**Conclusions**

- Zymuphen MP-TF is a highly sensitive and reproducible method for the measurement of MP-TF in plasma, and is an useful tool for assessing the clinical interest of this biomarker in pathology.
- Plasmas from LPS-induced blood release MP-TF which can be measured using Zymuphen MP-TF, in a TF-specific reaction.
- Synthetic liposomes, that mimicks TF-free microparticles, do not react with Zymuphen MP-TF.

**Materials and Methods**

Plasmas are prepared using a double centrifugation (15 min. at 1500g and 2 min. at 13000g) at room temperature to eliminate platelets.

**Specificity:** Either LPS-induced plasmas, or kit controls high and low, have been spiked with an anti-TF polyclonal Ab (HTI) at 2 µg/ml, or 0 µg/ml (controls), and incubated at 37°C for 2 hours before testing for MP-TF.

**Calibration and controls:** Full length recombinant Tissue Factor (1-263) (ADI) has been used as positive control.

**Reproducibility and repeatability**

<table>
<thead>
<tr>
<th>Inter-assay</th>
<th>Intra-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>N series</td>
<td>Mean (pg/ml)</td>
</tr>
<tr>
<td>CI [13-19 pg/ml]</td>
<td>7</td>
</tr>
<tr>
<td>CI [6-9 pg/ml]</td>
<td>7</td>
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</tbody>
</table>

**Normals**

<table>
<thead>
<tr>
<th>N</th>
<th>Mean Conc. (pg/ml)</th>
<th>Min (pg/ml)</th>
<th>Max (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>(below detection limit = 1 pg/ml)</td>
<td>0.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Increased in pathology: 2.0 to > 50.0 pg/ml (TF Eq.)*

**References**

Microparticles exposing Tissue Factor in human plasma

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Aim: Among microparticles (MP) those exposing Tissue Factor (TF) are of particular interest, for their critical role in the initiation of thrombosis. They may be a useful biomarker to identify an increased risk of thrombosis in various pathologies (e.g. cancer). We developed the Zymuphen MP-TF method, an ultra sensitive bio-immunoassay that allows the determination of MP-TF procoagulant activity in human plasma.

Method: Plasmas are prepared using a double centrifugation at Room Temperature (low and high speed) to eliminate platelets. MP-TFs are captured by a murine MoAb directed against the extracellular domain of TF, that does not inhibit TF activity. Following a washing step, FVIIa and FX are added into the reaction mixture. TF-FVIIa complexes form and activate FX into FXa (FXa) in presence of Ca++. FXa generation is dependent on TF and MP’s anionic phospholipids concentration. Then a FXa-specific substrate (CS 11(65)) is added, reacts with FXa, releasing pNA, which absorbance is recorded at 405nm. A lyophilized calibrator, containing rec.(h) TF relipidated with synthetic phospholipids, permits the standardization of the assay. Calibration is from 0 to 5 pg/ml of TF equivalent, with a ratio of 0.1 nM PS / 1 pg TF.

Results: Normals were < 0.2 pg/ml, while 2 pathological plasmas that were found to have high MP's concentration (9.8 and >63 nM PS) with Zymuphen MP Activity, were found at 0.95 pg/ml and > 5 pg/ml using Zymuphen MP-TF. Plasmas from LPS-stimulated whole blood following a 6 hour incubation were significantly higher than baseline (t=0h) with a MP-TF generation ranging from 5 to 22 µg/ml. Recombinant Truncated TF (1-219), when mixed at 100 pg/ml with synthetic saturating phospholipids (870nM PS), was < 0.2 pg/ml demonstrating the specificity for MP-TF.

Conclusion: Zymuphen MP-TF is a highly sensitive and specific method for the measurement of MP-TF in citrated plasma, and is a useful for assessing the clinical interest of this biomarker in pathology.