CHROMOGENIC SUBSTRATE ASSAY FOR FACTOR XI

This kit is designed for the measurement of factor XI (FXI) in human plasma. Plasma is treated with acetone to destroy inhibitors of αFXIIa and FXIa. The contact system of plasma is then activated with kaolin and FXI is converted to FXIa by FXIIa. Following the activation step, FXIIa and kallikrein are inhibited, and the FXIa level is determined by its ability to cleave a chromogenic substrate and liberate p-nitroaniline (pNA)[1,2]. This can be measured photometrically, and is proportional to the FXI concentration.

REAGENTS

The reagents should be stored at 4°C until reconstituted.

1. *Factor XIa Substrate*
10µmol/vial 2AcOH.H-D-Lys(Cbo)-Pro-Arg-pNA, plus mannitol. Dissolve in 10ml sterile distilled water. Stable for at least 6 months at 4°C if kept free from contamination. It may also be stored in aliquots at -20°C.

2. *Kaolin*
Suspend in 10ml buffer, shake well before use. Store at 4°C.

3. *Buffer Concentrate*
Dilute 1 part of buffer concentrate with 9 parts of sterile distilled water. This gives a buffer of 0.1M Tris-HCl, 0.15M NaCl, 3.4mM EDTA, pH 7.4. Store at 4°C.

4. *Kallikrein Inhibitor*
Dissolve in 10ml distilled water and dilute 1ml with 99ml buffer. Stable for 8 hours at 4°C and 6 months at -20°C.

5. *Corn Trypsin Inhibitor*
Dissolve in 10ml distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

6. *Standard Plasma*
Add 1ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 4°C.

Reagents required, but not provided:
50% acetic acid; acetone.

BLOOD COLLECTION AND PREPARATION OF PLASMA

Blood (9ml) is mixed with 0.106M Tri-sodium citrate (1ml) and centrifuged at 2000g for 15 minutes at room temperature. The plasma samples should be removed with plastic pipettes within two hours of blood collection and should be assayed immediately or stored frozen at -20°C.

ACETONE TREATMENT OF PLASMA

600µl Standard plasma (or 300ul test plasma) and 200µl acetone (100µl acetone for test plasmas) are pipetted into siliconised glass test tubes (80x10mm), or polypropylene tubes, mixed well and left for 15 minutes at 4°C, then kept on ice until assayed.

PREPARATION OF THE STANDARD CURVE

The acetone treated standard plasma is diluted with buffer as follows:

Standard %	Plasma	Buffer
150	150µl	850µl
100	200µl	1800µl
	From the 100% Standard prepare:	
75	600µl	200µl
50	400µl	400µl
25	200µl	600µl
0	Use buffer alone	

Dilute 100µl of each acetone treated test plasma with 900µl buffer.

ASSAY METHOD

Have the substrate at 37°C, shake the Kaolin well before use. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer	200µl
Add Kallikrein Inhibitor	200µl

Mix and incubate at 37°C for 2 minutes, add:

Kaolin	200µl

Mix well, incubate for 60 minutes at 37°C, add:

Corn Trypsin Inhibitor	200µl

Mix well, incubate for 10 minutes at 37°C, add

FXIa Substrate	200µl

Mix and record the change in optical density per minute at 405nm (rate assay), or incubate for exactly 60 minutes at 37°C, add:

Acetic acid (50%)	200µl

Mix and read optical density at 405nm (end point assay).

CALCULATION

With the end point assay, if the test plasmas have high bilirubin levels, are lipaemic or have visible haemolysis, blanks must be performed. For the blanks add 200µl of buffer instead of FXIa Substrate. The A_{405} values for the blanks are subtracted from the test values before reading the Factor XI values from the standard curve.

Plot the results as A_{405} against percentage FXI for the standard plasma dilutions and read the values for the test plasma from the standard curve. The values can be expressed either as a percentage or in units per ml (U/ml) by applying the formula:

$$\text{FXI (U/ml)} = \frac{\text{\% Activity x Potency of Standard}^{*}}{100}$$

*The potency value of the standard plasma (Lot UD-0137-0316) for FXI is 0.98 U/ml.

INTERPRETATION

Normal Range 0.65 - 1.45 U/ml

Factor XI levels may be reduced in congenital deficiency, and result in a haemorrhagic diathesis (haemophilia C).

HAZARD WARNING

All materials of human origin were tested and found negative for the presence of HBsAG and anti-HIV antibody. However, as with all preparations of human origin, these products cannot be assumed to be free from infectious agents and suitable precautions should be taken in their use and disposal.

REFERENCES

1. Scott CF, Sinha D, Seaman FS, Walsh PN, Colman RW. Amidolytic assay of human factor XI in plasma: comparison with a coagulant assay and a new rapid radioimmunoassay. Blood 1984; 63: 42-50.

2. Scott CF & Colman RW. A simple and accurate microplate assay for the determination of factor XI in plasma. J Lab Clin Med 1988; 111: 708-714.

NOTE

The recommended standard and test sample dilutions may vary between different batches of this kit owing to differences in the specific activity of some batches of reagents.

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