TECHNOTHROMBIN TGA

Measurement on Ceveron® alpha TGA

The Ceveron® alpha system is for Research Use Only in the US and Canada.
The fluorogenic substrate ZGGR-AMC is cleaved by formed thrombin over time.

The coagulation cascade is activated upon addition of different concentrations of:
- tissue factor and
- phospholipids

The fluorogenic substrate ZGGR-AMC is cleaved by formed thrombin over time.
TEST PRINCIPLE

Plotting the fluorescence we obtain the raw data curve of thrombin generation
Plotting the changes in fluorescence as a function of time (cnt/min), we obtain a "Thrombin Generation Curve"
The calibration curve (thrombin curve) enables conversion of results from cnt/min to nM thrombin.

The calibration curve is created separately from sample measurement.

For each lot of substrate only one calibration curve has to be created.
Thrombin Generation Curve in nM Thrombin displays the different phases of the coagulation reaction.
We recommend following trigger reagents for the determination of:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA RA</td>
<td>- measure the activity of <strong>microparticles</strong></td>
</tr>
</tbody>
</table>
| TGA RB and RC Low | - measure the **thrombophilic tendency**  
                      (preferentially with platelet poor plasma PPP)  
                      - measure the thrombogenity of microparticles |
| TGA RC High     | - measure the effect of an **anticoagulant**                           |
The composition of the different TGA trigger reagents are:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA RA</td>
<td>Low conc. of phospholipid micelles containing no rhTF in Tris-Hepes-NaCl buffer</td>
</tr>
<tr>
<td>TGA RB</td>
<td>Low conc. of phospholipid micelles containing low rhTF in Tris-Hepes-NaCl buffer</td>
</tr>
<tr>
<td>TGA RC Low</td>
<td>High conc. of phospholipid micelles containing low rhTF (same as in RB) in Tris-Hepes-NaCl buffer</td>
</tr>
<tr>
<td>TGA RC High</td>
<td>High conc. of phospholipid micelles (same as in RCL) containing high rhTF in Tris-Hepes-NaCl buffer</td>
</tr>
</tbody>
</table>
RB and RClow are TRIGGERs that

- activate the **extrinsic pathway** and forms a small amount of initial thrombin,
- this leads to formation of fibrin
- it is rapidly inactivated in a TF/FVIIA/FXa complex by TFPI
- activates by positive feedback the **intrinsic system**. This means, via factor XI, IX and VIII more FXa and thrombin are generated.
- when thrombin burst gets too big, differences in e.g., FVIII or FIX can’t be measured any more.
RChigh is a TRIGGER that

- activate the **extrinsic pathway** and forms such a big amount of initial thrombin, this **thrombin burst** gets so big, so that differences in e.g., FVIII or FIX can't be measured any more.
Reader/analyzer implication on thrombin generation measurement

Advantages of TGA Measurement on Ceveron alpha TGA

- Cuvette temperature is constant at 37°C
- Reagents are preheated in the tip and pipetted at 37°C
- LED temperature is constant resulting in standardized excitation intensity
- No influence of meniscus due to side measurement
- Check of fluorogenic channels with F-Standard
- Short assay time due to possibility of stopping at peak thrombin
- Possibility to run routine assays and TGA from the same sample in same run
Reader/Analyzer implication on thrombin generation measurement

For thrombin generation measurement 4 channels with special fluorometric TGA modules consisting of an UV LED (365nm) for excitation and a photodiode for measurement of the emitted signal.

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UNIQUE: Measurement can be stopped after Peak Thrombin has been reached

ADVANTAGE:
> Shorter Assay time 20 min
The calibration curve is created **separately from sample measurement**.

- For each **lot of substrate** only **one calibration curve** has to be created.
Reader/ analyzer implication on thrombin generation measurement

<table>
<thead>
<tr>
<th>Channel</th>
<th>Expected Counts</th>
<th>Measured Counts</th>
<th>Diff. %</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1489</td>
<td>1488</td>
<td>-0.07</td>
<td>ok</td>
</tr>
<tr>
<td>6</td>
<td>1484</td>
<td>1484</td>
<td>0.00</td>
<td>ok</td>
</tr>
<tr>
<td>7</td>
<td>1487</td>
<td>1486</td>
<td>-0.07</td>
<td>ok</td>
</tr>
<tr>
<td>8</td>
<td>1493</td>
<td>1492</td>
<td>-0.07</td>
<td>ok</td>
</tr>
<tr>
<td>Mean</td>
<td>1488.3</td>
<td>1487.5</td>
<td>-0.05</td>
<td>ok</td>
</tr>
</tbody>
</table>

Limits for checking:
- Single Channel: 2.5 %
- Mean: 1.5 %

F-Standard – for target value verification - barcoded

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