

MEASUREMENT OF PROCOAGULANT POTENTIAL OF BLOOD MICROPARTICLES CARRYING TISSUE FACTOR

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BACKGROUND:

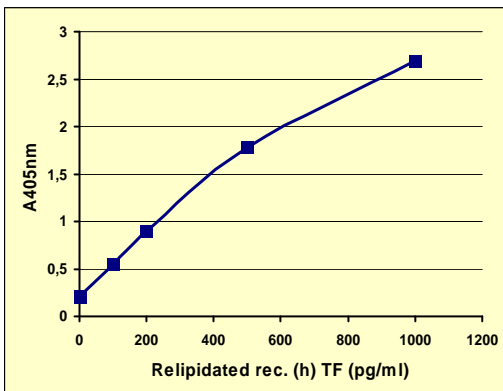
- Elevated levels of Tissue Factor (TF) are observed in patients with cardiovascular risk factors (hypertension, diabetes, dyslipidemia, and smoking) (5), but also in pathological states such as atherosclerosis, acute coronary syndrome, cancer, sepsis, inflammation, sickle cell disease. (1,2,3,4,5,6)
- 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.

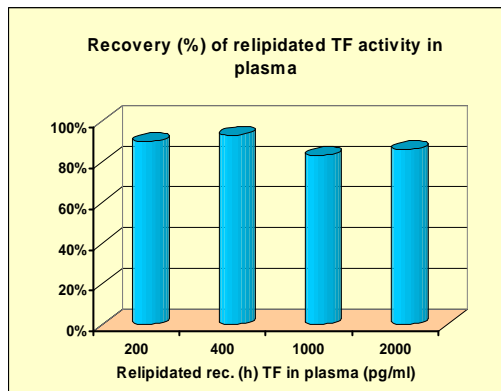
RESULTS

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

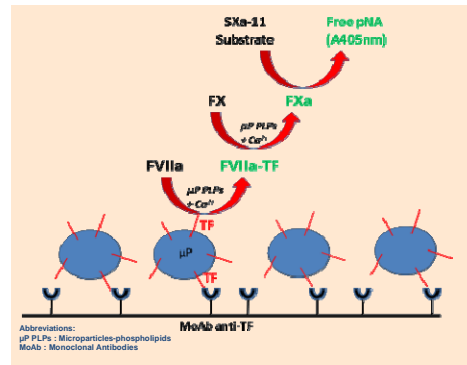
Recovery study of relipidated TF in plasma and specificity for TF
For recovery studies, relipidated TF is spiked into normal plasma and tested diluted 1:2 in sample buffer, assayed against a calibration curve obtained with relipidated TF diluted in assay buffer



Excellent recovery of relipidated TF spiked into plasma

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

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.

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.

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Measurement of procoagulant potential of blood microparticles carrying Tissue Factor.

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Blood microparticles (MPs) have high diagnostic and prognostic interest, not only for circulatory diseases, but also for inflammatory, malignant or infectious pathologies. These MPs objectivate blood cell activation, but they also contribute to the disease course worsening through their procoagulant effect. A special focus concerns MPs which expose Tissue Factor (TF), involved in thrombotic diseases. From the studies of JM Freyssinet's group, we adapted a new method which specifically measures the procoagulant activity of blood MPs carrying Tissue Factor (MP-TF). Plasma is obtained with the specific cautions required for avoiding ex-vivo cell (mainly platelets) activation, using a double centrifugation at Room Temperature. This plasma is then diluted and introduced into a microwell coated with a specific Anti-TF MoAb. Microparticles exposing TF are captured, and their activity is measured through Factor Xa generation. The revelation mixture contains Factor VII or (better) VIIa in a constant and in excess concentration, Factor X and calcium. There is a direct dose response relationship between MP-TF concentration and Factor Xa generated. This Factor Xa is then measured by its activity on a specific chromogenic substrate and colour development is measured at 405 nm. The assay is calibrated with recombinant TF, at a known concentration, which is relipidated at a well defined Phospholipids concentration (12.5 % PS) using liposomes, and spiked into normal plasma. The assay has a dynamic range from 20 pg/ml to 1,000 pg/ml TF, and is performed within less than 2 hours. Tested plasma can be used diluted 1:2 or at higher dilutions according to the expected MP-TF concentration in the assayed specimen. This assay introduces a new analytical tool for measuring an emerging marker of blood activation in circulatory or malignant diseases. The diagnostic and prognostic value of this marker is amplified by the nature of MP-TF, which are an indicator, but also a trigger for thrombotic pathologies. It offers promising perspectives for the early diagnosis of thrombotic or malignant diseases.