# Boston Medical Center
# Boston MA 02118
# Department of Pathology and Laboratory Medicine

## BARC PRO 014 BARC PRO 014 D-Dimer v1.1

### Copy of version 1.1 (approved and current)

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**Approval and Periodic Review Signatures**

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| Periodic review | Laboratory Director Review | 3/20/2018 | 1.1 | Chris Andry, PhD<br>Chris Andry | |
| Approval | Lab Director | 3/30/2017 | 1.1 | Chris Andry, PhD<br>Chris Andry | |
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**Version History**

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|---|---|---|---|---|---|
| 1.1 | Approved and Current | Minor revision | 3/30/2017 | 3/31/2017 | Indefinite |
| 1.0 | Retired | First version in Document Control | 3/23/2017 | 3/23/2017 | 3/31/2017 |

1.0 **PURPOSE**

1.1. The purpose of this standard operating procedure (SOP) is to describe the D-Dimer HS assay used in the Pathology and Laboratory Medicine Department of Boston Medical Center (BMC).

2.0 **SCOPE**

2.1. This SOP contains revised text from the clinical document control software.

2.1.1. PRINCIPLE:. The HemosIL D-Dimer HS test kit (Instrumentation Laboratory; Bedford, MA) quantitatively measures the plasma levels of clot degradation fragments containing D-dimer by a latex agglutination method. The IL D-Dimer HS latex reagent is a suspension of polystyrene latex particles of uniform size coated with the F(ab) fragment of a monoclonal antibody highly specific for the D-Dimer domain included in soluble fibrin degradation products. The use of the F(ab) fragment allows a more specific D-dimer detection, avoiding the interference of some endogenous factors like the Rheumatoid Factor. When plasma containing D-dimer is mixed with the latex reagent and the reaction buffer, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of the D-dimer antigen in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates. This is a turbidimetric immunoassay.

2.1.2. SPECIMEN REQUIREMENT: 1 ml aliquot of plasma from whole blood that was mixed with 3.2% sodium citrate. The plasma aliquot will be run the same day and at every time point. Each aliquot must have a TCP barcode label attached.

2.1.3.

Note: A sample with hemolysis, lipemia, bilirubinemia or rheumatoid factor will not affect the testing.

**3.0 RESPONSIBILITY**

3.1. Principal Investigator. It is the responsibility of the Principal Investigator (PI) at BMC to ensure that project personnel have been trained in accordance with this SOP, that the training is documented, and that this procedure is followed.

3.2. Project Personnel. It is the responsibility of the project lab personnel to ensure he/she has read, understands, and follows the SOP when working with blood samples and the data.

3.3. It is the responsibility of the project staff designated by the PI or BSS to ensure that all the required case report forms (CRFs) in the Comprehensive Data Resource (CDR) are completed.

3.4. Any planned deviation or change from this SOP, known prior to a collection, should be pre-approved by the Biospecimen Research Group-Quality Management (BRG-QM and the Technical Project Manager (TPM), and well-documented by the site following the QM-0006 and submitting Change Request Form, QM-0006-F2.

3.5. Any unplanned deviation that is unexpected or identified during or after a collection should be well documented by the site. Such deviations should be submitted to BRG QM and the TPM, following QM-0006, and submitting Deviation Report Form, QM-0006-F3.

## 4.0 **DEFINITIONS**

4.1. Definitions

4.1.1. N/A

4.2. Acronyms

4.2.1. **BMC** Boston Medical Center
4.2.2. **BRG-QM** Biospecimen Research Group – Quality Management
4.2.3. **BSS** Biospecimen Source Site
4.2.4. **CDR** Comprehensive Data Resource
4.2.5. **CRF** Case Report Form
4.2.6. **PI** Principal Investigator
4.2.7. **PPE** Personal Protective Equipment
4.2.8. **RPM** Revolutions Per Minute
4.2.9. **SDS** Safety Data Sheets
4.2.10. **SOP** Standard Operating Procedure

4.2.11. **TPM** Technical Project Manager

## 5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Precautions (CDC-1987) shall be used for blood handling.

5.2. Comply with institutional policies regarding blood borne pathogens and the use of appropriate Personal Protective Equipment (PPE) at all times.

5.3. Dispose of all contaminated supplies in the appropriate biohazard and sharps containers.

5.4. Handle all chemicals appropriately according to Safety Data Sheets (SDS).

## 6.0 MATERIALS/EQUIPMENT

### 6.1. Materials/Equipment

6.1.1. ACL TOP 500 Coagulation System

6.1.2. ACL TOP Cuvettes (# 0029400100)

6.1.3. ACL TOP Rinse Solution (# 0020009700)

6.1.4. ACL TOP Cleaning Agent A (# 0009831700)

6.1.5. Factor Diluent (# 0009757600)

6.1.6. Reagent grade deionized water: For QC & reagent preparation: Fisher Scientific product # 751-628 (20L/5Gal)

### 6.2. Reagent Preparation

6.2.1. D-Dimer HS Kit (# 0020007700)

6.2.1.1. Latex Reagent: Dissolve the contents of each vial with 2 mL of distilled water. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25°C for 30 minutes and gently invert to mix before use. Do not shake.

6.2.1.2. Reaction Buffer: Gently invert to mix before use. The reagent is ready for use. Do not shake.

6.2.1.3. D-Dimer Calibrator: Dissolve the contents of each vial with 1 mL of distilled water. Replace the stopper and swirl gently. Make sure of the complete

reconstitution of the product. Keep the reagent at 15-25°C for 30 minutes and gently invert to mix before use. Do not shake. Note: Avoid foam formation when homogenizing reconstituted reagents and calibrator. Bubbles on top of the liquids may interfere with the instruments liquid sensors.

6.3. **Reagent storage and stability**

6.3.1. Check dates on all vials on the ACL TOP 500 and replace any that are ready to expire.

6.3.2. Unopened reagents and calibrator are stable until the expiration date shown on the vial when stored at 2-8°C.

6.3.3. Latex Reagent - Stability after reconstitution: 1 month at 2-8°C in the original vial or 4 days at 15°C on the ACL TOP 500. Do not freeze.

6.3.4. Reaction Buffer - Opened reagent is stable 1 month at 2-8°C in the original vial or 4 days at 15°C on the ACL TOP 500.

PLEASE NOTE: *The D-Dimer HS Latex Reagent and Reaction Buffer have the same stability claims and should be used as a pair on the ACL TOP 500. If one vial is replaced the other component (ie- Latex Reagent or Reaction Buffer) should also be replaced at the same time regardless of the residual content in the vial.*

6.3.5. D-Dimer Calibrator - Stability after reconstitution: 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°C and gently mixed before use. Do not refreeze.

6.3.6. For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

7.0 **PROCEDURE**

7.1. **Quality Control**

7.1.1. **Summary and principle**: Plasmin, a serine protease, when free from inhibitors digests the insoluble cross-linked fibrin yielding a variety of soluble derivatives. Their molecular weights depend on the extent of the digestion. 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. The Low and High D-Dimer HS 500 Controls are prepared by means of a dedicated process and contain different concentrations of partially purified D-Dimer obtained by digestion of Factor XIIIa cross-linked human fibrin with human plasmin.

7.1.2. **Composition**

7.1.2.1. The D-Dimer Controls kit consists of:

7.1.2.1.1. Low D-D Control (Cat. No. 0020008610): 5 vials x 1 mL of a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers and preservative.

7.1.2.1.2. High D-D Control (Cat. No. 0020008610): 5 vials x 1 mL of a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers and preservative.

7.1.2.1.3. The material in this product was tested by FDA approved test methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), Anti-HCV and HIV 1/2 antibodies. Handle as if potentially infectious.

7.1.3. **Preparation**

7.1.3.1. Dissolve the contents of each vial with 1 mL of distilled water or equivalent. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the control at 15-25°C for 30 minutes and invert to mix gently before use. Do not shake.

Note: Avoid foam formation when homogenizing reconstituted controls. Bubbles on top of the liquids may interfere with the instruments liquid sensors.

7.1.4. **Reagent storage and stability**

7.1.4.1. Unopened controls are stable until the expiration date shown on the vial when stored at 2-8°C.

7.1.4.2. Stability after reconstitution: 1 month at 2-8°C, 8 hours at 15-25°C on-board the ACL TOP 500 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.

7.1.4.3. For optimal stability remove control from the system and store it at 2-8°C in the original vial.

7.1.5. Run Control 1 and Control 2 once per shift. Repeat any out of control values. Document all QC problems on the Coagulation QC Problem Log located in the QC logbook.

7.1.6. Once the controls are within tolerance limits, return the Dimer controls to the refrigerator, being mindful of the expiration date. These controls are stable for 1 month after reconstitution if stored refrigerated at 2-8°C.

7.2. **Procedure**

7.2.1. **Perform Calibration** (For new lot # or as needed)

7.2.1.1. Edit/Verify Lot Numbers and Enter Calibration Target Value

7.2.1.2. Choose Menu Bar ► Setup ► Material List.

7.2.1.3. Double click on D-Dimer HS Cal to open the IL Material Definition screen.

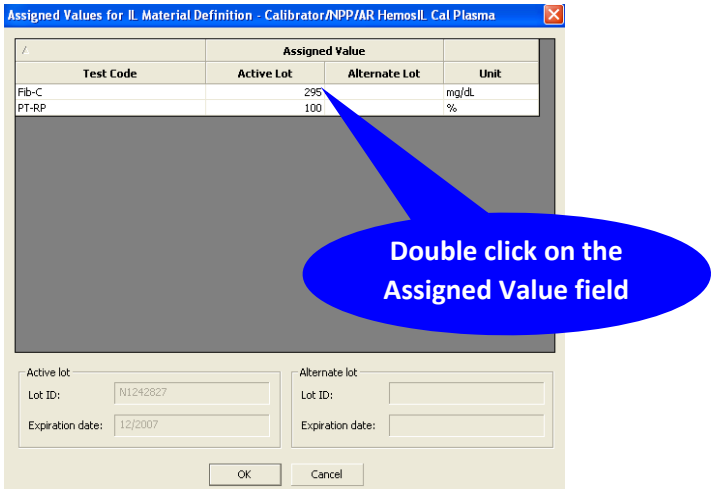
7.2.1.4. Single click the Lot Specific Information tab.

7.2.1.5. Enter/verify the lot number in the Lot ID: field and the expiration date in the Expiration date: field from the vial label.

7.2.1.6. If available, single click or touch Save  .

7.2.1.7. Single click or touch Assigned Values  .

7.2.1.8. Double click on the Assigned Value field to place the cursor to the field and enter the appropriate value from the Material Insert.

7.2.1.9. 


7.2.1.10. Choose OK.

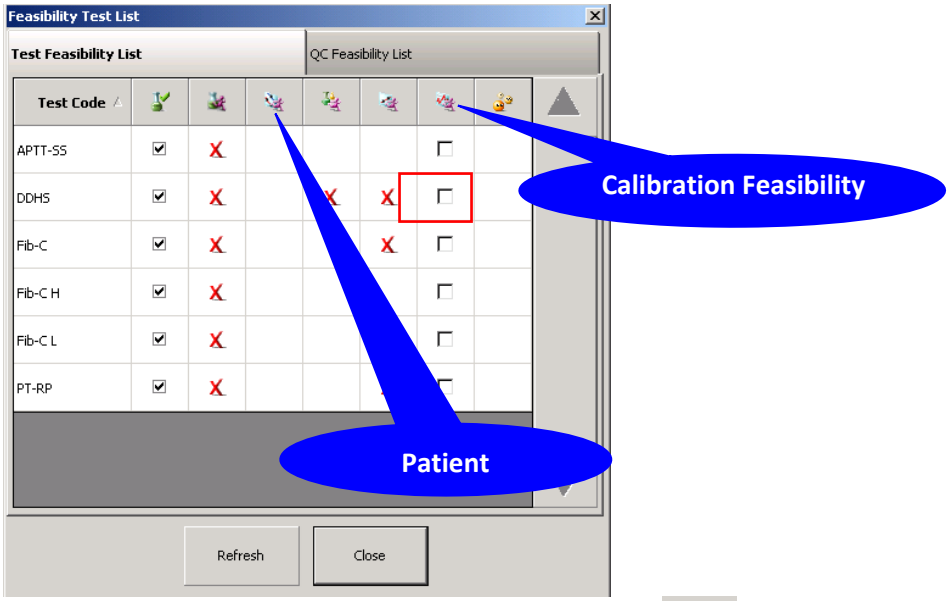
7.2.1.11. Single click or touch Previous Screen  to return to the Material List.

7.3. **Load Reagents**

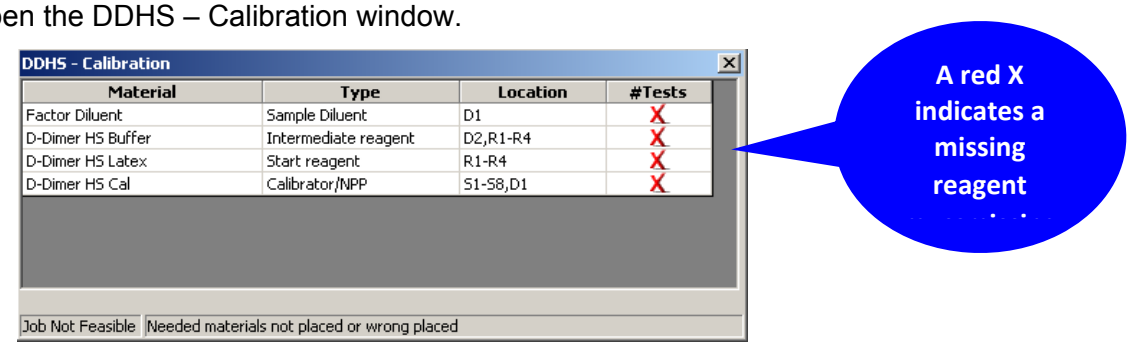
7.3.1. Single click or touch Reagent Area  to access the screen.

7.3.2. Single click or touch Feasibility Test List  .

7.3.3.




7.3.4. Single click on the X in the Calibration Feasibility column for the DDHS to open the DDHS – Calibration window.




7.3.5.

7.3.6. Use the DDHS Calibration Feasibility to sort missing materials by Location

7.4. **<u>Calibrate DDHS from the Calibration Status List</u>**

7.4.1. Choose Menu Bar ► Calibration ►Status List.

7.4.2. Double click on the DDHS test to open the Calibration Details screen.

7.4.3. Single click or touch Program .

NOTE: If Program is not available, place your mouse over it to display why the test is not feasible.

7.4.4. Choose OK at the Do you confirm the operation? prompt.

7.4.5. Single click or touch Previous Screen  to return to the Calibration Status List.

7.4.6. Verify the Job Status on the Calibration Status List for the DDHS is Active.

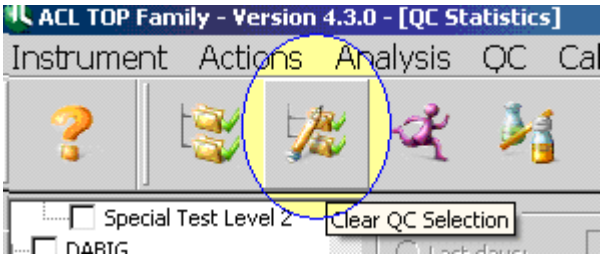
7.4.7. Once the calibration is complete, review calibration results. If there are no errors/failures, the controls are in range and the calibration is acceptable, choose the Validate icon to validate the calibration curve.

7.5. **Performing Quality Control**

7.5.1. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the TOP in the D1 diluent track.

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

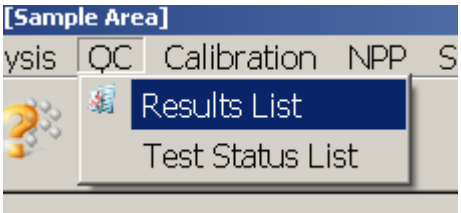
7.5.3. Double-click on any line item to reveal the QC Statistics screen. Select the Clear QC icon.

7.5.4. 


7.5.5. Select D-Dimer High Control and D-Dimer Low Control from the QC Tree listing and choose the Run icon. Repeat for any other required controls.

7.6. **Reviewing Quality Control**

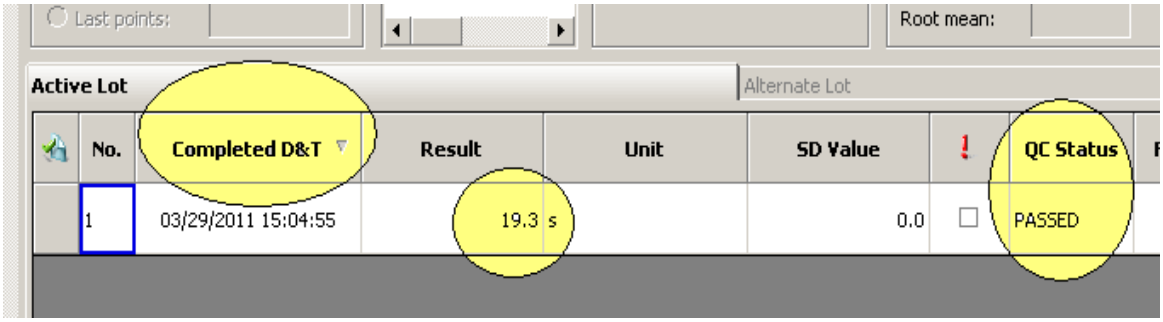
7.6.1. Select QC from the tool bar and single click on Results List. See picture below.

7.6.2. 


7.6.3. From QC Result List Screen, double click on DDHS. This will bring up the QC Statistics Screen and actual QC results.

7.6.4. From the QC Statistics Screen, select the first control of interest by clicking the control name itself on the QC tree and NOT the white box to the left.

7.6.5. Locate the control result by looking at the Completed D & T column and finding the particular time it was run. Look at the QC Status column to see if it PASSED or FAILED. See picture below.



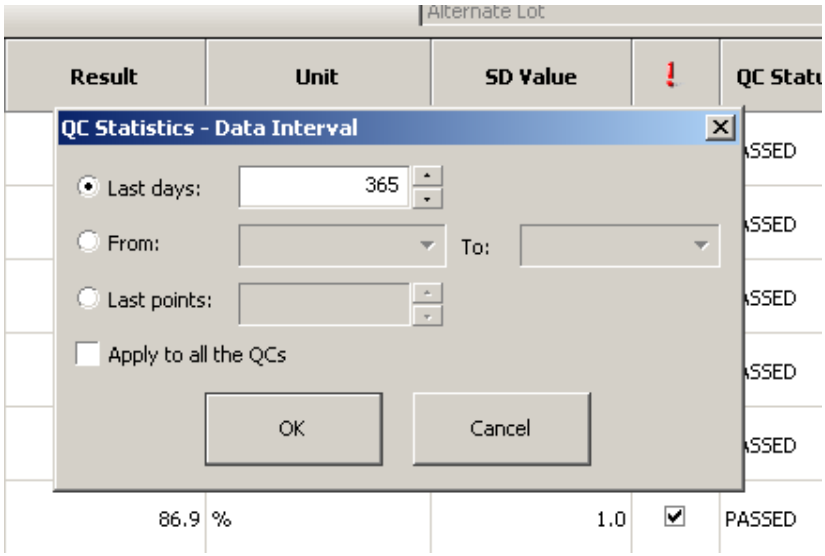

7.6.6.

NOTE: The QC Statistics screen has three different looks that can be toggled from QC values, Levy-Jennings charts or both. If the control values are not initially visible from this screen, try toggling from screen to screen by selecting one of the following icons located at the top of the screen.

7.6.7. If controls are still not visible, try adjusting the filter by selecting the filter icon located on the tool bar

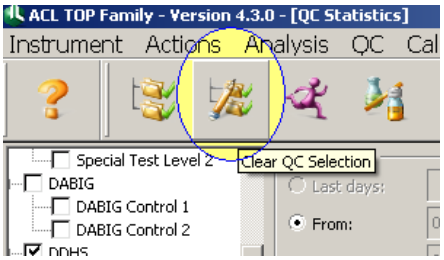
7.6.8. 

7.6.9. and selecting 365 days. .



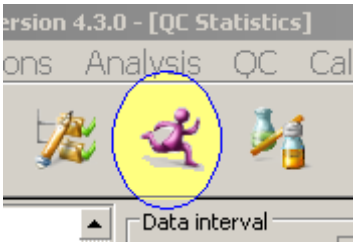

7.7. **Repeat any controls that FAILED**.

7.7.1. From the QC Statistics Screen, select the Clear QC icon.




7.7.2.

7.7.3. Locate the QC Tree and place a checkmark in the white box next to the control or controls that need to be run and select the Running Man icon.




7.7.4.

7.8. **Record control values on the appropriate TOP QC Log**.

7.9. **Corrective action**: Results of quality control must always be within acceptable range prior to reporting results. If results exceed expected range, begin troubleshooting

reagent and instrument including but not limited to, preparing new reagent and/or QC material, performing instrument maintenance, replacing de-ionized water aliquot, observing reagents for bacterial growth, etc. If results still exceed expected limits, discontinue testing and notify supervisor immediately. Review all patient results (if applicable) since the last acceptable QC. Refer to QC Manual for detailed quality control instructions. Always record all values and document corrective action taken on the TOP QC Review Problem Log.

7.10. **Sample Processing**:

7.10.1. Insert CAPPED patient sample tubes, or the aliquoted 12x75mm tubes with barcodes facing out on any sample rack that has a BLUE BAR. Load the sample rack onto the analyzer in any sample track.

NOTE: Be sure to use the correct sample rack for capped versus uncapped samples.

7.10.2. Single click or touch Sample Area from the Tool Bar to access the Sample Area screen.

7.10.3. Choose a sample track by pressing a track button on the front of the analyzer.

7.10.4. Once the barcode reader is in position, load the sample rack onto the analyzer until the light on the control panel turns green.

7.10.5. Review the Sample Area screen to ensure that all samples were identified.

7.10.6. If a sample has a question mark inside the blue circle, then the barcode on that Sample was not read and the sample was not identified. If the unit was unable to read a barcode, remove the rack and rescan in a slow and steady motion.

7.10.7. If the unit was unable to read the barcode, remove the rack, verify placement of barcodes, and replace the rack. Reprint barcode label if necessary.

7.10.8. Single click or touch the Run icon to start analysis. Notice that the Analyzer Status has changed to Busy.

7.10.9. On the bottom of the screen there will be a Time to Completion bar. This indicates the time until the end of the run (when the analyzer goes back to a Ready status).

7.11. **INTERPRETATION OF RESULTS**:

7.11.1. The determination of D-Dimer is becoming a widespread tool for diagnosing thrombosis and monitoring thrombolytic therapy.

7.11.2. 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 the normal pregnancy but very high levels are associated with complications.

7.11.3. 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.

7.12. **REPORTING RESULTS:**

7.12.1. Units: D-Dimer results are reported in ng/ml DDU. This is a change from our previously reported units of mg/L FEU.

7.13. **Measuring Range:**

7.13.1. The D-Dimer measuring range on the TOP is defined by the concentration of the calibrator used and is approximately 150 – 3680 ng/ml.

7.13.2. Samples initially outside the measuring range, on the high side will be automatically diluted on the TOP. The D-Dimer result is automatically multiplied by the dilution factor. This results in a measuring range of up to 69,000 ng/ml.

7.13.3. Values > 69,000 ng/ml will be reported as > 69,000 ng/ml.

7.14. **Procedure Notes**:

7.14.1. Specimens with values ≤150 ng/ml should be checked for bubbles, correct tube volume and checked for a clot by rimming the tube with two applicator sticks before being reported as <150 ng/ml with DMRNEG and RV attached.

7.14.2. Specimens with values > 69,000 should be checked for correct tube volume, repeated and checked for a clot by rimming the tube with two applicator sticks before being reported as will be reported as >69,000 with DMRNEG attached.

7.14.3. Any specimen with a Dimer that Fails with a repeat that Fails must be checked for a clot by rimming the tube with two applicator sticks. If a clot is found, credit the test in Misys, General Laboratory using the Without Result option and the reason

XCLOT. Call health care provider and document. If there is no clot, check sample integrity, re-spin and run again, check for instrument error codes, run controls again and/or call the Instrumentation Laboratories Tech Service line (posted on TOP). If specimen is unsatisfactory credit the test in Misys, General Laboratory using the Without Result option and the reason SUNSAT. Notify health care provider, document and request a new sample

7.14.4. If multiple tests are ordered on a sample and one test Fails, all results will be held so that none are auto verified. Find the Patient in the Sample List. Click on one of the results to open the Test information at the bottom of the screen. It is likely that a valid extended test will have been run automatically after the first result Failed. Select the tests to be reported by clicking on the empty square to the left (Selection Column) in the Test Information screen. A red check mark will appear. Do not select the Failed result. Click on the large green V (Validate icon) at the top of the screen and then click on the large yellow arrow that points to the right (Upload icon). This will send the valid results to Misys where they may be resulted in OEM.

7.15. **Computer Notes:**

7.15.1. The Misys Code for this test is DDE. The test name is D-DIMER, QUANTITATIVE.

7.15.2. Interfaced results can be answered using the OEM function using Misys SmarTerm.

7.15.3. All results >150 and ≤ 69,000 ng/ml will auto verify.

7.15.4. The DMRNEG comment will automatically be appended to the result. It translates to: "< 230 ng/ml is presumptive negative for venous thromboembolism in correlation with pre-test probability screening."

7.15.5. Results can be entered manually in the Misys SmarTerm:

7.15.5.1. Print the D-Dimer result from the TOP.

7.15.5.2. Print an SCO worksheet in Misys SmarTerm.

7.15.5.3. Answer the test using the MEH function, Worksheet: SCO, Test: DDE. Print an LHR (List Hold Results) sheet and release result using RHR (Release Hold Results).

7.15.5.4. The DMRNEG comment, <230 ng/ml is presumptive negative for venous thromboembolism in correlation with pre-test probability screening" will automatically attach to the results when answered in the SCO worksheet. If not answered in the worksheet, use code DMRNEG to attach the comment.

7.15.5.5. Staple the sheet to the worksheet and file

7.16. **Limitations of the Procedure:**

7.16.1. D-Dimer results are not affected by:

7.16.1.1. Hemoglobin up to 500 mg/dL

7.16.1.2. Bilirubin up to 18 mg/dL

7.16.1.3. Triglycerides up to 1327 mg/dL

**7.16.1.4.** Rheumatoid Factor up to 1400 IU/mL.

7.17. **NORMAL RANGE and NEGATIVE PREDICTIVE VALUE:**

7.17.1. Normal Range for D-Dimer is < 243 ng/ml. (accepted 9/11/2011, in effect 10/18/2011)

7.17.2. A D-Dimer result of < 230 ng/ml is considered presumptive negative for VTE in correlation with pre-test probability screening. (accepted 9/11/2011, in effect 10/18/2011)

7.18. **PROCEDURE NOTES:**

7.18.1. Result of this 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 result of D-Dimer test alone.

7.18.2. The measurement of D-Dimer should not be used as an aid in the diagnosis of VTE, in patients with the following conditions:

7.18.2.1. Therapeutic dose anticoagulant therapy for >24 hours

7.18.2.2. Fibrinolytic therapy within previous 7 days

7.18.2.3. Trauma or surgery within previous 4 weeks

7.18.2.4. Disseminated malignancies

7.18.2.5. Aortic aneurysm

7.18.2.6. Sepsis, severe infections, pneumonia, sever skin infections

7.18.2.7. Liver cirrhosis

7.18.2.8. Pregnancy

7.18.3. An elevated D-Dimer is not specific for DIC, but may be seen with other conditions including acute thrombosis, liver disease, postoperative state, eclampsia, cancer, sickle cell crisis and inflammatory conditions. The D-Dimer is most useful in the evaluation of DIC when analyzed in conjunction with the PT, platelet count and fibrinogen level (Toh et al., J Thromb Haemost 2007;5:604-06)

## 8.0 **REFERENCES**


8.1. HemosIL™ D-Dimer HS package insert issued 11/2010, Instrumentation Laboratory.

8.2. HemosIL™ D-Dimer Controls package insert issued 4/2010, Instrumentation Laboratory.

8.3. ACL TOP On-Line Help Manual Rev 2.2, Instrumentation Laboratory.

8.4. ACL TOP Training Manual 4/2010

8.5. Clinical and Laboratory Standards Institute (formerly NCCLS). Collection, Transport, and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays – 4th Edition; Approved Guideline. NCCLS document H21-A4, 2003.


## 9.0 **ATTACHMENTS**

| **INITIATION/REVISION HISTORY** | | | |
|---|---|---|---|
| **REV #** | **DESCRIPTION OF CHANGE** | **AUTHOR** | **EFFECTIVE DATE** |

| | | | |
|---|---|---|---|
| 1.1 | Reformatting clinical procedure into BARC format | Liz Duffy | 03/27/2017 |