COAGULATION
Global Tests

• **aPTT** – activated partial thromboplastin time

• **PT** - prothrombin time

• **Fibrinogen**

The Ceveron® alpha system is for Research Use Only in the US and Canada.

October 2015
The contact factor pathway is initiated by activation of contact factors of plasma, and can be measured by the *activated partial thromboplastin time (aPTT)* test.

The Tissue factor pathway is initiated by exposure of blood to "tissue factor" (a specific cellular lipoprotein), and can be measured by the *prothrombin time (PT)* test, also reported as an INR value.

The quantitative and qualitative measurement of fibrinogen is measured by the *thrombin time (TT)*.

Measurement of the exact amount of fibrinogen present in the blood is generally done using the Clauss method for fibrinogen testing.
The contact factor pathway is initiated by activation of contact factors of plasma, and can be measured by the activated partial thromboplastin time (aPTT) test.

The Tissue factor pathway is initiated by exposure of blood to "tissue factor" (a specific cellular lipoprotein), and can be measured by the prothrombin time (PT) test, sometimes reported as an INR value.

The quantitative and qualitative measurement of fibrinogen is measured by the thrombin time (TT).

Measurement of the exact amount of fibrinogen present in the blood is generally done using the Clauss method for fibrinogen testing.
COAGULATION
Global Tests

- aPTT – activated partial thromboplastin time
- PT - prothrombin time
- Fibrinogen

The Ceveron alpha system is for Research Use Only in the US and Canada.
The **partial thromboplastin time** (PTT) or **activated partial thromboplastin time** (aPTT) is a commonly performed test for coagulation, especially the *intrinsic pathway*. Apart from detecting abnormalities in blood clotting, it is also used to measure the effect of heparin.

aPTT is measured in seconds.
aPTT – Reagent Composition

aPTT REAGENT COMPOSITION

- Activator to convert FXII to FXIIa
- Phospholipid (replaces “in vivo” platelet surface on which coagulation reactions occur)
- CaCl2 – used to reintroduce calcium ions that were chelated by sodium citrate
- Referred to as “partial thromboplastin” since no Tissue Factor is used – two-stage assay (activation and recalcification)
INTENDED USE

- Intrinsic and severe common pathway factor deficiencies

- Assess impact of......
  - unfractionated heparin
  - other antithrombotic agents (direct thrombin inhibitors)
Dapttin® TC (lyophilized)

Dapttin® TC is a double activated aPTT reagent to be used:

- as a test for the intrinsic coagulation system.
- to detect deficiencies of Factors II, V, VIII, IX, X, XI, and XII.
- to measure heparin since it is sensitive to the presence of heparin.

Dapttin® TC measures the overall activity of the intrinsic coagulation system.

Dapttin® TC is sensitive to the factors XII, XI, X, IX, VIII, V and II, but insensitive to factors VII and XIII.
Siron LS (aPTT liquid)

Siron LS is an activated partial thromboplastin time (aPTT) reagent to be used:

- as a test for the intrinsic coagulation system
- for specific determinations of factors VIII, IX, X, XI and XII* when used in combination with the corresponding deficiency plasma
- for assessing heparin impact

Siron measures the overall activity of the intrinsic coagulation system. Siron is sensitive to the factors XII, XI, X, IX, VIII, V and II, but insensitive to factors VII and XIII.
Siron LIS (aPTT liquid)

Siron LIS is an activated partial thromboplastin time (aPTT) reagent to be used:
- as a test for the intrinsic coagulation system
- in the research evaluation of haemophilia A and B
- for specific determinations of factors VIII, IX, X, XI and XII* when used in combination with the corresponding deficiency plasma
- for control of heparin treatment

Siron measures the overall activity of the intrinsic coagulation system.
Siron is sensitive to the factors XII, XI, X, IX, VIII, V and II, but insensitive to factors VII and XIII.
# aPTT – activated partial thromboplastin time

<table>
<thead>
<tr>
<th>aPTT Reagents:</th>
<th>DAPTTIN® TC</th>
<th>SIRON LS (Lupus Sensitive)</th>
<th>SIRON LIS (Lupus Insensitive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activators</td>
<td>Lyophilized</td>
<td>Liquid</td>
<td>Liquid</td>
</tr>
<tr>
<td></td>
<td>Silica</td>
<td>Ellagic Acid</td>
<td>Ellagic Acid</td>
</tr>
<tr>
<td></td>
<td>Phospholipids</td>
<td>Phospholipids</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>On Board Stability</td>
<td>5 days</td>
<td>5 days</td>
<td>5 days</td>
</tr>
<tr>
<td>Normal Range</td>
<td>29—42 sec</td>
<td>24—36 sec</td>
<td>24—36 sec</td>
</tr>
<tr>
<td>Factor Sensitivity</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lupus Sensitivity</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Heparin Sensitivity</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

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aPTT – ordering informations

Dapttin® TC (lyophilised)

REF 5035060  Dapttin® TC 5x2 ml
REF 5035090  Dapttin® TC 6x10 ml
REF 5035100  Dapttin® TC 20x10 ml

Siron LS (aPTT liquid)

REF 5035105  Siron LS 2x4 ml
REF 5035107  Siron LS 10x4 ml
REF 5035109  Siron LS 10x10 ml

Siron LIS (aPTT liquid)

REF 5035118  Siron LIS 2x4 ml
REF 5035119  Siron LIS 10x4 ml
REF 5035121  Siron LIS 10x10 ml

CaCl₂ 25 mmol/L Solution

REF 5277015  Ca-Chloride solution 25 mmol/l  100 ml
REF 5277017  Ca-Chloride solution 25 mmol/l  25 ml
(vial suitable for Ceveron® alpha buffer position)

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Coagulation – Global Tests

• **aPTT** activated partial thromboplastin time
• **PT** - prothrombin time
• **Fibrinogen**
The **prothrombin time** (PT) and its derived measures of **prothrombin ratio** (PR) and **international normalized ratio** (INR) are measures of the *extrinsic pathway* of coagulation. They are used to determine the clotting tendency of blood, in the research of warfarin dosage, liver damage and vitamin K status.

PT is measured in INR and seconds as well as Ratio.
PT REAGENT COMPOSITION

- Tissue Factor (recombinant/human or animal brain)
- Lipid (source of phospholipid since platelets were removed from plasma)
- CaCl₂ – used to reintroduce calcium ions that were chelated by sodium citrate
PT –

Causes for prolonged PTs

TO RESEARCH PROLONGED PT:

- Deficiencies or abnormalities in:
  - FVII (Extrinsic Pathway)
  - FV, FX, FII (prothrombin), and FI (fibrinogen)
    - Both PT and aPTT will be prolonged
- Vitamin K antagonists
  - PT sensitive to reductions in three of four vitamin K-dependent procoagulant proteins: FVII, FX, and FII
    - FIX measured by aPTT
  - Pharmacologic anticoagulants that modify vitamin K-dependent proteins such that they do not bind calcium thereby reducing blood coagulability
- Liver
  - Site for synthesis of vitamin K-dependent proteins
  - Site for clearance of coumarins (warfarin) and coagulation proteins
PT can be reported in following units:

- **Clotting time seconds**
  - clotting times are reagent and instrument dependent

- **% of normal or Quick**
  - this is a calibrated value, therefore it is less instrument but only reagent dependent

- **INR (International Normalized Ratio)**
  - the INR values are the most comparable results between reagents and instruments for anticoagulation due to a lot specific ISI for each PT reagent
Technoclot PT Plus– Using the AK Calibrant Calibrator Set

Technoclot PT Plus

Technoclot PT Plus with provided ISI

The Ceveron alpha system is for Research Use Only in the US and Canada.
To start calibration load reagents
Technoclot PT Plus—Using the AK Calibrant Calibrator Set

Open Calibration menu to add calibration.
Select **Technoclot PT Plus 10ml %** or **Technoclot PT Plus 4ml %** protocol depending on loaded reagent vial size.

The Ceveron alpha system is for Research Use Only in the US and Canada.
Technoclot PT Plus—Using the AK Calibrant Calibrator Set

Information on loaded reagent lot to be calibrated is shown

Calibration screen opens (enter lot specific % values for the calibrators)

At this point you can make changes in the calibration settings. It is recommended to use the predefined settings.
Start calibration
After calibration has finished, the calibration curve of % of normal is displayed.

Press OK and calibration data will be saved. Don’t leave calibration menu with cancel – calibration data will be deleted.

Confirm with Yes(Ja)
Sample Measurement

Load all reagents necessary for PT sample measurement in menu ♦Loading♦
Go to ♦Work List♦ and enter sample IDs and tests (select test depending on vial size and calibration made)

Start measurement

After measurement has been competed validate the results. Results will be transferred in ♦Archive♦ and control results in ♦Quality Control♦
Technoclot PT Plus

TECHNOCLOT PT Plus is a standardised Ca-thromboplastin reagent obtained from rabbit brain which is characterized by sensitivity to coagulation factors II, V, VII and X. In addition, the reagent contains a heparin neutralizing agent.

<table>
<thead>
<tr>
<th>ISI</th>
<th>&lt;1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolve in:</td>
<td>solvent provided in the kit</td>
</tr>
<tr>
<td>Normal time:</td>
<td>approximate values: 10 – 14 seconds</td>
</tr>
<tr>
<td>Normal range in % of normal:</td>
<td>70 to &gt; 100%</td>
</tr>
</tbody>
</table>
Technoclot® PT Owren

TECHNOCLOT® PT Owren is a thromboplastin reagent modified according to Owren. The reagent is enriched with bovine plasma from which the prothrombin complex factors II, VII and X have been removed by adsorption. The bovine plasma remains a source of fibrinogen and factor V, which therefore cannot be detected by the test.

Dissolve in: aqua dest.
PT – Prothrombin time

Technoclot® PT Owren

| Factor II | Technoclot® PT Owren | R square 0.9980 |
| Factor VII | Technoclot® PT Owren | R square 0.9964 |
| Factor X | Technoclot® PT Owren | R square 0.9999 |

<table>
<thead>
<tr>
<th>Thrombotest® vs. TECHNOCLOT® PT Owren</th>
<th>Normotest® vs. TECHNOCLOT® PT Owren</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r</td>
<td>Pearson r</td>
</tr>
<tr>
<td></td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>0.993</td>
</tr>
<tr>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Technoplastin® HIS

TECHNOPLASTIN® HIS (HIS = Heparin InSensitive) is a standardized Ca-thromboplastin reagent obtained from rabbit brain which is characterized by sensitivity to coagulation factors II, V, VII and X.

In addition, the reagent contains a heparin neutralizing agent.

Dissolve in:    aqua dest.
Normal time:    approximate value, 13 to 17 seconds
Normal range in % of normal: 70 to >100%
Ceveron alpha PT and aPTT are research assays to determine:

- If warfarin or heparin are not present in sample, then:
  - Prolonged PT and normal aPTT = deficiency of FVII
  - Normal PT and prolonged aPTT = deficiencies in any of the intrinsic pathway factors (FVIII, FIX, FXI, or FXII)
  - Prolongation of both PT and aPTT = deficiencies of factors common to both pathways (FX, FV, FII, or fibrinogen)

- PT, via the INR, is used to measure oral anticoagulant (warfarin)
- aPTT is used to measure heparin anticoagulant
- aPTT is affected by inhibitors such as Lupus Anticoagulant
Coagulation – Global Tests

- **aPTT**  activated partial thromboplastin time
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- **Fibrinogen**

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**Fibrinogen** is converted into fibrin clot by thrombin. Fibrinogen levels <1 g/L contributes to coagulation dysfunction.
Technoclone standard test definitions for Fibrinogen

For each package size two protocols are defined:

- Fibrinogen
- Fibrinogen Curve

What is the reason for having a separate protocol for curve measurement?

- Normal fibrinogen values are ~ 3 g/L.
- To extend the calibration curve to 6 g/L, sample dilution in curve protocol is different from that in sample measurement protocol.
Fibrinogen on Ceveron

Fibrinogen curve

The Ceveron alpha system is for Research Use Only in the US and Canada.
Fibrinogen on Ceveron

Fibrinogen

In sample measurement protocol:

- the test for calibration is defined
- dilution ratio is defined and automatically considered for result calculation
Fibrinogen calibration

To start calibration load the reagents
Fibrinogen on Ceveron

Fibrinogen calibration

• Open Calibration menu to add calibration.
• Select **Fibrinogen Curve** 2mL or 5mL protocol depending on the loaded reagent.
Fibrinogen on Ceveron

Fibrinogen calibration

Information on loaded reagent lot is displayed. Confirm with OK. Calibration screen opens.....

At this point you can make changes in the calibration settings. It is recommended to use the predefined settings. Start calibration.....
Fibrinogen on Ceveron

Fibrinogen calibration

After calibration is finished, the curve is displayed.

You can validate the results after measurement of controls.

Press OK and calibration data will be saved.

Don’t leave calibration menu with cancel – calibration data will be deleted.
Fibrinogen Sample Measurement

• Load all reagents necessary for Fibrinogen sample measurement in menu ♦Loading♦.

• Go to ♦Work List♦ and enter sample IDs and tests: Fibrinogen 2mL or Fibrinogen 5mL.

• Start measurement.

• After measurement has been competed validate the results.

• Results will be transferred in ♦Archive♦ and control results in ♦Quality Control♦.