Department of Pathology
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D-Dimer Procedure

Sta Compact Max

I. PRINCIPLE

The test principle of 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 whose D-dimer level is to be assayed. 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, the latter being measured photometrically. The increase in absorbance is a function of the D-dimer level present in the test sample.

II. CLINICAL SIGNIFICANCE

Elevated D-dimer levels are observed in all diseases and conditions with increased coagulation activation, e.g. thromboembolic disease, disseminated intravascular coagulopathy (DIC), acute aortic dissection, myocardial infarction, malignant diseases, and 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.

III. SPECIMEN

A. 2.7 ml or 1.8 ml Blue Top Tube. Mix nine parts of freshly collected blood with one part of 0.11 mol/L (3.2%) sodium citrate anticoagulant. Invert the tube gently three or four times immediately after venipuncture to ensure proper mixing of blood an anticoagulant.
   - If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 mL saline and the first 5 mL of blood or six dead space volumes of the catheter discarded.

B. The citrate concentration must be adjusted in patients who have hematocrit values above 55%. See STA Compact Max Start Up Operating Procedure.

C. Specimens that are clotted, collected in the wrong tube, have visible hemolysis or have less than a 90% fill or >10% overfill are rejected.

D. It is unacceptable to combine the contents from separate under-filled sodium citrate tubes.

E. Handling/ Storage Conditions:
   1. The whole blood specimen is checked for clot formation by gently inversion and observation.
   2. Centrifuge the capped blood specimen as soon as possible after collection for 2 minutes at 7200 RPM (3500 x g) in the Stat Spin Express 3 or S/P ® Brand Stat-60.

a. Testing requires produce platelet-poor plasma (platelet count <10x10^9/L).
b. The plasma may remain on the packed cells if testing immediately or separated if freezing.
c. To separate plasma, use a plastic transfer pipette; remove the plasma to a properly labeled polypropylene/plastic tube until ready to test.

3. If testing is not completed within 4 hours, the plasma must be removed, using a plastic transfer pipette. Remove the plasma to a properly labeled polypropylene/plastic tube and freeze.
4. If testing is further delayed, the plasma may be held for 8 hours at room temperature, or 4 weeks at –20 °C. A frost-free freezer should not be used. Thaw the sample rapidly at 37 °C, allow sufficient time to obtain complete thawing.
5. Specimens should be stored at room temperature after testing. Once removed from the analyzer, caps must be removed if additional testing is ordered.

IV. REAGENTS

A. STA® - Liatest® D-Di kit
1. Reagent 1: Tris buffer
2. Reagent 2: 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).
3. Preparation: Allow Reagent 1 and 2 to stand at room temperature (18-25 °C) for 15 minutes before use. Mix the reagents by gentle swirling of the vials without creating any bubbles. Then, place a new STA® - mini Reducer and the perforated cap on each vial.
4. Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.
With the STA® - mini Reducer and perforated cap in place the stability of Reagents 1 and 2 after opening and in their original vials is 15 days on STA® Compact.

B. STA® - Liatest® Control N + P kit: provides a normal plasma and an abnormal plasma intended for the quality control of the following antigenic assays by the immuno-turbidimetric method:
D-dimer assay on STA Compact® with STA® - Liatest® D-Di
1. Reagent 1: STA® - Liatest® Control, citrated normal human plasma, lyophilized.
2. Reagent 2: STA® - Liatest® Control, citrated abnormal human plasma, lyophilized.
3. Preparation: Reconstitute each vial of Reagent 1 or 2 with exactly 1 ml reagent grade Nerl water. Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes. Then, swirl the vial gently before use.
4. Storage: The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C, do not freeze. Once reconstituted, they remain stable for 8 hours on STA Compact®.

C. STA® - Owren-Koller is a buffer solution intended for use as a diluent for reagents and patient samples in coagulation tests. STA® - Owren-Koller: buffered solution* of pH approximately 7.35.

1. Preparation: Allow the reagent to stand at room temperature (18-25 °C) for 30 minutes before use.
2. Storage: The buffer solution in intact bottles is stable until the expiration date indicated on the box label, when stored at 2-8 °C. After opening, it remains stable for 3 days on STA Compact®

D. STA – DESORB U is a decontaminating solution for use with the STA Compact® It is designed as an integral part of the STA® analyzer system.
   1. Preparation: Install a new STA® - maxi Reducer) and the perforated cap on a freshly opened bottle of STA® - Desorb U before loading it into the analyzer. N.B.: a fine white sediment may be observed in the bottom of the bottle; this has no effect on the performance of the product on STA® Compact model, place one bottle in the product drawer
   2. Storage: 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.
      Once opened, the STA® - Desorb U with STA® - maxi Reducer and perforated cap in place, is stable for: 5 days on board STA Compact®
   3. The STA® - Desorb U reagent contains KOH, a corrosive chemical at the concentration provided (< 1 %).
      Danger:
      1. Causes severe skin burns and eye damage.
      2. Wear protective gloves/protective clothing/eye protection/face protection.
      3. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
      4. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

      CAUTION:
      1. Store reagent kits at 2-8 °C.
      2. For in vitro diagnostic use only.

V. INSTRUMENTATION/EQUIPMEN

A. STA-R®, STA Compact
B. STA® - mini Reducer
C. Cuvette roll – 1000
D. Distilled Water
E. Pipettes & tips

VI. CALIBRATION

A. Pre-calibration protocol
Kit reagents are pre-calibrated: this pre-calibration is valid for all the kits of the same lot. To enter the calibration on the analyzer, scan the barcode printed on the Assay Value insert across the barcode reader of the instrument. The calibration values for the lot of reagents being used will subsequently be validated after the two D-dimer control levels

have been determined.
The calibration curve can be examined on the screen of the analyzer in the "Calibration" menu (see the Reference Manual).

B. Calibration Verification
   1. Find a patient with a D-Dimer between 3.0 and 3.6 µg/mL FEU.
   2. Run the D-Dimer test. Once this test is complete, and the results are between 3.0 and 3.8 µg/mL FEU, add on the following dependent tests
      a. D-Di 1:2
      b. D-Di 1:4
      c. D-Di 1:8
      d. D-Di 1:15
      e. D-Di 1:20 (optional)
   3. Print the test results.
   4. To calculate the linearity, (D-Dimer are performed at 1:1 dilution)
      a. Use the Calibration Template for D-Dimer : S:\Intranet Lab - Has forms for every dept\Lab Forms\Hemo\Calibration Template for D-Dimer.doc
         i. Fill out form.
         ii. Insert the STA D-Dimer results in the Column marker STA D-Dimer.
         iii. D-Dimer Calibration Verification should be
            - Range 0.0 -1.0 µg/mL ± 0.15
            - Range 1.0 -4.0 µg/mL ± 0.15
      b. Use the -Dimer Calibration Verification Template: S:\Intranet Lab - Has forms for every dept\Lab Forms\Hemo\D-Dimer Calibration Verification Template.com
         i. Insert the Values for the Compact results and the expected results
         ii. Graph the points
         iii. Acceptable Verification is R values 0.95-1.00 and slope 0.90-1.10
   5. If the excel sheets or program is not available, send the Results to the TSS to calculate the linearity.
   6. If your Verification fails, call the hotline for solutions or to call for service engineer. DO not run D-Dimers until Calibration Verification is satisfactory.

VII. QUALITY CONTROL

A. STA® - Liatest® Control N + P
   1. It is necessary to run controls to ensure accuracy and reproducibility of the results. Two different levels of controls should be used. Prepare the controls and transfer to the instrument. These controls are used undiluted.
   2. Onboard stability is 8 hours.

B. <u>Liatest D Dimer Control N and P schedule:</u>
   1. Liatest controls must be tested at least every 8 hours of testing and for each vial of reagent for the respective measurement range to ensure that the system if functioning properly

2. QC will be run every 6 hours manually by the tech. This is to ensure there is no point that the QC is expired when testing needs to be done, and to minimize QC material used.
3. 09:00-09:30 - Make up new QC and any reagents needed
   - 09:30-10:00 - Load and manually run QC and check results
   - 15:30-16:00 - Manually run QC and check results
4. 21:00 – 21:30make up new Liatest D dimer controls
   - 21:30- 22:00 - Load and manually run QC and check results
   - 03:30-04:00 – Manually run QC and check results

C. If Quality Control is not within acceptable range, check that all the components of the test system are functioning correctly, i.e., reagents, assay conditions, etc. If necessary, repeat the tests.

VIII. PROCEDURE:

A. Refer to START-UP procedure for the analyzer before running patient and QC specimens at the start of each shift.
B. There are two modes to load samples.
   1. Automatic Mode:
      a. Instrument is connected to a LIS and can download testing
      b. Load sample onto instrument and the tests will auto-populate with test ordered in LIS
   2. Manual Mode used for LIS downtime or Interface downtime:
      a. Instrument is either not linked to a LIS, or sample is not ordered in the LIS
      b. Load sample onto instrument, then select the test(s) to be performed
C. Patients' plasmas are tested undiluted.
D. The D-dimer assay of the plasmais an optical assay reading aat 540 nm. If any of the patient results falls above the working assay rangeof 4 ug/mL, the instrument automatically retests the sample with a 1:5 dilution with Owrens Koller buffer.
E. Results below the determined assay range of 0.27 ug/mL have an "*" on the result and a message- ALARM STA-LIATEST D-DI RESULT VALUE in primary units skewed. These results are reported as < 0.27 ug/ML

IX. REPORTING RESULTS

A. Report results using interface/manual result entry in the LIS system.

Reference interval for UPH-Pekin for D-Dimer's: 0.00-0.50 µg/ml FEU's
Cut off for Exclusions of DVT and PE for D-Dimer is 0.00-0.50 µg/ml FEU's

Analytical Measurement Range: 0.27-20.0 µg/ml FEU's

D dimer is useful for excluding the diagnosis of venous thromboembolism when results are combined with clinical information including pretest disease probability. Use of age-

adjusted dimer cutoff with probability assessment can be used to rule out suspected PE in emergency department patients and is associated with low likelihood of subsequent symptomatic VTE.

D-dimer levels are expressed in initial fibrinogen equivalent units (FEU). By definition, one FEU is the quantity of fibrinogen initially present that leads to the observed level of D-dimer. The actual quantity of D-dimer is approx. half of an FEU.

B. Results below 0.27 µg/ml turn out as <0.27
C. Results above 20.00 µg/ml turn out as >20.0.

X. LIMIT OF DETECTION

The limit of detection was assessed according to CLSI guideline EP17-A. The limit of detection on STA Compact® is 0.27 µg/ml (FEU)

XI. LIMITATIONS OF THE PROCEDURE

A. Cloudy plasmas may lead to an under-estimation of the D-dimer level. A note should be entered for any patient with a lipemia that D-Dimer level may be falsely decreased due to lipemia.
B. Concentrations of fibrinogen degradation products greater than 15 µg/ml may lead to an over-estimation of the D-dimer level.
C. The presence of rheumatoid factor at a level greater than 50 IU/ml may lead to an over-estimation of the D-dimer level.
D. The STA® - Liatest® D-Di is insensitive to the following substances: hemoglobin (up to 2 g/L), conjugated bilirubin (up to 290 mg/L), unconjugated bilirubin (up to 200 mg/L), unfractionated heparin (up to 2 IU/mL), and low molecular weight heparin (up to 2 anti-Xa IU/ml).
E. The presence of anti-bovine albumin and/or anti-mouse antibodies in certain subjects may lead to an over-estimation of the D-dimer level.
F. Patient with distal DVT may have a normal D-dimer level.
G. D-dimer assay should not be used in patients with high Wells' PTP score, an assessment score for DVT risk.
H. The D-dimer level increases during pregnancy and with age.

XII. REFERENCES

A. STA Compact Max® Reference Manual June 2016.
B. STA Compact Max® User Guide November 2015.
C. STA Compact Max® Software version 106.08.01.00

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

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