COATEST® Heparin - 25 5539 63

ENGLISH - Insert revision 12/2002 CE

Intended use of the kit

For the 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 —FXa—> 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
   Chromogenic substrate (Bz-Ile-Glu-(g-OR)-Gly-Arg-pNA·HCl) 15 mg with mannitol added as a bulking agent. Reconstitute with 20 mL sterile water to obtain a concentration of 1 mmol/L. The solution is stable for 6 months at 2-8°C.
2. **Factor Xa** 1 vial
   Bovine Factor Xa 71 nkat. Reconstitute with 10 mL sterile water. The reconstituted Factor Xa is stable for 1 month at 2-8°C or 6 months at -20°C or below.
3. **Buffer, stock solution** 1 vial
   Tris 0.5 mol/L, pH 8.4, 10 mL. An opened vial of stock solution is stable for 2 months at 2-8°C. Before use dilute accordingly: 1 volume of stock solution with 9 volumes of sterile water.
4. **Antithrombin** 1 vial
   Lyophilized human Antithrombin, 10 IU. Reconstitute with 10 mL sterile water to obtain a concentration of 1 IU/mL. The reconstituted Antithrombin is stable for 1 month at 2-8°C or 6 months at -20°C or below.
5. **Normal Plasma (human)** 4 vials
   Lyophilizied plasma. Reconstitute with 1.00 mL sterile water. The reconstituted plasma is stable for two weeks at 2-8°C or 1 month at -20°C or below.

PRECAUTIONS AND WARNINGS
Each donor unit used in the preparation of human source reagent has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and anti-bodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borned diseases, the handling and disposal of human source reagents from this product should be made with care.[8]

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.

Reagents not provided
— Sterile water
— Acetic acid 20% or citric acid 2%
— NaCl 0.9% (saline)
— Heparin

Material required but not provided
— Photometer, 405 nm
— Heating device, 37°C ± 0.2°C
— Calibrated pipettes
— Plastic test tubes
— Semi-micro cuvettes
— Mixer
— Stopwatch
— Centrifuge, 2000 x g

Specimen collection

Blood (9 volumes) is 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 24 hours at 2-8°C or 6 months at -20°C or below.

Quality control

Two levels of heparin controls, calibrated against International Standards, are recommended for a complete quality control program.[9] Each laboratory should establish its own mean and standard deviation and should establish a quality program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice. Refer in Westgard et al for identification and resolution for out-of-control situations.[10]

Procedure

All conditions included in this package insert are referred to Microplate method and ACL 3000. Detailed instrument settings including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix.

Calibration
A standard curve is required for each new lot of Coatest Heparin. For preparation of the 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.

Heparin IU/ml plasma	Heparin dilution 0.1 IU/mL µL	Buffer working solution µL	Human Normal Plasma µL	AT µL
0.1	100	700	100	100
0.3	300	500	100	100
0.5	500	300	100	100
0.7	700	100	100	100

b)

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

c) Assay

Add in a plastic tube	Sample	Sample blank
Diluted test plasma or standard	200 µL	200 µL
Incubate at 37°C (3-4 minutes)		
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%	300 µL	300 µL
Water		300 µL
Mix		

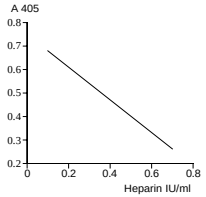
Transfer the content to a semi-micro cuvette and read the absorbance of the sample and the sample blank at 405 nm. The colour is stable for at least 4 hours.

NOTE: The assay can also be performed in the initial rate mode. Dilute S-2222 with 10 mL sterile water to obtain a concentration of 2 mmol/L. Care should be taken to keep the reaction conditions described above, implicating e.g. proportional volume changes when called for.

Results

Substract 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.

Standard curve



Limitations of the procedure

In some pathological states, plasma alone may hydrolyze the chromogenic substrate S-2222. This interference can be determined by substituting FXa with an equal volume of buffer.

Expected results
To obtain an optimal effect with minimum risk of bleeding or thromboembolic complications the heparin activity should be in the range recommended by the manufacturer.

Performance Characteristics

Precision
Coefficient of variation (CV) between series is 2.6% and within series 2.3% at the 0.7 IU/mL level.

Detection Limit
The assay allows detection of 0.05 IU/mL of heparin.

Measuring Range
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.

Sensitivity:

System	
ACL 3000	ΔAbs per 1IU/mL of Heparin activity 6.2 Abs

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.

Bibliography / Literatur / Bibliografía / 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

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

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