Tests of Global coagulation
Thromboelasatometry
Thromboelastography
Thrombin generation

Steve Kitchen
• **Prothrombin time** –
  - Tissue factor at around 2000pm
  - Sensitive to ‘extrinsic coagulation pathway’

• **Thrombin Generation Tests** –
  - Tissue factor is often used at 1 to 5pm
  - Sensitive to all coagulation factors but (?) and XIII
Low level tissue factor is often the trigger of choice

- TF-VIIa activates factor IX to IXa

IXa can be used as trigger of thrombin generation

Other triggers: e.g. contact factor activators
Value of Thrombin Generation Tests

- May be sensitive to:
  - All clotting factor levels apart from XIII
  - Natural anticoagulants (including TFPI)
  - Pharmacological anticoagulants (e.g. LMWH)
  - Hypercoagulability – predict who will thrombose?
  - Hypocoagulability - prediction of who will bleed?
Thrombin Generation: Pre analytical variable

- Anticoagulant and concentration (+/-CTI)
- Material tested (PPP, PRP, frozen PRP)
- Platelet contamination (including microparticles)
- Contact activation
- Sample quality etc etc
Thrombin Generation Tests: a family of tests that detect thrombin formation

TG assays vary:

- **Trigger** e.g. IXa, TF
- Phospholipid
- **Sub sampling or continuous monitoring** to detect thrombin
- Fibrinogen, chromogenic or fluorogenic substrate
- **Units to recorded** (e.g. AU, total thrombin, peak thrombin)
- **Calibration system** may be used to measure the thrombin
- **Material tested** (e.g. defibrinated plasma or plasma or PRP)
α2-macroglobulin bound thrombin

- α2- macroglobulin-thrombin does not cut natural substrates

- But does cleave small chromogenic and fluorogenic substrates
The Thrombinoscope assay uses an algorithm to discount this influence, but not all systems do.

The Thrombinoscope assay uses α2-macroglobulin-thrombin as reference material to standardise the assay.
Thrombinsoscope TG assay is carried out on a Fluorometer.
Test wells:

The trigger (e.g. 5pm Tissue factor) + calcium chloride + fluorogenic substrate is added to a set of wells (may be in triplicate)

Thrombin formation is continually monitored by detecting fluorescence and converting this into nM thrombin (requires a calibrator)
The green line shows the graph obtained when testing a subject’s plasma with the ‘calibrator’

This data is needed for each subject, to calculate the results

The Calibrator
A ‘calibrator’ + calcium + fluorogenic substrate is added to a further pair of wells, to allow calculation of thrombin levels, and to compensate for:

- Substrate depletion
- Sample turbidity
- Inner filter effect
Thrombin Generation Test Curve Parameters (Thrombogram)

- Peak thrombin
- Lag time
- Area Under the Curve (ETP)
- Time to Peak

‘Calibrator’ trace from Calibrator wells – used to calculate nmol thrombin

Traces from Test wells
Thrombinoscope TG assay and the calibrator

- Each sample is normally measured in triplicate and the calibrator is tested in duplicate.
- The thrombin calibrator (thrombin-α2-M complex) allows calculation of thrombin levels in the sample.
- The calibrator compensates for: sample turbidity, the inner filter effect, and substrate depletion.
Contact activation may influence thrombin generation at low TF concentration (<=2pmol).

Contact activation of XII can occur during venepuncture or sample storage.

Contact activation reduces sensitivity of assay when <=2pm TF is used.
Influence of tissue factor concentration on CTI effect

Corn Trypsin Inhibitor (CTI) and Tissue Factor concentration in the ETP assay

(Joost van Veen, 2005)
Thrombinsoscope Thrombin Generation Assay: Factor II deficient plasma supplemented with normal plasma

Normal Plasma (100% II)

Decreasing concentrations of factor II
Many assay principles

Many assay formats

A lot of publications with in-house assays

A lot of publications with in-house/hybrid assays

Not a lot of standardisation

Is it time to standardise the assays?

Working group on thrombin generation
TEG® Analyser

Cup rotates pin is stationary
ROTEM ® Device

Pin rotates Cup is stationary
## Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ROTEM</th>
<th>TEG</th>
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<tbody>
<tr>
<td>Time for clotting to begin</td>
<td>CT (sec)</td>
<td>R (min)</td>
</tr>
<tr>
<td>Time until clot is 20 mm</td>
<td>CFT (sec)</td>
<td>K (min)</td>
</tr>
<tr>
<td>Rate of clot growth</td>
<td>Angle (degrees)</td>
<td>Angle (degrees)</td>
</tr>
<tr>
<td>Clot firmness - trace width</td>
<td>MCF (mm)</td>
<td>MA (mm)</td>
</tr>
</tbody>
</table>
ROTEM / TEG analysis

- Clotting time (CT / r) [sec]
- Clot formation time (CFT / k) [sec]
- Maximum clot firmness (MCF / MA) [mm]
NEQAS Survey data ROTEM® - Intem CT
(n= 7 -10)
Citrated Whole Blood Normal range 100 - 240 sec

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
<th>CV</th>
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<tbody>
<tr>
<td>Normal (1)</td>
<td>147 sec</td>
<td>117 - 161</td>
<td>10%</td>
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<tr>
<td>Normal (2)</td>
<td>145 sec</td>
<td>129 - 197</td>
<td>15%</td>
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<tr>
<td>FXI &lt;1 U/dl</td>
<td>950 sec</td>
<td>596 - 1562</td>
<td>37%</td>
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NEQAS Survey data TEG® R
(n = 13 - 14)

<table>
<thead>
<tr>
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<th>Median</th>
<th>Range</th>
<th>CV</th>
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</thead>
<tbody>
<tr>
<td>Normal (1)</td>
<td>5.6 min</td>
<td>4.8 – 7.6</td>
<td>15%</td>
</tr>
<tr>
<td>Normal (2)</td>
<td>5.8 min</td>
<td>4.2 – 7.2</td>
<td>19%</td>
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<tr>
<td>FXI &lt;1 U/dl</td>
<td>No clot</td>
<td>-</td>
<td>-</td>
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### Agreement between results in different centres - Clot Firmness

<table>
<thead>
<tr>
<th></th>
<th>ROTEM (Extem)</th>
<th>TEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF (mm)</td>
<td>MA (mm)</td>
</tr>
<tr>
<td>Range</td>
<td>CV</td>
<td>Range</td>
</tr>
<tr>
<td>Normal (1)</td>
<td>36 -47</td>
<td>42-53</td>
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<tr>
<td>Normal (2)</td>
<td>24-27</td>
<td>16-61</td>
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<tr>
<td>FXI &lt;1 U/dl</td>
<td>30-36</td>
<td>No clot</td>
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</table>

Fibrinogen in normal 2 2.5 g/l versus 5 g/l in normal 1