**SUMMARY AND EXPLANATION**

Coagulation Factor X (Stuart Prower Factor, FX) is a vitamin K dependent protein produced by the liver. It has a central position in the coagulation cascade. Factor X is activated both by the extrinsic and intrinsic systems before exerting its effect on the conversion of prothrombin to thrombin. Patients who need anticoagulation therapy can be treated with warfarin, which is a vitamin K antagonist. This treatment leads to a decrease in all vitamin K dependent clotting factors and thus a prolonged clotting time of blood. The therapy requires careful monitoring since it is essential to find an optimal balance between risk of thrombosis and risk of bleeding for each patient. The DiaPharma Factor X Kit is a useful tool in the management of patients with lupus inhibitors receiving warfarin therapy.

**MEASUREMENT PRINCIPLE**

1. FX \( \xrightarrow{RVV} \) FXa
2. Chromogenic substrate \( \xrightarrow{FXa} \) Peptide + pNA (color)

The method is based on a two-stage principle. In stage one, Factor X is activated in the presence of calcium to Factor Xa (FXa) by the activator Russell’s Viper Venom (RVV). In stage two, the generated FXa hydrolyses the chromogenic substrate, thus liberating the chromophoric group, pNA. The color is then read with a spectrophotometer at 405 nm. The generated FXa and thus the intensity of color is proportional to the FX activity in the sample.

**REAGENTS**

- **Chromogenic Substrate**
  - Preparation: 25 mg lyophilized FXa chromogenic substrate with mannitol added as a bulking agent. Reconstitute substrate with 20 ml sterile water.
  - Storage & Stability: The reconstituted substrate is stable for 6 months at 2 - 8°C.

- **Russell’s Viper Venom**
  - Preparation: FX activating protein from Russell’s Viper Venom. Reconstitute the RVV with 15 ml sterile water.
  - Storage & Stability: The reconstituted activator is stable for 1 month at 2 - 8°C.

- **CaCl\(_2\)**
  - Preparation: 20 ml of 0.1 mol/L calcium chloride solution. Before use, mix 1 volume of RVV with 1 volume of CaCl\(_2\). The mixture is stable for 48 hours at 2 - 8°C.
  - Storage & Stability: The solution is stable at 2 - 8°C until the expiry date printed on the label.

- **Buffer**
  - Preparation: 100 ml buffer solution containing 0.05 mol/L Tris, pH 7.8 and 20 mg/L Polybrene\(^{\text{TM}}\) (hexadimethrine bromide). Ready for use.
  - Storage & Stability: The buffer is stable at 2 - 8°C until the expiry date printed on the label.

The sealed reagents are stable at 2 - 8°C until the expiry date printed on the label. Contamination by microorganisms should be avoided once the vials are opened.

**QUALITY CONTROLS**

Normal and abnormal controls are recommended for a complete quality control program. Each laboratory should establish its own mean and standard deviation and should establish a quality controls program to monitor laboratory testing. Controls should be analyzed at least every 8 hour shift in accordance with good laboratory practice. Refer to Westgard et al for identification and resolution for out of control situations.

**RESULTS**

Factor X results are reported in activity (%).

**SPECIMEN COLLECTION**

Nine parts of freshly drawn venous blood are collected into one part 3.2% trisodium citrate. Cen trifugation: 2000 x g for 10 – 20 minutes at 20 - 25°C. Refer to NCCLS document H21-A3 for further instructions on specimen collection, handling, and storage.

**RECOMMENDED MEASURING RANGE/DETECTION LIMIT**

The FX concentration is linear between 10% of normal (detection limit) and 130% of normal.

**CALIBRATION**

A standard curve is required for each DiaPharma Factor X kit. Five standards must be included in each test run. For standardization, use commercially supplied hemostasis calibration plasma, or a pool of calibrated fresh frozen normal plasma obtained in the same manner as the plasma to be tested.

<table>
<thead>
<tr>
<th>% FX</th>
<th>Normal Plasma, µl</th>
<th>Buffer, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>75</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Further dilute the standards as follows:

- Standards: 50 µl
- Buffer: 1000 µl

**PROCEDURE**

**Microplate Method**

**Predilution of samples and controls:** The same predilution is performed on the plasma samples and controls as was performed on the standards. The predilution is performed to prevent interference from inhibitors, lipemic samples, icteric or hemolysed samples.
Plasma samples, and controls: 50 μl
Buffer: 1000 μl

PROCEDURE – CONTINUED

Kinetic Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount / Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add pre-diluted Sample</td>
<td>50 μl</td>
</tr>
<tr>
<td>Incubate at 37°C</td>
<td>3 – 4 min.</td>
</tr>
<tr>
<td>Add chromogenic substrate</td>
<td>50 μl</td>
</tr>
<tr>
<td>Mix well and add within</td>
<td>30 sec</td>
</tr>
<tr>
<td>RVV + CaCl₂ (20 - 25°C)</td>
<td>50 μl</td>
</tr>
<tr>
<td>Mix well</td>
<td></td>
</tr>
</tbody>
</table>

Transfer the microplate to a microplate reader immediately after addition of the RVV + CaCl₂ and measure the absorbance change in a microplate reader at 405 nm. The microplate reader must be pre-incubated at 37°C.

If performing the acid-stopped method, mix and incubate at 37°C for 3 minutes after addition of RVV + CaCl₂, then add 50 μl of 20% Acetic acid. Read the absorbance of the samples at 405 nm. The color is stable for at least 4 hours.

DETERMINATIONS PER KIT

Microplate method: approximately 400
Automated methods: approximately 200 – will vary according to analyzer

CALCULATION

Plot the change in absorbance per minute (ΔA/min) for the standard samples against their concentration of FX. Read the %FX value for the corresponding absorbance for the unknown sample from the standard curve.

PERFORMANCE CHARACTERISTICS

A linear regression of the FX clotting versus chromogenic assay was performed, yielding:
FX Chromogenic = 0.76⋅FX Clotting + 21.77 with a correlation of R² = 0.90

Confidence Interval for Slope & Intercept:

<table>
<thead>
<tr>
<th>FX Clotting (%)</th>
<th>FX Chromogenic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidence Level (95.0%)</td>
<td>8.25</td>
</tr>
<tr>
<td>Lower 95.0%</td>
<td>18.77</td>
</tr>
<tr>
<td>Upper 95.0%</td>
<td>24.78</td>
</tr>
<tr>
<td>X Variable</td>
<td>0.72</td>
</tr>
</tbody>
</table>

The central tendency for within-run precision of the DiaPharma Factor X kit is approximately 7 %CV.

ACCUACY

Clinical studies were done with a total of 241 individuals (92 normals + 149 OAC patients) across three sites. Citrated plasma samples from each individual were assayed by the FX Kit and by the routine prothrombin method at each site. The data were compared by log-log linear regression, and showed a correlation coefficient (“r”) of 0.89.

LIMITATIONS AND INTERFERING FACTORS

• Bilirubin, hemoglobin, and plasma from hyperlipemic patients give difficulties in absorbance readings. In these cases individual plasma blanks are necessary.
• Heparin concentrations below 30 U/ml are neutralized by Polybrene® and do not influence the assay.
• Temperature control and incubation times are critical.
• Proper mixing is important to be sure that the reaction mixture is homogenous, but avoid vigorously shaking, as the proteins may precipitate in the foam.

EXPECTED VALUES

Investigations have been made of concentrations in plasma obtained from:
1) Normal subjects
DiaPharma Factor X has been evaluated in a study with 65 subjects. The samples were tested according to standard procedure, and the range of results (90% limits) was 130% to 59%. These limits corroborate data in the literature.
2) Anticoagulant therapy patients
DiaPharma Factor X has been evaluation in a study of 149 patients undergoing oral anticoagulant. The range of Factor X concentrations in plasma of anticoagulant therapy patients was 13 to 79%, with a mean of 30%.

REFERENCES

3) Coatest® FX Package Insert. Chromogenix/Instrumentation Laboratory.

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