IMUCLONE® D-Dimer ELISA

Product No. 602

Sekisui Diagnostics, LLC
500 West Avenue, Stamford, CT 06902
Tel. (203) 602-7777 Fax (203) 602-2221

INTENDED USE

The IMUCLONE® D-Dimer ELISA allows for the quantitative measurement of D-dimer, crosslinked fibrin degradation product (XL-FDP), in human plasma from patients with thrombotic disorders such as disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism (PE). This ELISA is limited to Research Use Only in the United States.

EXPLANATION OF THE TEST

During early clot formation, thrombin is activated, cleaving fibrinopeptides from the soluble plasma protein, fibrinogen. Molecular polymerization occurs with the formation of soluble fibrin that is then stabilized with covalent cross-linking by FXIIia activity to produce an insoluble fibrin clot. This stabilized fibrin network is immediately degraded by the fibrinolytic enzyme, plasmin, which process is known as fibrinolysis. Under normal physiological conditions, excess plasmin is rapidly neutralized by alpha-2-antiplasmin within the region of the clot. A variety of XL-FDPs are formed, depending on the extent of fibrinolysis. The smallest fragment is the plasmin resistant species, D-dimer. Detection of D-dimer therefore indicates this sequence of events: thrombin activation, clot formation and subsequent clot lysis.

Elevated XL-FDP indicates reactive fibrinolysis and is seen in a number of arterial and venous occlusive disease states including acute myocardial infarction (MI), deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC) and other coagulation disorders. Elevated XL-FDP has also been reported in surgery, trauma, sickle cell disease, liver disease, severe infection and sepsis, inflammation and malignancy, obstetric complications, and hyperfibrinolysis. The persistence or heightened elevation of XL-FDP post-operatively may precede thromboembolic episodes. Determinations of XL-FDP levels have been used to monitor the course and response to thrombolytic therapy. Plasmin rarely will degrade other coagulation proteins such as fibrinogen, and form fibrinogen degradation products (FDP). Determination of FDP levels does not reflect clot lysis.

PRINCIPLE OF THE PROCEDURE

The IMUCLONE D-Dimer ELISA uses a monoclonal antibody against human D-dimer coated to plastic microwells. During an incubation period, D-dimer in the test sample binds to the antibody coated microwells. Following a step during which extraneous plasma proteins are washed away, a horseradish peroxidase (HRP) conjugated monoclonal antibody recognizing the bound D-dimer is added, completing the formation of the antibody sandwich complex. Following another washing step, TMB (3, 3', 3, 5' – tetramethylbenzidine), a substrate, is added to the microwells and its subsequent reaction with the HRP creates a blue colored solution. The enzyme substrate reaction is stopped by the addition of sulphuric acid, turning the solution color yellow. D-dimer levels are quantified by measuring the solution absorbances at 450 nm and comparing the values with those from a standard curve.

REAGENTS

12 strips of 8 antibody coated microwells in a frame holder plus storage bag
2 vials of Sample Diluent, ready to use (50 mL)
3 vials of D-Dimer Calibrator, see flyer for concentration (lyophilized)
1 vial of D-Dimer Plasma Control I, High Control (lyophilized)
1 vial of D-Dimer Plasma Control II, Low Control (lyophilized)
3 vials of Anti-Human D-Dimer-HRP Immunoconjugate (lyophilized)
1 vial of Conjugate Diluent, ready to use (25 mL)
1 vial Wash Solution, 20 fold concentrate (50 mL)
1 vial of Substrate, TMB containing hydrogen peroxide (25 mL)
1 vial of Stop Solution, 0.45M Sulfuric Acid, ready to use (6 mL)

WARNING

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Limited for research use only in the United States. For in vitro use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when properly stored at 2°-8°C.

1. Antibody Coated Microwell Strips: Once removed from the aluminium pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture, and stored in the provided storage bag.

2. Sample Diluent: Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C.

3. D-Dimer Calibrator: Reconstitute each vial with 2 mL of Sample Diluent to generate a standard of “C” ng/mL. See the enclosed flyer for the exact concentration. The calibrator is stable for at least 8 hours at room temperature.

4. D-Dimer Plasma Control I and Control II: Reconstitute each vial with 0.5 mL of 0.22 µm filtered deionized H2O. The controls are stable for 8 hours at room temperature, 24 hours at 2°-8°C or for 2 months at −20°C.

5. Anti-Human D-Dimer-HRP Immunoconjugate: Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted immunoconjugate is stable for at least 8 hours at room temperature or for at least 4 weeks at 2°-8°C.

6. Conjugate Diluent: Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.

7. Wash Solution: If solids are present, incubate the vial for 15-30 minutes in a 37°C water bath. Shake the vial and dilute the amount required 1:20 in distilled water (the entire vial is sufficient to prepare 1 Liter of Wash Solution). The Wash Solution may be used for up to 4 weeks after opening when stored at 2°-8°C in its original vial. Diluted Wash Solution may be used for up to 7 days when stored at 2°-8°C.

8. TMB Substrate: Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.

9. 0.45M Sulfuric Acid: It is ready to use. Warning: Sulphuric acid is caustic. Handle with care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling.

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A3, Vol. 18, No. 20, December 1998. Plasma collection should be performed as follows:

(...)over
1. Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 1,500 rpm for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at −20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

The D-Dimer Controls and test sample must be tested diluted 1:50 in the Sample Diluent. For expected D-Dimer concentrations >10 μg/mL, samples may be assayed at a higher dilution, 1:100, 1:200 or higher. If low D-Dimer concentrations are expected, samples may be assayed at lower dilutions, 1:20, 1:10 or 1:5.

**PROCEDURE**

**Materials Provided** – See Reagents

**Material Required But Not Provided**
- 0.22 µm filtered deionized H₂O
- 50-300 µL eight channel multi-pipette
- 0-200 µL, 200-1000 µL single pipettes
- Microwell plate reader for reading absorbance at 450 nm
- Microwell plate washer (optional)

**Preparation of the Standards**

Using the 200 ng/mL D-Dimer Standard provided, prepare the following standard solutions:

<table>
<thead>
<tr>
<th>D-Dimer Standard</th>
<th>C ng/mL</th>
<th>C/2 ng/mL</th>
<th>C/4 ng/mL</th>
<th>C/10 ng/mL</th>
<th>C/20 ng/mL</th>
<th>0 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. of D-Dimer Standard</td>
<td>1 mL</td>
<td>0.5 mL</td>
<td>0.25 mL</td>
<td>0.1 mL</td>
<td>0.05 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td>Vol. of Sample Diluent-F</td>
<td>0 mL</td>
<td>0.5 mL</td>
<td>0.75 mL</td>
<td>0.9 mL</td>
<td>0.95 mL</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Mix each standard gently to ensure complete mixing. The standard dilutions are stable for at least 6 hours at room temperature.

Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Place the strips in the frame provided. Add the reagents to the microwells and perform the various assay steps as indicated on the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer Standard, diluted test sample or diluted control</td>
<td>200 µL</td>
<td>Add the standard or diluted test samples to an appropriate microwell. Incubate for 1 hour at 18°-25°C</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>300 µL</td>
<td>Wash the wells 5 times.</td>
</tr>
<tr>
<td>Anti-Human D-Dimer HRP Conjugate</td>
<td>200 µL</td>
<td>Add the conjugate to each microwell. Incubate for 1 hour at 18°-25°C</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>300 µL</td>
<td>Wash the wells 5 times.</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>200 µL</td>
<td>Add the substrate immediately after washing the wells. Incubate for exactly 5 minutes at 18°-25°C</td>
</tr>
<tr>
<td>0.45M H₂SO₄</td>
<td>50 µL</td>
<td>Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45M H₂SO₄. Wait for 10 minutes to allow the color to stabilize and measure the absorbance at 450 nm.</td>
</tr>
</tbody>
</table>

**RESULTS**

Construct a standard curve by plotting the mean absorbance value for each D-Dimer standard versus its corresponding concentration in ng/mL. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

**CALCULATIONS**

From the standard curve obtained, directly interpolate the D-Dimer concentration in samples tested. The concentration must be multiplied by the actual dilution factor used, i.e. if the sample was diluted by 1:50, multiply the measured concentration by 50. Alternatively, an ELISA software (i.e. Dynex, etc.) can be used for the calculation of concentrations.

**LIMITATIONS OF THE PROCEDURE**

There are no known limitations for the assay. Blood activation ex vivo should be avoided such that the assay is specific for fibrin degradation in vivo.

**EXPECTED VALUES**

The D-Dimer concentration in normal human plasma is usually < 400 ng/mL. D-Dimer concentrations are elevated in cases of trauma, inflammation, infection and pregnancy.

**PERFORMANCE CHARACTERISTICS**

- **Sensitivity**: The lower limit of detection has been found to be 2-4 ng/mL.
- **Precision**: The intra-assay variation this ELISA is < 10%. Typically, for a given lot, the intra-assay variation has been found to be < 6%.

**BIBLIOGRAPHY**