZEN (-70°C) for longer periods.
4. Frozen plasma specimens should be rapidly thawed at 37°C (using the Blood Bank water bath) for approximately 5 minutes. After thawing, each sample should be gently mixed and tested immediately.

SAMPLE PROCESSING:1. Before centrifugation, check the whole blood sample for gross clot formation by gentle inversion and observation. *This is the preferred method for detecting clots when using analyzers with cap piercers.*
2. Alternately, to check for clot formation, you may remove the cap and insert two wooden sticks into the sample, then remove the sticks and observe for clots. *Note: removal and replacement of the cap may cause errors with a cap-piercing probe. If the sample cap has been removed, you may have to pour off the centrifuged plasma into a sample cup and run it in a rack designated for uncapped samples.*
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| **Equipment/ Materials** | This test is intended for use on the ACL TOP 500. |
| **Reagents** |

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| **Item** | **Part No.** | **Storage** | **Packaging/Use** |
| Latex Reagent | 0020500110 | 2-8º C | **Open expiration = 7 days loaded on the TOP analyzer** |
| Reaction Buffer | 0020500120 | 2-8º C | **Open expiration = 7 days loaded on the TOP analyzer** |
| DDHS500 (L) control  | 0020500210 | 2-8º C | See stability below |
| DDHS500 (H) control | 0020500220 | 2-8º C | See stability below |
| Factor Diluent | 0009757600 | 15-25º C | **Replace/refresh analyzer aliquot every 7 days**; open bottle good until expiration printed on bottle |
| Nerl Reagent Grade Water | IM#21895 | 4D-135 | **Open expiration = 30 days** |

**PREPARING REAGENTS:**1. Latex Reagent: gently swirl several times to mix before use.
2. Reaction Buffer: gently mix several times before use.
3. Factor Diluent: discard old diluent and pour a fresh 5 mL aliquot every 7 days during weekly maintenance.

***Note: bubbles on top of the liquids may interfere with the instrument’s liquid sensors.*** |
| **Quality Control** | Low and High D-Dimer HS 500 controls are analyzed at least once every 8 hour shift of patient testing. Typical shifts and analyzer usage is defined in the chart below. In the event of an analyzer downtime, QC must be run every 8 hours of patient testing on the testing analyzer.

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| **Control Material:** | **Performed on the** **Heme ACL TOP:** | **Performed on** **the Stat ACL TOP:** | **Stability:** |
| *LOW –* *DDHS500 Control**(white cap)* | By DAY SHIFT (0800-1600) | By EVENING SHIFT(1600-2400) | 8 hrs on the TOP;***30 days when removed from TOP and stored at 2-8º C.*** |
| By NIGHT SHIFT(MIDNIGHT-0800) |
| *HIGH –* *DDHS500 Control**(red cap)* | By DAY SHIFT (0800-1600) | By EVENING SHIFT(1600-2400) | 8 hrs on the TOP;***30 days when removed from TOP and stored at 2-8º C.*** |
| By NIGHT SHIFT(MIDNIGHT-0800) |

**PREPARING CONTROLS:**1. Reconstitute the contents of each vial with 1 mL of Nerl reagent grade water.
2. Replace the stopper and swirl gently.
3. Write on each vial the 30-day refrigerated expiration date/time.
4. Make sure each vial is completely reconstituted.
5. Keep the control at 15-25º C (room temperature) for 30 minutes.
6. Invert to mix gently before use. DO NOT SHAKE.

***Note: bubbles on top of the liquids may interfere with the instrument’s liquid sensors.*****PROCESSING & STORING D-DIMER CONTROLS:**1. Immediately after analyzing D-Dimer controls, replace stopper and cap vials, being careful not to mix up the stoppers/caps.
2. Store in refrigerator at 2-8º C for up to 30 days.
3. QC shall be run with the first patient of each shift (as defined above). QC may be removed from the refrigerator and tested immediately. There is no ‘warm up’ period required for the QC material.
4. If more than two failures are obtained on a given vial, it may be evidence that the QC has been removed from refrigeration for too long. Repeat QC using the vials available in the alternate laboratory area before reconstituting new vials.
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| **QC Corrective Action** |

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| **IF:** | **QC IS:** | **THEN:** |
| First run | Acceptable | Proceed with patient testing |
| First run | Unacceptable | *Repeat affected QC material* |
| Second run | Acceptable | Proceed with patient testing |
| Second run | Unacceptable | *Check all reagents and QC vials for expiration, degradation, discoloration, or other abnormal findings. If found, repeat QC using in-date/normal reagents/materials.* |
| Third run | Acceptable | Proceed with patient testing |
| Third run | Unacceptable | *Discontinue patient testing and notify supervisor.* |

* **Document all QC corrective action in the ACL TOP software by following these steps:**
1. Select the failed data point in the Levy-Jennings chart by clicking directly on the point. Selected points will appear with a circle around them.
2. Click on the COMMENT icon  at the top of the screen.
3. Enter an appropriate **corrective action comment** and **your initials** in the pop-up box then choose OK.

  |
| **Calibration** | Calibration and storage of a valid D-Dimer HS 500 calibration curve are required to obtain D-Dimer results.**D-dimer calibration is performed:*** With every change of reagent lot numbers
* At least every 6 months for calibration verification
* As needed based on QC shifts/trends
* After major parts replacement, as determined by vendor service

**PREPARING CALIBRATORS:**1. Reconstitute the contents of calibrator vial with 1 mL of Nerl reagent grade water.
2. Replace the stopper and swirl gently.
3. Write on each vial the 30-day refrigerated expiration date/time.
4. Make sure each vial is completely reconstituted.
5. Keep the calibrator at 15-25º C (room temperature) for 30 minutes.
6. Invert to mix gently before use. DO NOT SHAKE. ***Note: bubbles on top of the liquids may interfere with the instrument’s liquid sensors.***

**PERFORMING CALIBRATION:**1. Choose **Setup, Materials List.**
2. Double-click on the DDHS500 Calibrator to open the **Materials Definition** screen.
3. Choose the **Lot Specific Information** tab and enter the calibration plasma lot # and expiration date.
4. Enable **Lot Management** from the Lot Specific Information tab.
5. Select the **Save** icon to store the lot #. Once the lot number is saved, the Assign Values icon becomes available.
6. Select the **Assign Values** icon.
7. Enter the calibration value from the D-Dimer HS 500 package insert. Press **OK**.
8. Choose the **Previous Screen** icon to exit.
9. Load the D-dimer HS 500 Latex, Buffer, Calibrator, and Factor Diluent onto the analyzer.
10. Select **Calibration, Status List.**
11. Double-click on the DDHS500 test to open the **Calibration Details** screen.
12. Choose the **Run** icon.
13. Select **OK** at the “Do you confirm the operation?” prompt.
14. Choose the **Previous Screen** icon to exit.
15. Verify the Job Status for the DDHS500 test code says **Active**.
16. Once the calibration is complete, review the calibration results with the Hematology Supervisor. This calibration uses a spline curve fit, therefore there are no slope, intercept or r2 values displayed.
17. If the calibration is acceptable, choose the **Validate** icon to validate the calibration curve.
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| PROCEDURE | **Step** | **Action** |
|  | 1 | * Place a properly labeled sample tube in a sample rack with the barcode facing outward:
1. Racks with BLUE handles are for capped tubes when using the cap-piercer on the ACL TOP 500.
2. Racks with plain BLACK handles are for open tubes when the cap-piercer is not available, or for SAMPLE CUPS, when needed.
 |
|  | 2 | * Select an available sample track and load the sample rack when the barcode reader is in position.
* Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually from the test and programming window.
 |
|  | 3 | * Click on the RUN icon if the ACL TOP is not currently running.
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|  | 4 | * Refer to the procedure *HCO 200 – ACL TOP 500 Automated Coagulation Analyzer* for instructions about how and when to perform analyzer and D-dimer related maintenance.
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| **Results Interpretation/ Expected Results** | * D-Dimer HS 500 results are reported in ng/mL of Fibrinogen Equivalent Units (FEU).
* **Normal patients will have results ≤500 ng/mL.**
* The assay result should be used with other information, including the clinical context, in forming a diagnosis.
 |
| **Critical Values** | * + - **Results >500 ng/mL should be called to the provider or designee.**
		- Refer to *HAD 010 – Hematology Laboratory Critical/Panic Value Reporting* for more information.
 |
| **Results Reporting** | * + - D-dimer HS 500 results are interfaced directly to the LIS (Vista):
1. Access the automated result entry routine in Vista (EA – enter/verify data, auto instrument).
2. Choose the BCOAGULATION worklist (BCO).
3. Enter the accession # for the sample you wish to verify.
	* + ***Note: certain analyzer flags will prevent results from crossing the interface. Investigate all results that do not cross the interface, as they may be in need of further action.***
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| **Method Performance Specifications** | The linearity and reportable range for D-Dimer HS 500 results on the ACL TOP are indicated in the chart below:**Linearity/Reportable range:**

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| Without dilution: | With automatic analyzer dilution: |
| 215 – 7,650 ng/mL | 215 – 128,000 ng/mL |

When the rerun capability of the instrument is activated, the instrument makes an on-board dilution and corrects the final result for the dilution factor, thereby expanding the test range to 128,000 ng/mL. If the result still exceeds the expanded range, report as >128,000 ng/mL. |
| **Method Limitations** | 1. D-dimer results are not affected by:
* Hemoglobin up to 500 mg/dL
* Bilirubin up to 18 mg/dL
* Triglycerides up to 1327 mg/dL
* Rheumatoid factor up to 1400 IU/mL
1. The monoclonal antibody (MA-8D3) used in 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 overestimation 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.
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| **Technical Support/****Service Information** | **Instrumentation Laboratories Technical Support Hotline (24/7)****1-800-678-0710*****Document all instrument troubleshooting/service and store all troubleshooting/ service records in the ACL TOP 500 analyzer Maintenance Log binder.***Important analyzer information:**ACL TOP 500 – Hematology Laboratory**Serial #: 09080763**ACL TOP 500 – Stat Laboratory**Serial #: 09080761 |
| **References & Attachments** | * D-Dimer HS 500, package insert. Instrumentation Laboratory. Bedford, MA 01730-2443.
* CLSI. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition.* CLSI Document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
* CLSI. *Quantitative D-Dimer for the Exclusion of Venous Thromboembolitic Disease; Approved Guideline.* CLSI Document H59-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
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| *Version #:* | ***1.0*** |

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| **Prepared by:** | **Date Adopted:** | **Approved by:** |
| Heather Crum, MT(ASCP) |  |  |

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| **Review Date:** | **Revision Date:** | **Reviewed/Revised by:** |
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| ***“I, the undersigned, do hereby certify that I have read this new/revised procedure. I understand the instructions contained within and have had the opportunity for any/all of my questions to be answered by the Hematology Supervisor and/or the Medical Director. I agree to govern myself accordingly.”*** |
| **Name:** | **Signature:** | **Date Read:** | **Comments/Notes:** |
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