INTENDED USE:
BIOPHEN™ Factor XIII kit is a chromogenic method proposed for in vitro quantitative determination of Factor XIII activity in human citrated plasma.

SUMMARY AND EXPLANATION:
Factor XIII (FXIII) protransglutaminase circulates in plasma as A2B3 tetramer, the A subunit being the functional form. When activated by thrombin and calcium to FXIIIa, it acts in the last step of the coagulation cascade and contributes to fibrin crosslinking and clot stiffness. FXIII deficiency may be congenital, or acquired as a result of hyperconsumption or presence of autoantibodies. Low FXIII levels have been associated with bleeding complications, eg in situations such as trauma or surgery. FXIII is also involved in various other processes such as wound healing and maintenance of pregnancy.

Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be stored under vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

PRINCIPLE:
Factor XIII (FXIII), in the tested sample, is converted into activated Factor XIII (FXIIIa) by thrombin in presence of calcium++. The conversion of NADPH into NADP+ by thrombin is proportional to the concentration of FXIII in the tested sample.

HYPHEN BioMed

REFERENCE:
D750-02/BI/7005/v3
**PROCEDURE:**
The kit can be used in kinetics mode on automated methods. Perform the test at 37°C and read the absorbance at 340 nm.

**Automated methods:**
See the specific application and specific precautions for each analyzer (provided on request for various instruments according to availability; contact your local distributor for CS-series applications).

**Assay method:**
1. Reconstitute the calibrators and controls as indicated in the specific instructions. For preparing the calibration curve, dilute the calibrator in physiological saline to calibrate from approximately 0 to 150% FXIII. The 1:2 working dilution in physiological saline (in the schema below) corresponds by definition to 100% for a normal plasma pool, or C% FXIII for a commercial calibrator.

2. Establish the calibration curve and test it with the quality controls. The exact control and control concentrations for each batch are indicated on the flyer provided with the kit.

3. As an example, the here below showed the schema for application on CS-series. Dispense the following to the reaction cuvettes incubated at 37°C (directly managed by the analyzer):

<table>
<thead>
<tr>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen, calibrator or control</td>
</tr>
<tr>
<td>Physiological saline</td>
</tr>
<tr>
<td>R1: Thrombin reagent, pre-incubated at 37°C</td>
</tr>
<tr>
<td>Mix and incubate at 37°C for exactly 110 seconds, then add the following:</td>
</tr>
<tr>
<td>R2: Detection reagent, pre-incubated at 37°C</td>
</tr>
<tr>
<td>Mix, incubate at 37°C, and measure (kinetics mode) the optical density (OD)/min at 340 nm between 200 and 500 seconds</td>
</tr>
</tbody>
</table>

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

**CALIBRATION:**
The BIOPHEN™ Factor XIII assay can be calibrated for the assay of FXIII activity in plasma.

Using a linear scale:
- The test is linear from 5 to 150% of FXIII on Sysmex CS-5100 (at the standard dilution).

The calibration curve shown below, obtained on Sysmex CS-5100 analyzer, is given as an example only. The calibration curve established for the assay series must be used.

**QUALITY CONTROL:**
The use of quality controls serves to validate method compliance, along with between-run reproducibility for a given lot of reagents. Include quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be defined, preferably for each test series, and at least for each new reagent lot, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range.

Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

**RESULTS:**
- On the Sysmex CS-series analyzer, the calibration curve is obtained in Lin-Lin scale, with the OD/min at 340 nm along the Y-axis and the FXIII concentration, expressed as %, along the X-axis.
- The concentration of Factor XIII in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in percentage.

**LIMITATIONS:**
- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for Heparin concentration up to 2 IU/mL, bilirubin concentration up to 60 mg/dL, hemoglobin concentration up to 250 mg/dL, intrinsic fibrinogen concentration up to 250 mg/dL, ammonium concentrations up to 0.5mM, and fibrinogen concentrations from 0.8 up to 6 g/L by plasma overload tests. For high concentrations, an additional (eg 1:3) pre-dilution could be used and the result multiplied by the complementary dilution factor).

**EXPECTED VALUES:**
The reference range established on healthy adult subjects (n=120) using Sysmex CS-5100 (Central 90%, 95% percentile) was measured between 60 and 146 %.

However, each laboratory has to determine its own normal range.

**PERFORMANCES:**
- The lower analyzer detection limit depends on the analytical system used (0.5% on Sysmex CS-5100).
- On Sysmex CS-series, the measuring range is from about 5 to 300% of FXIII.
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and triplicates within each series for a control level. The following results were obtained:

<table>
<thead>
<tr>
<th>Control</th>
<th>Intra assay</th>
<th>Inter assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean</td>
<td>CV%</td>
</tr>
<tr>
<td>Normal</td>
<td>40</td>
<td>102 3</td>
</tr>
<tr>
<td>Abnormal</td>
<td>40</td>
<td>28 8</td>
</tr>
</tbody>
</table>

**REFERENCES:**
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". 2008.

**SYMBOLS:**
Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.