Quantitative D Dimer (XDP)
Using the ACL TOP™ Analyzer Technical Procedure 1594.t

Principle

Coagulation activation results in the formation of fibrin which aggregates and is cross-linked by Factor XIII to produce a fibrin clot. Upon activation of the fibrinolytic system, plasmin cleaves fibrinogen and fibrin into D and E fragments, resulting in fibrin degradation products with cross-linked D domains as the smallest unit. The detection of cross-linked degradation products, or D-Dimers are indicative of reactive fibrinolysis. 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. The 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 fibrin soluble derivatives. 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, which contains D-Dimer, is mixed with the Latex Reagent and the Reaction Buffer included in the D-Dimer 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 (turbidimetric immunoassay).

Specimen

Whole blood, anticoagulated with 3.2% sodium citrate at a 1:9 ratio. Centrifuge immediately on receipt until sample is platelet-poor (less than 25,000/mm3). D-dimers are stable in patient samples for:

- 4 hours at 15-25^{o}C, 12 hours at 2-8^{o}C.
- 1 month at <-18^{o}C. Frozen plasma is thawed for 5 minutes in a 37^{o}C Waterbath and testing must be performed with 2 hours. Specimens should not be refrozen.

Citrate tubes that are underfilled, patients with high hematocrits (>55%), hemolyzed samples or clotted samples may be unacceptable for testing. Please refer to administrative procedure #1058 for further clarification.

Equipment and Materials

- ACL TOP 700 LAS® Coagulation Analyzer with waste, rinse, and disinfectant canisters
- Cuvettes, 2400 cuvettes-600 strips (4 cuvettes/strip), Part No. 29400100, Beckman Coulter

- Rinse fluid, 1 x 4000 ml, Part No. 20009700, Beckman Coulter
- Clean A, hydrochloric acid 100 mmol/L acid cleaning solution, 4 X 500 ml, Part No 9831704, Beckman Coulter
- Clean B, hypochlorite solution, 1 x 80 ml, Part No 9832700, Beckman Coulter
- Specimen cups (IL 2ml sample cups, Beckman Coulter only)
- Waterbath
- Vortex

Reagents

- The D-Dimer HS kit (pn20007700) consists of:
  - Latex Reagent: 3 x 2 mL vials of a lyophilized suspension of polystyrene latex particles coated with F(ab')2 fragment of a mouse antibody (MA-8D3) directed against D-Dimer. The reagent contains bovine serum albumin, buffer, stabilizers and preservative.
    - Dissolve the contents of each vial with 2 mL of sterile water. Replace the stopper and swirl gently. Ensure the complete reconstitution of the product. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use. Do not shake.
    - Stable after reconstitution 1 month at 2-8°C in the original vial or 4 days at 15°C on the ACL TOP. Do not freeze
  - Reaction Buffer: 3 x 8 mL vials of phosphate buffer containing bovine serum albumin, stabilizers and preservative.
    - Gently invert to mix before use. The reagent is ready for use. Do not shake.
    - Stable when opened 1 month at 2-8oC in the original vial or 4 days 15°C on the ACL TOP.
  - 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.

- Dissolve the contents of each vial with 1 mL of sterile water. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25oC for 30 minutes and invert to mix before use. Do not shake.

- Stable after reconstitution 3 days at 15-25oC, 1 month at 2-8oC or 2 months at –20oC in the original vial. Frozen Calibrator may be thawed at 37oC and gently mixed before use. Do not refreeze

- The D-Dimer Controls kit (pn20008610) consists of:

  - WARNING: Human source material. Treat as potentially infectious. The materials from which these products have been produced were tested and found nonreactive for the presence of HBsAg and antibody to HIV. No known test method can offer complete assurance that infectious agents are absent. Therefore all human blood-based products should be handled with proper laboratory practices.
  - Low D-D 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.
  - High D-D 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.

    - Dissolve the contents of each vial with 1 mL of sterile water. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25oC for 30 minutes and invert to mix before use. Do not shake.

    - Stable for 30 days at 2-8oC, 8 hours on the ACL TOP, or 60 days frozen at -70oC

- Sterile water available from pharmacy.
- Factor diluent, PN 09757600

Calibrator and Calibration

Calibrator is provided in the D Dimer HS kit and has a value of around 3480 ng/ml.
See Operating the ACL TOP for Calibration procedure. Calibration is done every lot change or every 6 months.

Controls

- Controls should be tested at the initiation of testing at least once at the beginning of each 8 hour shift and upon reagent changes.
- Control Tolerance limits: The range is calculated based on +/- 2.0 standard deviations (SD) from the mean control value. Corrective action when tolerance limits are exceeded:

  - Rerun out of control material.
  - Verify reagent performance.
  - Check instrument performance.
  - Document actions taken to identify and correct the problem before reporting any patient results.

Procedure

See Operating the ACL TOP Analyzer for performing patient testing.

Reporting Results

The D-Dimer concentration in ng/ml is calculated automatically by the analyzer based on the reference curve. The D-Dimer level in ng/ml is expressed in D Dimer units. Some manufacturers express D-Dimer results in FEU (Fibrinogen Equivalent Unit). The equivalence between these two measurement units is approximately 2 FEU = 1 ng/mL.

Values exceeding the upper curve limit of 3680 ng/ml will be reflexed and diluted to achieve an upper reporting range of 69,000 ng/ml. Those samples that result in a failed curve on the dilution mode or show a value of above 69,000 ng/ml should be manually diluted offline (times 100 with factor diluent per IL recommendation) and rerun to assure that the value is truly above 69,000ng/ml. Report at >69,000 for those samples that have results over 69,000ng/ml.

Limitations and Interferences

- Manufacturer indicates that there is no interferences with testing for
  - 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
- The monoclonal antibody (MA-8D3) used in the D-Dimer HS 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.
- Specimens from patients who have received 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. HemosIL D-Dimer HS Reaction Buffer contains a blocking agent against HAMA to minimize this interference on the assay results.

Reference Range

The normal range for D Dimer is less than 230 ng/ml. The cutoff value for DVT/PE is 230 ng/ml for an individual 50 years of age or less. For above 50 years the cutoff value is an age related calculation of Age x 4.6 ng/ml. This value is automatically calculated in Beaker and listed in the cutoff value component of the test report for D Dimer only. This calculation is not included nor reported on D Dimer included in a DIC panel.

Performance Characteristics

Measurement range extends from 150-69000 ng/ml when reflex rules are turned on. The detection limit is at 21ng/ml per manufacturer. The HemosIL DDHS was evaluated prospectively in patients suspected of venous thromboembolism to determine the sensitivity and verify manufacturers cut-off values in patients undergoing definitive diagnostic studies for pulmonary embolism (PE) and deep vein thrombosis (DVT). The HemosIL DDHS has sensitivity, specificity and negative predictive values of 97%, 44.6%, 99.2% for VTE.

References

- HemosIL™ D-Dimer HS package insert issued 06/2005, Instrumentation Laboratory.

- HemosIL™ D-Dimer Controls package insert issued 4/2004, Instrumentation Laboratory.
- ACL TOP On-Line Help Manual Rev 2.2, Instrumentation Laboratory.
- CLSI Document Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Ed. H21-A5, 2008

Procedure History

Date	Written by	Revision	Approved by	Approval date
6/2010	L Freeman	New Procedure	D Dwyre MD	08/17/10
		Biannual Review	D Dwyre MD	8/24/12
		Biannual Review	D Dwyre MD	10/3/14
12/2014		Updated for >69000 results	D Dwyre MD	12/3/14
		Biannual Review	D Dwyre MD	10/18/16
		Biannual Review		
10/2018		Update age related cutoff		