

MEASUREMENT OF PROCOAGULANT POTENTIAL OF BLOOD MICROPARTICLES CARRYING TISSUE FACTOR

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BACKGROUND:

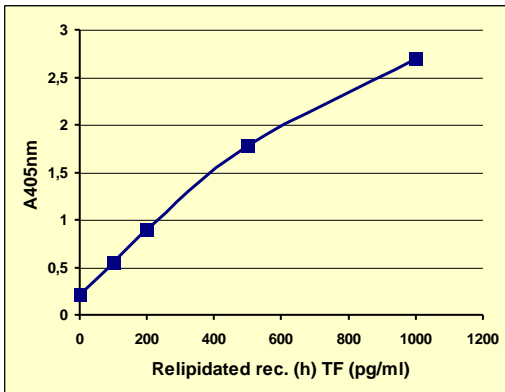
- Elevated levels of Tissue Factor (TF) are observed in patients with cardiovascular risk factors (hypertension, diabetes, dyslipidemia, and smoking) (5), but also in pathological states such as atherosclerosis, acute coronary syndrome, cancer, sepsis, inflammation, sickle cell disease. (1,2,3,4,5,6)
- Within the atherosclerotic plaque, sequestered microparticles (MPs) constitute the main reservoir of TF activity, promoting coagulation following plaque erosion or rupture.(4)
- Microparticles carrying TF (MP-TF) are a marker of thrombogenicity.
- Initiation of blood coagulation pathways in diseases is assumed to be triggered, at least in part, by recruitment of MP-TF and decryption of TF, at the site of injury.(2,4)

OBJECTIVES OF THE ASSAY:

- To develop a simple and specific method to detect the procoagulant potential of MP-TF in plasma or in cell culture supernatants.
- To avoid any non-specific signal due to MPs which do not carry Tissue Factor and which could interfere in the assay.
- Dynamic range from 0 to 1000 pg/ml (0-22pM) of relipidated TF. Detection of less than 100 pg/ml (2.2pM) of relipidated TF in plasma.

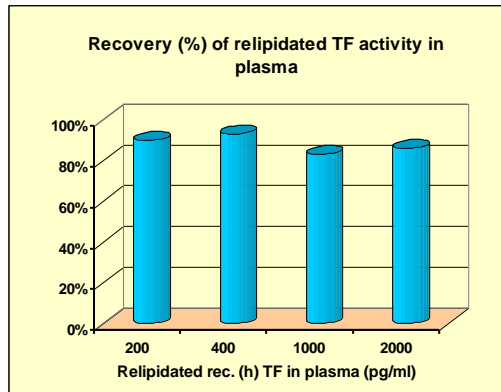
RESULTS

Dose-response curve using relipidated human recombinant Tissue Factor diluted in assay buffer
(Recombinant Tissue Factor from American Diagnostica relipidated with PS/PC/PE/Cholesterol Liposomes from Hyphen BioMed)



Assay range: 50 to 1000 pg/ml
Detection threshold: < 50pg/ml

Recovery study of relipidated TF in plasma and specificity for TF
For recovery studies, relipidated TF is spiked into normal plasma and tested diluted 1:2 in sample buffer, assayed against a calibration curve obtained with relipidated TF diluted in assay buffer



Excellent recovery of relipidated TF spiked into plasma

DISCUSSIONS

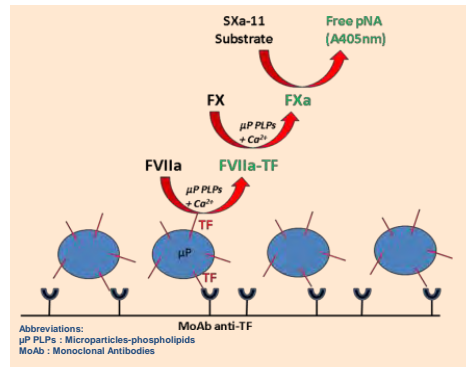
- Assay specific for TF procoagulant activity in plasma: double specificity combining capture with anti-TF MoAb and revelation through FXa generation.
- Recovery in plasma: about 90% of relipidated TF activity when spiked into plasma tested diluted 1:2 in assay buffer.
- Improved Specificity: specificity for TF has been verified by assaying TF-free liposomes.
- Procoagulant activity assay: the revelation system mimics the physiological initiation of coagulation and thus permits to evaluate the real procoagulant activity of the TF-exposing microparticles that are captured onto the solid phase.
- High Sensitivity: the method is sensitive to concentrations of relipidated TF lower than 100pg/ml (2.2pM) in plasma.
- Can be applied to cell culture supernatants: cell culture supernatants (from Dr JM Freyssinet's group) have been tested successfully using this technique.

CHARACTERISTICS OF THE METHOD:

- This assay does not provide any information on the cell origin of microparticles.
- Truncated Tissue Factor (soluble TF) is not measured by this assay.

MATERIAL AND METHOD

- Principle:** MP-TF are captured through an anti-(h)-TF MoAb coated onto a micro ELISA plate. The assay is carried out in presence of calcium ions, phospholipids being supplied by tested microparticles themselves. FVIIa-TF complexes are formed and activate FX into FXa onto MPs surface. Subsequent cleavage of a FXa specific substrate releases pNA. Absorbance read at 405nm is directly proportional to the quantity of MP-TF present in the sample.



Test Procedure:

- 100µl of sample diluted 1:2 in assay diluent
- 1h at 37°C
- Washing step
- 100µl of Reagent 1 : Human Factor VIIa (NovoSeven®), Human Factor X (Hyphen BioMed) in Tris-NaCl-CaCl₂ Buffer
- 1h at 37°C
- 50µl of Reagent 2 : SXa-11 FXa specific Substrate (Hyphen BioMed)
- 1h at 37°C
- 50µl of citric acid 2%
- Read Absorbance at 405nm

- Specificity** : No reactivity (<100pg/ml) was found when TF-free liposomes were spiked into normal plasma. (Recovery=0%)

- Normal range** : MP-TF were assayed in 14 normal individuals. Plasma samples were obtained following a double centrifugation at room temperature (15min. at 2500g, then 2min. at 13000g).

Mean (N=14): < 100pg/ml

Assay of TF-expressing THP-1 cell culture supernatants
Malignant cells supernatants were kindly provided by Drs Toti F and Freyssinet JM

Dilution	1:40	1:80	1:160	Mean concentrations in supernatants
THP-1 "R3" cells (pg/ml)	4,500	2,270	1,080	534,400
THP-1 "R7" cells (pg/ml)	3400	1,700	940	422,400

CONCLUSIONS

- Fully TF-dependent assay principle (Capture and Detection) ensures maximum specificity for the assay.
- Sensitivity of less than 50pg/ml, may be enhanced, if required, by optimizing the detection system.
- Combined with Zymuphen MP Activity (Assay of microparticles coagulant activity in plasma), offers a useful tool for investigating MPs generation in plasma and their role in plasma thrombogenicity.
- Important clinical applications can be developed in all pathologies where TF triggers procoagulant pathways.

References:

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