

Intended use of the kit

For the *in vitro* photometric determination of the heparin or LMW heparin activity in human plasma.

Measurement Principle

1. LMW Heparin + AT \longrightarrow [LMW Heparin · AT]
- 2 a. [LMW Heparin · AT] + FXa (excess) \longrightarrow [LMW Heparin · AT · FXa]
- 2 b. S-2732 + FXa \longrightarrow Peptide + pNA (yellow) + FXa (residual)

LMW Heparin (e.g. Fragmin) or Heparin is analyzed as a complex with Antithrombin (AT) present in the plasma sample. Activated Factor X (FXa) is added to a mixture of plasma sample and the chromogenic peptide substrate S-2732 in a buffer. Two competing reactions then start. One is the inhibition of FXa by the [LMW Heparin · AT] complex, the other is the FXa catalyzed release of pNA from the substrate. After a certain period of time most of the FXa is inhibited and the release of pNA has essentially declined. Further release of pNA is stopped by the addition of acetic acid and the absorbance at 405 nm is measured. The correlation between absorbance (A 405) and LMW Heparin/Heparin activity is linear in the 0.1–1.0 IU/mL range when plotted in a log-lin scale.

Reagents

The sealed reagents are stable at 2-8°C until the expiration date printed on the label. Avoid contamination by microorganisms of the reagent.

1. **S-2732** 1 vial
Chromogenic substrate (Suc-Ile-Glu(g-Pip)-Gly-Arg-pNA), 6 mg, with mannitol as bulking agent. Reconstitute with 2.6 mL water to a concentration of 2.9 mmol/L. The solution is stable for 6 months at 2-8°C.
2. **Factor Xa** 1 vial
Bovine Factor Xa, 13 nkat. Reconstitute with 10.4 mL water. The solution is stable for one month at 2-8°C.
3. **Buffer, 20 mL** 1 vial
Tris 50 mmol/L, EDTA 7.5 mmol/L, pH 8.4, I=0.2. Once opened, the buffer solution is stable for two months at 2-8°C.
4. **LMW Heparin Standard, 1mL** 1 vial
A LMW Heparin (Fragmin) standard, 100 IU/mL. (Calibrated against the International Standard for LMW Heparin, established by the WHO, using an anti-Factor Xa method). Once opened, the solution is stable for 6 months at 2-8°C.

Reagents required but not provided

- Deionized water, filtered through 0.22 µm or NCCLS type II water.⁸
- Saline (0.9% NaCl).
- Pooled normal human plasma taken on ice and prepared according to "SPECIMEN COLLECTION." A lyophilized preparation is available from Chromogenix AB or subsidiaries.
- Stopper solution: acetic acid 20% or monosodium citrate 20%.

PRECAUTIONS AND WARNINGS

Harmful if swallowed (R 22).
Avoid contact with skin and eyes (S24/25).
Do not empty into drains (S29).
Wear suitable protective clothing (S36).
This product is for *in vitro* diagnostic use.

Materials required but not provided

- Photometer, 405 nm
- Semi-micro cuvette (1cm)
- Centrifuge, 2000-4000 x g
- Stopwatch
- Disposable plastic tubes
- Pipettes, calibrated

Specimen collection

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C.

Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.

Procedure – Manual Technique

Precautions

Proper mixing is important to make sure that the reaction mixture is homogenous, but avoid vigorous mixing as the proteins may precipitate in the foam. The method is designed for room temperature and should be kept within ±2°C from the temperature used when the standard curve was established.

Calibration

A standard curve is required for each new lot of the Coatest LMW Heparin/Heparin.

LMW Heparin

Add 10 µL of the LMW Heparin standard (100 IU/mL) to 1.0 mL of pooled normal plasma and mix carefully to obtain a plasma containing 1.0 IU/mL. Prepare standards by mixing this 1.0 IU/mL plasma solution with pooled normal plasma according to the table below.

Heparin

For the determination of heparin, the standard curve must be made up by using a heparin standard of known concentration (not provided). Dilute the heparin with saline in order to obtain 100 IU/mL and proceed according to the instructions for LMW Heparin above.

Plasma Standards IU/mL	Spiked plasma 1.0 IU/mL µL	Pooled normal plasma µL
0.1	25	225
0.3	75	175
0.5	125	125
0.8	200	50
1.0	250	0

These standards can be kept at -20°C or below for 6 months or at 2-8°C for two days. For routine purposes larger amounts can be produced and kept frozen in suitable aliquots.

Quality control

It is suggested that each time a test is performed one should include a LMW Heparin/Heparin plasma sample preparation, other than that used to create the standard curve. This could be a single sample obtained from any source and at a known concentration about midrange within the standard curve.

Method

Perform at room temperature (20–25°C).

S-2732 + Buffer solution

Mix 1 volume of substrate with 3 volumes of buffer. The solution is stable for at least 24 hours at room temperature or one week at 2-8°C.

Add in a test tube	Sample or standard	Plasma blank (Note 1)
S-2732 + buffer	200 µL	–
Buffer	–	400 µL
Standard or test plasma	25 µL	25 µL
Factor Xa	200 µL	–
Mix and incubate for 8 min	–	–
Stopper solution (Note 2)	200 µL	200 µL
Mix.	–	–

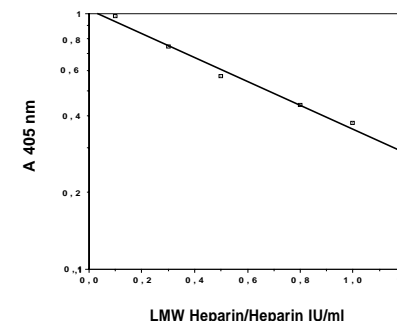
Read the absorbances at 405 nm. The colour is stable for at least 4 hours. Subtract the absorbance of the plasma blank from the sample.

Note 1. If the sample is not significantly more opaque than the standard plasma and if the bilirubin or haemoglobin content are not high enough to interfere, the pooled normal plasma blank can be used for all samples.

Note 2. The volume of the stopper solution can be chosen between 200–1000 µL in order to fit the cuvette.

Calculation

Plot the absorbance (A 405) for the standards (Y axis) after subtracting the plasma blank against their respective concentration of LMW Heparin/Heparin (X-axis) on log/Lin graph paper. Check whether A 405 for the two control samples correspond with the standard curve for the lot number of the kit. (Each laboratory must set its own guideline for control of assay quality). Read the IU/mL value for the unknown plasma from the standard curve after subtracting the A 405 for the plasma blank.



Limitations

1. Valid determination of activities below 0.05 IU/mL may be difficult due to influence from heparin antagonists released from the platelets. Such low levels are, however, generally considered to be of limited clinical relevance.
2. If the sample contains more than 1.0 IU/mL, dilute 1:3 in pooled normal plasma and repeat the assay. Multiply the result by 3.

Expected results

To obtain an optimal effect with minimum risk of bleeding the LMW Heparin/Heparin activity should be in the range recommended by the producer. Suitable time point for sampling (also stated by the producer) must be considered.

Precision

The coefficient of variation (C.V.) within series (n=15) was 0.8% (0.8 IU/mL) and 3.3% (0.4 IU/mL). The following table shows the C.V. between series at various activities. 15 assays were performed during 4 consecutive weeks with reconstituted reagents kept at 2-8°C.

IU/mL	0.2	0.4	0.6	0.8	1.0
Heparin C.V.	6.9%	4.2%	5.7%	6.9%	8.0%
Fragmin C.V.	6.5%	5.9%	3.9%	5.7%	7.2%

Accuracy

The assay correlates well with Coatest Heparin. Plasma samples drawn from healthy volunteers after intravenous injection of Fragmin gave a correlation coefficient of 0.98 in the activity range 0–3.5 IU/mL (n=439). In patients treated with s. c. Fragmin activities in the range 0–1.2 IU/mL gave a correlation coefficient of 0.98 (n=56).

Sensitivity

The assay allows detection of 0.05 IU/mL of LMW Heparin/Heparin. To increase the accuracy in the range below 0.1 IU/mL a 0.05 IU/mL standard is recommended.






Specificity

The assay measures specifically the anti-Factor Xa effect of LMW Heparin/Heparin. The method is slightly dependent on the patients antithrombin concentration, since antithrombin is essential for the effect of LMW Heparin/Heparin. If the result obtained deviates from the expected activity, measurement of patients antithrombin level is recommended (Coamatic Antithrombin available from Chromogenix or subsidiaries).

Bibliography / Literatur / Bibliografia / Bibliographie / Bibliografia / Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

<p>IVD</p> <p><i>In vitro</i> diagnostic medical device <i>In-vitro</i> Diagnostikum De uso diagnóstico <i>in vitro</i> Dispositif médical de diagnostic <i>in vitro</i> Per uso diagnostico <i>in vitro</i> Dispositivo médico para utilização em diagnóstico <i>in vitro</i> "in vitro" diagnostisk udstyr <i>In vitro</i> diagnostisk medicinsk produkt Προϊόν για διαγνωστική χρήση <i>In vitro</i></p>	<p>LOT</p> <p>Batch code Chargen-Bezeichnung Identificación número de lote Désignation du lot Numero del lotto Número de lote Batch nr. Tillverkningskod Αρ. Παρτίδας</p>	<p></p> <p>Use by Verwendbar bis Caducidad Utilisable jusqu'à Da utilizzare prima del Data limite de utilização Anvendelse Användning Χρήση έως</p>	<p></p> <p>Temperature limitation Festgelegte Temperatur Temperatura de Almacenamiento Températures limites de conservation Limiti di temperatura Limite de temperatura Temperatur begrænsninger Temperatur gräns Περιορισμοί θερμοκρασίας</p>	<p></p> <p>Consult instructions for use Beilage beachten Consultar la metódica Lire le mode d'emploi Vedere istruzioni per l'uso Consultar as instruções de utilização Se vejledning for anvendelse Ta del av instruktionen före användning Συμβουλευτήτε τις οδηγίες χρήσης</p>	<p>CONTROL</p> <p>Control Kontrollen Control Contrôle Controllo Controllo Kontroll Kontroll Υλικό ποιοτικού ελέγχου</p>	<p></p> <p>Biological risks Biologisches Risiko Riesgo biológico Risque biologique Rischio biologico Risco biológico Miljø oplysninger Biologiska risker Βιολογικοί κίνδυνοι</p>	<p></p> <p>Manufacturer Hergestellt von Fabricado por Fabricant Prodotto da Fabricado por Producent Tillverkare Κατασκευαστής</p>	<p>EC REP</p> <p>Authorised representative Bevollmächtigter Representante autorizado Mandataire Rappresentanza autorizzata Representante autorizado Leverandør Auktoriserad representant Εξουσιοδοτημένος αντιπρόσωπος</p>
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