TFPI (Tissue Factor Pathway Inhibitor, type - I)

Determination of TFPI activity in plasma with S-2222 (microplate method) .

Background

Human TFPI is a modular protein synthetized primarily by the vascular endothelium under normal physiologic conditions; small amounts are also expressed by monocytes and macrophages.
The regulatory activity of TFPI is directed towards the extrinsic initiation pathway of the coagulation cascade involving binding and direct inhibition of factor Xa. It also inhibits the Tissue Factor/ factor VIIa complex in a factor Xa dependent fashion. TFPI expression can be modulated in several cell types in response to various inflammatory stimuly.
In vivo TFPI is distributed in three pools: 80-85% is associated with endothelial cell-surface, and it is released into plasma after injection of heparin; 10% circulates in plasma primarily in association with the lipoproteins and a small amount in free form, and 3% is found in platelets.
TFPI levels are elevated in pregnancy and lower in the new-born than those in the adult, while recently a significant reduction was found in woman taking combined oral contraceptives, suggesting a potential underlying cause for an increased risk for thrombosis. The role of TFPI in several thrombotic, inflammatory and malignant conditions is already established.

Measurement Principle

TFPI activity assay is based on the ability of TFPI in the sample to inhibit TF/FVIIa catalytic activity, in presence of FXa. Plasma is incubated for a prolonged time, allowing the formation of inctive TF/FVIIa/TFPI/FXa complexes.
Fibrin polymerization inhibitor, I-2882, is added to prevent formation of cross-linked fibrin.
After first incubation, residual TF/FVIIa catalytic activity is determined by the addition of FX and a selective chromogenic sustrate, S-2222.

Reagents

1. TBS/BSA/Polybrene® Buffer
(0.05 M Tris-HCL, 0.15 M NaCl, pH 7.5, supplemented with 2.0 mg/ml BSA and 2.0 µg/ml Polybrene®)
Dissolve 6.057 g Tris base, 8.766 g NaCl, 2.0 g BSA, and 2.0 mg Polybrene® in 900 ml H_2O, adjust the pH to 7.5 with 2 M HCl and top up to 1000 ml with H_2O

2. Acetic acid (50 % (V/V)
Add 125 ml 100 % Acetic acid to 125 ml H_2O

3. $CaCl_2$ (50 mM)
Dissolve 0.7351 g $CaCl_2$ - $2H_2O$ in 100.0 ml H_2O

4. I-2882 (10mg/ml) Art.No. 82 38 15 10
Dissolve the vial content (40 mg) with 4.0 ml H_20

5. Factor Xa, bovine (7,1 nkat/ml) Art.No. 82 09 85
Dissolve the vial content (71 nkat) with 10.0 ml H_20

6. Recombinant Human Tissue Factor
(RecombiPlasTin, Instrumentation Laboratory
Art.No. 49 73 27 50)
Dissolve the vial content with 5.0 ml diluent

7. Recombinant Factor VIIa (30 kIE/ml)
(NovoSeven, Novo Nordisk)
Dissolve the vial content (240 kIE = 4.8 mg) with 8.5 ml diluent

8. Factor X, bovine (2 U/ml) Art.No. 82 22 39
Dissolve the vial content (2 U) in 1.0 ml H_2O

9. S-2222 (2.7 mM) Art.No. 82 03 16
Dissolve the vial content (25 mg) in 12.49 ml H_2O

10. HCl 2M

11. Normal plasma

Additional Material

Fridge or ice-bath
Thermostat at 37°C
Water bath at 37°C
Microplates, flat-bottom
Lids for microplates or Parafilm®
Spectrophotometer 405 nm

Storage Conditions

Store the Reagents 1 and 3-7 at 2-8°C or on ice until use it.
Reagents 8 and 9 can be prepared during the first incubation step (30 min) and should have room temperature.
All the reconstituted reagents can be used over a period of at least three weeks at 2-8°C. Avoid contamination by microrganism.

Specimen Collection

Blood (9 volumes) is mixed with 0.1 mol/l sodium citrate (1 vol) and centrifuged at 2000xg for 20 minutes at 20-25°C. Separate plasma carefully from the blood cells. Plasma can be stored in aliquots at -70°C.

Standard Curve

1. Dilute Normal Plasma 1:10 with ice cold TBS/BSA/Polybrene® Buffer (Reagent 1)
2. Dilute this 1:10 dilution (= 10 %) further to 7.5 %, 5.0 %, 3.75 %, 2.5 %, 1.25 %, 0.625 % and 0.313 % with ice cold TBS/BSA Buffer/Polybrene® (Reagent 1).

Note: an aliquot of 100 µl of each dilution (10 % - 0 %) is sufficient for the standard curve.

Sample Dilution

Normal Samples 10 µl Plasma + 390 µl ice cold TBS/BSA/Polybrene® Buffer (Reagent 1)
Heparinized Samples 10µl Plasma + 790µl ice cold TBS/BSA/Polybrene® Buffer (Reagent 1)

Preparation of the Combined Reagent

(sufficient for 1 plate)

1. Factor VIIa	10µl
2. Tissue Factor	125µl
3. Factor Xa	125µl
4. I-2882	125µl
5. CaCl2	2500µl
6. TBS/BSA Buffer	7115µl

Preparation of the Substrate Reagent

(sufficient for 1 plate)

1. Factor X	0.65 ml
2. TBS/BSA Buffer	1.95 ml
3. S-2222	2.60 ml

Microplate Assay Procedure

Generally, the determination of double values is recommended

Standard or Sample Dilution	**25µl**
Combined Reagent	**100µl**
Incubate the microplate covered with a lid for 30 min. at 37 °C	
Substrate Reagent	**50µl**
Incubate the microplate covered with a lid for 30 min. at 37 °C	
Acetic acid	**50µl**

Blank: For the Blank determined with the 10%-standard, 50 µl acetic acid are added to the wells before the Substrate Reagent.

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