Intended Use
This D-Dimer assay is used for the quantitative determination of the fibrin degradation product D-dimer in human plasma. This application is intended to be used on the TECO Comango automated instrument or automated instrument (such as Hitachi), which have an endpoint detection at 405 nm. D-dimer containing moieties are formed by plasmin degradation of factor XIIIa cross-linked fibrin. 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 rise during pregnancy and high levels are associated with complications. The assay contains antibody-coated latex particles. In the presence of the D-dimer, the particles aggregate to larger aggregates and light scattering decreases. The change of light is proportional to the amount of D-dimer in the sample. The latex particles are coated with a monoclonal antibody reacting with fibrin D-dimer or fragment D of fibrinogen but not with fibrinogen, allowing D-dimer determination in human plasma.

Contents & Determinations

<table>
<thead>
<tr>
<th>Product</th>
<th>Blue D-Dimer Kit-60</th>
<th>Blue D-Dimer Kit-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>3x1 mL</td>
<td>2x1 mL</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>1x7 mL</td>
<td>2x1 mL</td>
</tr>
<tr>
<td>IBS Buffer</td>
<td>2x15 mL</td>
<td>2x15 mL</td>
</tr>
<tr>
<td>TECal DD</td>
<td>1x1 mL</td>
<td>1x1 mL</td>
</tr>
<tr>
<td>TECcontrol DD low</td>
<td>1x1 mL</td>
<td>1x1 mL</td>
</tr>
<tr>
<td>TECcontrol DD high</td>
<td>1x1 mL</td>
<td>1x1 mL</td>
</tr>
<tr>
<td>Determinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator M</td>
<td>60 Det.</td>
<td>120 Det.</td>
</tr>
<tr>
<td>Calibrator A</td>
<td>70 Det.</td>
<td>140 Det.</td>
</tr>
</tbody>
</table>

Further materials required:
- TECO Coatron 400 nm
- Calibrated pipettes, Pipette tips, Test tubes, 2mL

Preparation

- **Latex**: Liquid, ready to use
  - Contains microparticle coated with monoclonal antibodies MA-8D3 suspended in a buffer solution containing stabilizers, detergent and preservatives. Agitate the latex by repeated vortexing after storage. Allow the latex to equilibrate at the working temperature of the instrument for 30 min before use.

- **Reaction Buffer**: Liquid, ready to use
  - Contains stabilizers, detergent and preservatives.
- **IBS Buffer**: Imidazole Buffered Saline, Ready to use
  - IBS Buffer: contains lyophilized human plasma enriched for D-dimer, reconstituted with 1 mL distilled water.

Storage & Stability

Unopened reagents and controls should be used within expiration date and should be stored at 2-8°C. Opened reagents:
- **Latex & Buffer**: 4 weeks
- **ECal & EControl**: 24 hours

Split D-Dimer reagents: Store aliquots of two or more vials (discontinued glass) immediately, if longer stability is required after opening. Dispersed plasma can be refrigerated only once in aliquots (120-150µL). Stored at -20°C in closed polypropylene tubes, the aliquots must be used within 30 days.

Precautions

- Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV, HCV. However products from human blood should be considered as potentially infectious.

- **Specimen collection and storage**
  1. Obtain venous blood by clean venous puncture.
  2. Immediately mix 9 parts blood with 1 part sodium citrate (3.2% or 3.8%) and mix well.
  3. Centrifuge the specimen at 1500g for 15 min. (platelet <30000/mL)
  4. Separate plasma after centrifugation and store in plastic or glass clot activator.
  5. Use plasma within 8 hours, otherwise store frozen and thaw just prior to use.

Procedure

- **A. Automated Method: Coatron A4**

  1. Obtain diluted plasma.
  2. Mix 1:1 plasma: Reaction Buffer and add 100 µL per well.
  3. Place 9 wells for patient sample and 1 well for dilution control.
  4. Place 9 wells for patient sample and 1 well for dilution control.
  5. Load suspension in each well and incubate for 30 min, avoid light.
  6. Wash 4 times.
  7. Load suspension in each well and incubate for 30 min, avoiding light.

- **B. Manual Method: Coatron M Series**

  System setup
  
<table>
<thead>
<tr>
<th>Component</th>
<th>System M</th>
<th>System L</th>
<th>System D</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer</td>
<td>100 ng/mL</td>
<td>150 ng/mL</td>
<td>200 ng/mL</td>
</tr>
<tr>
<td>Calibrator</td>
<td>500 ng/mL</td>
<td>750 ng/mL</td>
<td>1000 ng/mL</td>
</tr>
<tr>
<td>Control</td>
<td>150 ng/mL</td>
<td>250 ng/mL</td>
<td>300 ng/mL</td>
</tr>
</tbody>
</table>

  1. Pipette 25 µL plasma to cuvette(s).
  2. Add 100 µL Reaction Buffer.
  3. Incubate for 2 - 10 min.
  4. Add 50 µL prewarmed Latex and mix at least 15x with pipette.

Expected Results

The D-dimer concentration in healthy normal subjects is below 200ng/mL. PE has been shown to cause increased levels of D-dimer. Patients with confirmed deep venous thrombosis (DVT) have D-dimer concentrations of 200 ng/mL and above. High levels are found in case of DIC and the circulating half-life of D-dimer is about 12 hours. Elevated D-dimer levels can therefore persist for some time after the active process has ceased. The results may be reported either in D-dimer units or in fibrinogen equivalent units (FEU); 1000ng/mL is equivalent to about 2 mg/L of FEU.

Quality Control

- **TEControl DD low**
  1. Use only plastic tubes or glass coated.
  2. Contain prewarmed Latex and mix at least 15x with pipette.

Performance Characteristics

- **Calibration**
  1. Calibrator DD low CV. <5.0% (within run)
  2. Calibrator DD high CV. <5.0% (within run)

- **Precision**
  1. Appropriately measured, appropriate dilution should be used. Use Saline Solution.

- **Accuracy**
  1. Appropriately measured, appropriate dilution should be used. Use Saline Solution.

- **Sensitivity**
  1. 200ng/mL 99% 62% 99% 41%

- **Specificity**
  1. 1 ng - 1 ng - 42 µl

- **Validation**
  1. 200µL TECal DD - 38 µl

Warranty

This product is warranted to perform in accordance with its labelling and literature. Any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

References