INTENDED USE OF THE KIT
For the kinetic, photometric determination of heparin in plasma.

MEASUREMENT PRINCIPLE
1. Heparin + AT (excess) → [Heparin - AT]
2. [Heparin - AT] + Fxa (excess) → [Heparin - AT - Fxa] + Fxa (remaining)
3. S-2222 → Peptide + pNA

Heparin is analysed as a complex with Antithrombin (AT) present in the sample. The concentration of this complex is dependent on the availability of AT. In order to obtain a more constant concentration of AT, purified AT is added to the test plasma. Fxa (in excess) is neutralized in proportion to the amount of heparin, which determines the amount of [Heparin - AT] complex. The remaining amount of Fxa hydrolyses the chromogenic substrate S-2222 thus liberating the chromophoric group, pNA. The colour is then read photometrically at 405 nm.

REAGENTS
When kept at 2-8°C the sealed reagents are stable until expiry date printed on the label. Avoid contamination by microorganisms in opened vials.

1. S-2222 1 vial
2. Factor Xa 1 vial
3. Buffer, stock solution 1 vial
4. Antithrombin 1 vial
5. Normal Plasma (human) 4 vials

SPECIMEN COLLECTION
Blood (9 volumes) mixed with 0.1 mol/L sodium citrate (1 volume) and preferably cooled immediately on ice to minimize release of heparin antagonists from blood cells. Centrifuge at 2000 x g for 20 minutes at low temperature and as soon as possible after blood collection. The plasma is stable for 2 hours at 2-8°C or 6 months at -20°C or below.

PROCEDURE
Calibration
A standard curve is required for each new lot of Coatest Heparin. For preparation of standards, a heparin standard of known concentration must be used (not provided). Two standards (e.g. 0.1 and 0.7 IU/mL) must be included in each test run.

a) Preparation of standards
Use a two-step procedure for dilution of heparin: 1. Dilute with saline to obtain 10 IU/mL. 2. Make a 100-fold dilution with buffer to obtain 0.1 IU/mL.

The 0.1 IU/mL heparin solution is further diluted according to the table below to obtain different standard concentrations.

<table>
<thead>
<tr>
<th>Heparin IU/mL plasma</th>
<th>Heparin dilution 0.1 IU/mL µL</th>
<th>Buffer working solution µL</th>
<th>Human Normal Plasma AT µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>700</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.3</td>
<td>300</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>700</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>500</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>0.7</td>
<td>700</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

b) Dilution of samples
Test plasma (kept on ice) 100 µL
Antithrombin 200 µL
Buffer, working solution 800 µL
Mix well

C) Assay
Add in a plastic tube
Diluted test plasma or standard incubate at 37°C (3-4 minutes) 200 µL
Fxa (20-25°C) 100 µL
Mix and incubate at 37°C for 30 sec S-2222 (37°C) 200 µL
Mix and incubate at 37°C for exactly 3 min Acetic acid 20% or citric acid 2% Water 300 µL Mix

RESULTS
Subtract the respective blank activities for the standards from their absorbances (A) at 405 nm. Plot the corrected A for the standards against their concentrations of heparin on a linear graph paper. Read the heparin value for the corresponding A for the unknown sample from the standard curve after due correction for the sample blank activities.

Performance characteristics

- Sensitivity: The assay allows detection of 0.05 IU/mL of heparin. At heparin concentrations above 0.7 IU/mL, dilute the sample 1:5 with Human Normal Plasma. Multiply the obtained result by 5. Accurate blood sampling and plasma treatment is a prerequisite for valid determination of heparin levels below 0.2 IU/mL.
- Accuracy: When comparing the Coatest Heparin assay with Activated Partial Thromboplastin Time (APTT) Assay, in patients undergoing heparin therapy (N=25) and heparin administration in healthy volunteers (N=40), the correlation coefficient obtained were 0.90 and 0.91 respectively.
- Specificity: No drug interference has been reported. The present method is less sensitive to heparin antagonists (platelet factor 4) than APTT and thrombin time methods. Teien et al. (2) found the present method insensitive to FDP levels in pathological plasmas.

Alternative procedures
Method applications on automated instruments are available on request from Chromogenix AB.
REFERENCES


PROCEDURE ALTERNATIVE

Applicazioni del metodo usando strumenti automatici sono disponibili su richiesta dalla Chromogenix AB.