STAGO SATELLITE D-DIMER PROCEDURE

PRINCIPLE

The specific degradation of fibrin (i.e., fibrinolysis) is the reactive mechanism responding to the formation of fibrin. Plasmin is the fibrinolytic enzyme derived from inactive plasminogen. Plasminogen is converted into plasmin by plasminogen activators. The main plasminogen activators are tissue plasminogen activator (tPA) and pro-urokinase which is activated into urokinase (UK) by, among others, the contact system of coagulation.

In the bloodstream, plasmin is rapidly and specifically neutralized by a_2– antiplasmin, thereby restricting its fibrinogenolytic activity and localizes the fibrinolysis on the fibrin clot.

On the fibrin clot plasmin degrades fibrin into various products, (i.e., D-Dimers). Antibodies specific for these products, which do not recognize fibrinogen, have been developed. The presence of these various fibrin degradation products, among which D-Dimer is the terminal product, is the proof that the fibrinolytic system is in action in response to coagulation activation.

Clinical applications for this test are as follows: Disseminated Intravascular Coagulation (DIC), negative predictor for the diagnosis of a thrombotic episode (i.e., DVT, PE), efficacy of treatment for a thrombotic episode and screen for possible re-occurrence (MI), and screen for other activation states of coagulation (i.e., post-operative, cancer, cirrhosis).

This assay is based on the change in turbidity of a microparticle suspension that is measured by photometry. A suspension of latex microparticles, coated by covalent bonding with monoclonal antibodies specific for D-Dimer, is mixed with the test plasma. An antigen-antibody reaction takes place, leading to an agglutination of the latex microparticles which induces an increase in turbidity of the reaction medium. This increase in turbidity is reflected by an increase in absorbance, which is measured photometrically. The increase in absorbance is a function of the D-Dimer level present in the test plasma.

SPECIMEN: SPECIMEN REQUIREMENT

1. Patient Preparation: No special preparation is required.
2. Whole blood specimen collected in a 9:1 ratio of blood to anticoagulant **3.2% (0.109M) Na Citrate** tube – blue top. No other anticoagulant is acceptable.
3. It is not necessary to draw a discard tube prior to filling a blue stopped tube in routine circumstances. If you are using a butterfly needle to draw you will need to draw another tube first as the tube will not properly fill because of the air in the tubing.
4. **If blood is drawn from an indwelling catheter**, the line should be flushed with saline and the first 5 ml of blood discarded.
5. D-dimer samples are stable for 24 hours if left unspun and stoppered at room temperature or refrigerated
6. Plasma is also stable for up to 8 hours at 2 – 8 ^{0}C
7. Plasma may be frozen immediately at a temperature of < -20 ^{0}C for up to one month if testing is delayed.

8. NCCLS guidelines require a 90% fill volume. If the specimen is below the fill line, it must be rejected and redrawn. If the specimen appears to be over filled, it also needs to be rejected and redrawn.
9. Specimens that are clotted, collected in the wrong tube or have visible hemolysis must be rejected.
10. Specimens should be centrifuged at 1000 rcf for 10 minutes or as ascertained by ensuring that the plasma platelet count is less than 10,000/ul (platelet poor plasma). Check plasma platelet counts quarterly and/or after change or repair of centrifuge – see separate procedure.
11. After specimen centrifugation, visually inspect the plasma for any clots

Warning: Treat all specimens as potentially infectious. Wear gloves and protective clothing.

EQUIPMENT AND MATERIALS

Equipment:

Stago Satellite Coagulation Analyzer
Cuvette roll (contains 220 cuvettes)
Centrifuge
Pipette tips
STA® Reducer (Cat. No. 00797 or 00801)
Magnetic Stir Bars (Cat. No. 27425)
Pipettes

Materials:

STA® Reagent 1 – Tris buffer
STA® Liatest® Control Ⓝ+Ⓟ (Cat. No. 00526):
STA® Desorb U Solution
STA® Reagent 2 – Latex
STA® Wash Solution
NERL reagent grade deionized water

NOTES

- Reconstituted controls must equilibrate at room temperature for 30 minutes before loading on the Stago Satellite
- All reagents and controls need to equilibrate on the analyzer for 5 minutes after the products have been loaded on the product carousel.

PREPARATION OF REAGENTS AND CONTROLS

A. STA Reagent 1: Tris Buffer

1. Ready to use.
2. Allow the reagent to stand at room temperature for 15 minutes.
3. Mix gently without creating bubbles. Remove the rubber stopper; place a STA® mini Reducer (Cat. No. 00797) in the vial, and replace the perforated cap.
4. Stability
    a. On board the STA Satellite® is 15 days
    b. Until expiration date if kept at 2 – 8 ^{0}C protected from light.

B. STA Reagent 2: Latex

1. Ready to use. Suspension of microlatex particles coated with two different mouse monoclonal anti-human D-Dimer antibodies (8D2 and 2.1.16) then stabilized with bovine albumin.
2. Allow reagent to stand at room temperature for 15 minutes.
3. Mix gently without creating bubbles. Remove the rubber stopper; place a STA® mini-reducer (Cat. No. 00797) in the vial, and replace the perforated cap.
4. Stability
    a. On board the STA Satellite® is 15 days.
    b. Until expiration date if kept at 2 – 8 ^{0}C protected from light.

C. STA® Liatest® Control Ⓝ+Ⓟ Latex

1. Citrated human control plasmas containing different levels of D-Dimer (normal and pathologic), freeze-dried. Intended to be used as controls for the immuno-turbidometric method. Cat No. 00526
2. Reconstitute each vial with 1.0 ml NERL deionized water.
3. Let sit 30 minutes at room temperature.
4. Swirl gently.
5. Reconstituted stability on board the STA Satellite®
    a. On board the STA Satellite® is 8 hours
    b. stored with rubber stopper at 2-8°C is 8 hours

D. STA® Desorb U

1. A decontaminating solution for use the instruments of the STA® Satellite and is designed as in integral part of the system.
2. Contains Potassium Hydroxide (KOH < 1%)
3. No special preparation is needed
4. Stability
    a. The reagent in intact bottles is stable until the expiration date indicated on the box label, when stored at 2-8°C and protected from light. DO NOT remove bottles from box or cut the top off the box.
    b. On board the STA Satellite® is 14 days

LOADING BAR-CODED BUFFER AND LATEX REAGENTS

1. Select (UN)loading from the main screen
2. Arrow down (or press the F2 function key) to “products vial” then press the <ENTER> key
3. Open the Product Cover
4. Press any key (other than ALT and Symb)
5. Position the cursor on the line corresponding to the vial position (use the ↓↑ keys) and [Enter]; the analyzer places the selected position in front of the barcode window.
6. Put the vial in the presented position. The barcode label must face the barcode reader window. The STA Satellite® will detect the vial.
7. Confirm the volume and stability of the reagent by using the [Enter] key.
8. To load additional bar-coded reagents, repeat steps 4-7.
9. To finish loading, press [esc] and [enter], close the product cover, and then press any key (other than ALT and Symb)

CALIBRATION

1. The kit reagents are pre-calibrated: this calibration is identical for all reagents of each lot.
2. To enter the data for the calibration curve: the database of the STA Satellite® monitors all reagent lot numbers. When the operator scans a new lot of Liatest® D-Dimer reagent, the STA Satellite® will request the operator to scan the barcode printed on the barcode insert across the STA Satellite® barcode reader.
3. The calibration curve will be validated for the lot being used once the two Liatest® D-Dimer controls have been run. If the validation controls are outside the assayed range, The STA Satellite® will not run patient samples.
4. Examine calibration curve on screen: Through the MAIN MENU under CALIB/CONTROL select CALIBRATION. Move the cursor to D-Dimer and [Enter]. The curve will appear on the STA Satellite® screen.
5. Print calibration curve: While examining the curve on the STA Satellite® screen, press ESC key for options. Select print, then [Enter]. Select Execute and [Enter] to execute the print command. The curve will print along with the information on reagents and control lot numbers. The STA Satellite® cannot print a calibration curve while it is running.

CALIBRATION VERIFICATION, AMR REVALIDATION, LIS TRANSMISSION AND CALCULATION VERIFICATION

- Calibration Verification, AMR Revalidation, LIS transmission and Calculation verification are performed together every six months. This is coordinated regionally using previously tested patient samples.
- Instructions:
    - Select at least three previously tested patient samples (low, mid-point, and high). In addition select two samples that are below (<220 ng/ ml) and above (>4000 ng / ml) the stated linearity to verify the AMR range and LIS transmission and calculations. Establish a 2SD acceptable range for each sample. Aliquot the samples into seven capped plastic tubes and freeze for up to on year. Distribute samples and worksheet to all Medical Office Laboratories that perform this test.
    - Place frozen samples in the 37 C incubator to thaw for 30 minutes upon receipt – set a timer.
    - Ensure that there is enough D-dimer reagent to run 10 samples
    - Order a D-dimer test for each sample using ZZTEST patients (use CAP ZZ patients for your location). This is done to ensure that results will cross the interface into the LIS.
    - Run D-dimer QC and verify the results in the LIS. QC must be within limits.
    - Once the samples have thawed, place in a micro cup and perform D-dimer on the Stago Satellite.
    - Write the results on the “Biannual Coagulation Calibration Verification, AMR Revalidation and LIS Transmission and Calculation Verification” worksheet. This is found on the forms section of the Laboratory website.
    - Submit worksheet to the supervisor for review and signature.
- See each specific section below for additional details.

A. CALIBRATION VERIFICATION

1. Calibration verification is performed every six months or with the following conditions
   - QC fails to meet established criteria
   - After major preventive maintenance or change of critical instrument component
   - If recommended by the manufacturer
2. It is not necessary to perform calibration verification with reagent lot number change. Running QC and ensuring that they are within the established and acceptable range is sufficient.
3. Lot to lot comparison is performed with every reagent or quality control lot number changes and serves the purpose of calibration verification – See lot to lot section below.
4. Review the results from above and ensure that they are within the 2SD established limits. If they are not within limits, re-run the samples. Call the regional coordinator if samples are still out of limits on re-run samples. It may be necessary to contact technical support.
5. The kit reagent is pre-calibrated by the manufacturer. The calibration is verified when previously tested patient samples that span the reportable range for d-dimer fall within the established acceptable 2SD limits.

B. AMR REVALIDATION

1. The AMR is revalidated every six months when the calibration verification is performed.
2. At least three previously patient samples (low, midpoint and high) are used for the revalidation procedure. Samples selected are near the stated AMR.
3. Review the results from above and ensure that they are within the 2SD established limits. If they are not within limits, re-run the samples. Call the regional coordinator if samples are still out of limits on re-run samples. It may be necessary to contact technical support.

C. LIS TRANSMISSION AND CALCULATION VERIFICATION

1. LIS transmission and calculation of the Stago Satellite is verified every six months when the calibration verification is performed.
2. LIS Transmission Verification – Review the results above and ensure that they transmitted from the Stago Satellite into the LIS. Manually print chart copies from the LIS and attach to the worksheet.
3. LIS Calculation Verification – Review the results above and ensure that the LIS has applied the correct calculation from ug / ml on the analyzer to ng / ml. Multiply the result from the analyzer (ug / ml) by 1000 to calculate the ng / ml value that is reported in the LIS.
4. Ensure that <0.2 ug/ml result from the analyzer is posted as <220 ng/ml in the LIS.
5. Ensure that >4.0 ug/ml result from the analyzer is NOT posted in the LIS.

LOT TO LOT COMPARISON OF REAGENTS AND CONTROLS

1. New QC lot number
   a. A three day lot to lot comparison is performed with QC lot number changes.
   b. Run current and new QC lot number twice per day (once during the morning startup and again in the afternoon).
   c. Attach print out to the D-dimer lot to lot worksheet, submit to the supervisor for review and file in the lot to lot section of the Stago maintenance binder.
2. New Reagent lot number

a. Lot to lot comparison is not necessary for reagent lot numbers. Before switching to the new reagent lot, print the calibration curve of the current lot number in use. After placing the new reagent lot, print that calibration curve as well and file both calibration curve printouts in the Reagent section of the Stago maintenance section.
b. To enter the data for the calibration curve: the database of the STA Satellite® monitors all reagent lot numbers. When the operator scans a new lot of Liatest® D-Dimer reagent, the STA Satellite® will request the operator to scan the barcode printed on the barcode insert across the STA Satellite® barcode reader.
c. Run both levels of QC after placing the new reagent lot on the analyzer. Results must be within the established and acceptable limits. If they are not, reconstitute new set of controls. If QC is still out after the second try, call technical support for assistance.

QUALITY CONTROL

1. QC can be run automatically at pre-set intervals (defined in Test Set-up) or by ordering manually from the Quality Control Menu – see stepwise instructions below.
2. All control ranges are monitored automatically by the STA Satellite®. If any controls are outside the either barcode or entered site specific range, the instrument will audibly and visually alarm the operator. Otherwise, the results can be found in the Daily Control file and the individual QC files. Control results are automatically filed in the STA Satellite® QC file. All results for a 24-hour period will be reduced to a "mean" value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean.
3. To print all the QC data points for the PT test, perform the following procedure prior to midnight. From the MAIN MENU under CAL/CONTROL select **Daily Controls.** Press F6 to print the screen (instrument must be idle).
4. To print individual QC data,
    a. From the MAIN MENU, select QUALITY CONTROL, and [enter]. Cursor to the D-Dimer test and [Enter] to view the Levy Jennings chart
    b. Press **F1** to view the results in tabular form.
    c. Press **F6** and Select 'Execute' then [Enter] to print the individual values under current controls
    d. Press [ESC] key to exit (back to graph).
    e. Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.
5. Quality Control is automatically sent to the LIS.

6. Accept QC in the LIS per the QC procedure and document and take appropriate action for any data that is out of limits. Each location has been assigned the following QC accession number:

Location	Liatest N accession number	Liatest P accession number
Arapahoe	0-QC-086601	0-QC-086602
East Denver	0-QC-066601	0-QC-066602
Franklin	0-QC-026601	0-QC-026602
Lakewood	0-QC-036601	0-QC-036602
Rock Creek	0-QC-426601	0-QC-426602
Westminster	0-QC-076601	0-QC-076602

7. Quality control is reviewed at least monthly by the Supervisor or designee.
8. Quality control may also be evaluated internally within Kaiser Permanente Colorado Region as inter-lab comparison data on an "as needed" basis.

MANUALLY REQUESTING QUALITY CONTROL

1. At the main menu, select CALIB/CONTROL
2. Select QUALITY CONTROL and press <ENTER>
3. Arrow down to D-dimer
4. Press the F1 function key
5. Press the F10 function key to confirm
6. When requested, type CQ as the password and press <ENTER> to confirm
7. Press the <ESC> key to exit. The Status Screen will appear and the instrument will begin testing once the test status screen appears.

LOADING PATIENT SAMPLES – Simple sample loading

1. Access the Sample Loading menu by pressing **F1.** The STA Satellite® moves the carousel to the first free position. If the sample has been transferred to a microtainer, press **F8** before proceeding.
2. Put the sample into the open position. If the tube is bar-coded, the barcode must face the reader window. The barcode is read by the instrument.
3. If the tube must be manually identified, type in the patient identification (accession number or name) and confirm by pressing the <ENTER> key. Then place the sample tube in the open position in the sample carousel.
    a. In MANUAL MODE, the operator must order the test(s) from the Selection menu, or from the Recorded Profile(s). Cursor to the test and press <**Enter>** to select. When all tests are ordered, press F10 to save.
    b. In AUTO MODE, the STA Satellite® will automatically order the test(s) selected in the AUTO MODE profile.
    c. If TELELOADING is selected as the AUTO MODE profile, the STA Satellite® will query the host computer and download the test(s) as well as assign the status (i.e. stat).

4. When loading is complete, press the [ESC] key. Select [QUIT] from the drop down Menu, and then [YES].

5. The TEST STATUS screen will appear. If there is not enough reagent(s) to run the test(s), the suspect reagent(s) will appear in red with the amount of deficiency. This deficiency will BLOCK the SAMPLE PIPETTING. When this occurs, add the necessary reagent(s) to run the samples by responding N (NO) to the warning message 'NEW TESTS

ARE DELAYED – Do you wish to reactivate?' Reagents can then be loaded into the reagent carousel. By responding Y (YES) to the warning message 'NEW TESTS ARE DELAYED – Do you wish to Reactivate?' the instrument will continue to perform all tests for which there is sufficient reagent (i.e. while waiting for reagents to stabilize after reconstitution).
6. All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the SYSTEM STATUS menu.
7. For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and press enter. This will display the FILE PROCESSING screen. Follow the options on the left-hand side of the screen (i.e. **F3** - rerun test).

CALCULATIONS

1. The STA Satellite® automatically plots the results in delta OD off of a standard curve and converts the results to µg/ml FEU.
2. The assay uses the sample undiluted. If the result is greater than the measurable range (4.00 µg/ml) the STA ®Satellite will NOT automatically re-dilute the sample.
3. A calculation is used in CERNER (x 1000) since D-dimer results from the STA Satellite® are reported in µg/ml on the analyzer. D-dimer results are reported in CERNER and in Health Connect in ng / ml.

REFERENCE RANGE

Less than 500 ng/ml FEU

REPORTING RESULTS

1. The results for a D-dimer are reported in whole numbers in ng/ml (Example: 489 ng/ml or 3153 ng/ml).

2. Results are verified in the LIS under the "ARE" application using the COXX STA service resource (Example COFR STA).

3. Critical results are called to the ordering provider and documented in the LIS.

Test	Critical Low	Critical High
D-dimer	None	>500 ng/ml

4. D-dimer results >4,000 ng/ml are reported as >4,000 ng/ml. Results $\geq$ 4.0 µg/ml on the analyzer will NOT be transmitted to the LIS and will not be printed and a **"VMAX"** result will be displayed on the analyzer screen. The operator will need to enter a result 4.1 in the Instrument Result field of the "ARE" application of CERNER and the LIS will convert this value as >4000 ng/ml.

5. D-dimer result <220 ng/ml is reported as <220 ng/ml.

LIMITATIONS AND INTERFERING SUBSTANCES

1. FDP concentrations greater than 15 µg/ml may lead to an over-estimation of the D-Dimer level.

2. The presence of rheumatoid factor at a level greater that 50 IU/ml may lead to an over-estimation of the D-Dimer level.

3. The STA® Liatest® D-Dimer is insensitive to fibrinogen and the E fragment. A low cross-reactivity is observed with the D fragment.

4. The STA® Liatest® D-Dimer is insensitive to the following substances: hemoglobin (up to 5 g/l); bilirubin (up to 200 mg/l); unfractionated heparin (up to 2 IU/ml0; LMWH (up to 2 anti-Xa IU/ml).

NOTES

1. The detection threshold of the STA® Liatest® D-Dimer on the STA Satellite® is 0.22 µg/ml FEU. The printout limits are pre-set at 0.22 – 4.00 µg/ml FEU.

2. The STA® Liatest® D-Dimer results are expressed in FEU, Fibrinogen Equivalent Units. By definition, an FEU is the quantity of fibrinogen initially present that leads to the observed level of D-Dimer. In general, the actual quantity of D-Dimer is approximately half of an FEU.

3. A V>MMax for STA Satellite® result indicates a result that is greater than 4.00 µg/ml FEU

4. When you have issues on the Satellite on D-dimers (i.e. QC failure and can't run pt samples):
   a. Try to run the test twice (no more than 3 times)
   b. If unable to resolve the problem, send to another KP Medical Office Laboratory
   c. Another option is to send the test directly to ESJH or EGSM. When sending D-dimer to SJH do the following:

- Indicate on the paper requisition, "Please run DDITT test" (this is our equivalent of the D-dimer that is run on the Satellite)
- You must also write "Call result to KDC at 303-344-7828 if result is >500" or "Call result to Dr. XX at XXX-XXX-XXXX if result is >500." ESJH and EGSM do not have critical results on D-dimer and will NOT call high result to anyone unless we specifically request it.

REFERENCES

1. STA® Liatest® D-Di, Immuno-Turbidimetric Assay of D-Dimer. Package insert #23296 -revised November 2005.
2. STA® Liatest® Control Ⓝ+Ⓟ (Cat. No. 00526): Control Plasmas for Immuno-Turbidimetric Assays of vWF and D-Dimer on the STA® Analyzers. Package insert #23189 – revised November 2004.
3. STA Satellite® Operator Manual. STA Satellite® Software Version 421.05

For additional information, please refer to the manufacturer's package insert.