Putting the patient first: monitoring anticoagulant therapy with the INR test

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**Coagulation Reference Laboratory (CRL) Leiden**

verifies PT/INR performance of POC test strip lots and clinical lab tests for several IVD-manufacturers.

CRL also assigns reagent/instrument specific values to frozen plasma’s used by External Quality Assessment scheme organizers.
I. Introduction on PT/INR testing

II. Clinical Case - real world experience

III. Need for a global Reference Measurement System for PT/INR tests

IV. Conclusions
Prothrombin Time or PT:

i. invented by Armand Quick in 1935

ii. misnomer!

iii. scientific name: tissue factor-induced coagulation time

PT is the time in seconds for the fibrin clot to form.

Measures function of the tissue factor (extrinsic) and common pathways.
Vitamin K-Dependent Clotting Factors

Recycling of Vitamin K is blocked by Vitamin K Antagonists
Background on PT/INR testing

- Vitamin K-antagonists for oral anticoagulation therapy;
- Mechanism: ↓ plasma levels of functional coagulation factors II, VII, IX and X;
- Monitoring is mandatory;
- “Prothrombin Time” (PT): Tissue Factor-induced coagulation time;
- **PT test:** citrated plasma sample + Tissue Factor (Thromboplastin) + Ca\(^{2+}\);
- PT is prolonged in case of reduced levels of factors II, VII, IX and X;
- Standardization of preanalytical conditions;
- Many different thromboplastins and instruments to measure PT;
- INR: normalization of PT results by calibration of thromboplastin/instrument.
To achieve HARMONIZATION of the PT, it is possible to express PT results on a common scale, i.e. the International Normalized Ratio (INR), if the ISI of the thromboplastin is known.

\[
\text{INR} = \left( \frac{\text{Patient’s PT in Seconds}}{\text{Mean Normal PT in Seconds}} \right)^{\text{ISI}}
\]

INR = International Normalized Ratio
ISI = International Sensitivity Index
INR monitoring is required for the patient who are on vitamin K antagonists.

The dose of VKA is adapted based on INR results so that it remains in the therapeutic range to prevent thrombosis from subtherapeutic INR or hemorrhagic complications from supratherapeutic INR.
II. Clinical Case Scenario – INR discrepancies

- National Network of Thrombosis Services who treat and monitor patients on VKA.
- Referral of patients to hospitals in case of medical procedures, e.g. cardioversion.
- Hospital X uses an INR target range of 2,5 – 3,5 for prophylaxis during and after **CARDIOVERSION in case of ATRIAL FIBRILLATION**.
- INR measured by hospital X was **SYSTEMATICALLY LOWER** than INR measured by Trombosis Service Y.
- **Impact?** about 3% of the planned cardioversions had to be postponed as patients had too low hospital INR (<2,5) after at least 4 weeks of follow-up by Thrombosis Service Y.
**Cardioversion** is a medical procedure by which an abnormally fast heart rate (tachycardia) or other cardiac arrhythmia is converted to a normal rhythm using electricity or drugs.
Discrepancies in INR between Hospital X and Thrombosis Service Y are caused
• by difference in sensitivity of the used PT reagent/instrument systems?
• by instable setting of the Vitamin K anticoagulated patients?

Origin of the discrepancies? Possible corrective measures?

What is the magnitude of INR differences generated by different commercial blood collection tubes and/or different PT-systems in VKA patients?
Study design I for assessing INR differences

- Collect fresh citrated plasma from VKA-patients in **BD Vacutainer Tubes**.
- Aliquot each plasma into three parts.
- Transport identical sets of specimens to **three routine medical labs with different PT/INR systems**.
- Analyze INR test results from the three labs.
- Perform a local calibration* with value-assigned INR plasma sets, for enabling direct comparability of INR test results among all 3 labs.

*Local calibration of PT systems, which is based on the utilization of a set of assigned INR plasma’s (either deep-frozen or freeze-dried), depends on the commutability of these plasma’s.
<table>
<thead>
<tr>
<th>Laboratories</th>
<th>Tromboplastin reagent</th>
<th>Analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab 1</td>
<td>Hepato Quick</td>
<td>STA-R Evolution</td>
</tr>
<tr>
<td>Lab 2</td>
<td>Innovin</td>
<td>Sysmex CA-1500</td>
</tr>
<tr>
<td>Lab 3</td>
<td>Recombioplastin 2G</td>
<td>ACL-9000</td>
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All VKA patients (n = 165)

<table>
<thead>
<tr>
<th>Tromboplastin reagent</th>
<th>Analyzer</th>
<th>Average INR (reported)</th>
<th>Average INR (after local calibration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepato Quick</td>
<td>STA-R Evolution</td>
<td>3,24</td>
<td>3,24</td>
</tr>
<tr>
<td>Innovin</td>
<td>Sysmex CA-1500</td>
<td>2,82 (-13,0 %)</td>
<td>2,94 (-9,3 %)</td>
</tr>
<tr>
<td>Recombioplastin 2G</td>
<td>ACL-9000</td>
<td>2,78 (-14,2 %)</td>
<td>2,96 (-8,6 %)</td>
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</table>

Maximum bias for all patients amounted to 0.46 INR (14%)
### Stable VKA patients (n = 84)

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<tr>
<td>Hepato Quick</td>
<td>STA-R Evolution</td>
<td>3.33</td>
<td>3.33</td>
</tr>
<tr>
<td>Innovin</td>
<td>Sysmex CA-1500</td>
<td>2.87 (-13.8%)</td>
<td>2.98 (-10.5%)</td>
</tr>
<tr>
<td>Recombiplastin 2G</td>
<td>ACL-9000</td>
<td>2.82 (-15.3%)</td>
<td>3.01 (-9.6%)</td>
</tr>
</tbody>
</table>

Maximum bias for stable VKA patients amounted to 0.51 INR (15%)
Reported INR in VKA patients

All VKA patients (n = 165)

Stable VKA patients (n = 84)

Log INR Difference plot: Commercial PT system vs. WHO-based INR

Allowable bias, WHO-based: ± 10% in the therapeutic range.

Study design II for assessing INR differences

- Collect fresh citrated plasma from VKA-patients in Sarstedt Monovette Tubes.
- Aliquot each plasma into three parts.
- Transport identical sets of specimens to three routine medical labs with different PT/INR systems.
- Analyze INR test results from the three labs.
- Perform a local calibration* with value-assigned INR plasma sets, for enabling direct comparability of INR test results among labs.

*Local calibration of PT systems, which is based on the utilization of a set of assigned INR plasma’s (either deep-frozen or freeze-dried), depends on the commutability of these plasma’s.
### Workplan II: 153 patients

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<th>Tromboplastine</th>
<th>Analyzer</th>
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<td>2.91</td>
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<td>3.07</td>
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<tr>
<td>Recombiplastin 2G</td>
<td>ACL-9000</td>
<td>2.73 (- 4.9 %)</td>
<td>2.91 (+ 0.3%)</td>
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Part II: 153 VKA-patients

\[
\text{INR} = (\frac{\text{PT}}{\text{MNPT}})^{\text{ISI}}
\]

**Innovin:** \( \text{INR} = \exp(-1.66 + 0.79 \times \ln \text{PT}) \)

*Model of Tomenson*

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Monitoring anticoagulant therapy/JCTLM 2019

Analysis of Mg\(^{2+}\) in citrated solutions from blood tubes

<table>
<thead>
<tr>
<th>Mg(^{2+}) concentration in citrate solution</th>
<th>BD Vacutainer</th>
<th>Sarstedt Monovette</th>
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<tr>
<td>Colorimetric method</td>
<td>2.76 - 2.53 mmol/L</td>
<td>0.27 – 0.25 mmol/L</td>
</tr>
<tr>
<td>Atomic Absorption Spectrometry</td>
<td>2.99 – 2.67 mmol/L</td>
<td>0.30 – 0.26 mmol/L</td>
</tr>
</tbody>
</table>
Magnesium ions can shorten the prothrombin time, both in the presence and absence of citrate. Butenas et al. showed that magnesium ion can enhance the amidolytic activity of factor VIIa. A similar effect of magnesium ion on factor VIIa activity during the PT assay may explain the shortening of the PT.

Effect of magnesium chloride in the presence or absence of citric acid/sodium citrate on the prothrombin time of dialysed coumarin plasma. By adding 0.09 mL of citric acid/sodium citrate (0.105 mol/L) to 0.5 mL of dialysed coumarin plasma, a final citrate concentration of 0.016 mol/L was achieved. The concentration of magnesium chloride given along the horizontal axis is the final concentration in the plasma.

Thromb Haemost 2001; 85: 647–50
Ensures that the measurements will be equivalent to those made using different reagents/instruments from different suppliers.
Urgent Need of a PT/INR Reference Measurement System

<table>
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<th>Metrological traceability chain</th>
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<tr>
<td><strong>Primary Reference Standards</strong></td>
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<tr>
<td>Well defined WHO Thromboplastin: rTF/16?</td>
</tr>
</tbody>
</table>

and standardized preanalytics

Commutable and value-assigned Secondary Reference Materials

RBT/16?

Metrological traceability of PT/INR test results - worldwide
• **INR discrepancies** that impact patient management could largely be explained by the influence of blood collection tubes.

  • Systematic differences in INR between Hepato Quick versus Innovin/Recombiplastin 2G were mainly caused by Mg^{2+}-contamination from rubber stoppers of citrated blood tubes.
  • The systematic differences were undiminished present in stable VKA-treated patients.
  • The average INR difference between Hepato Quick and Innovin/Recombiplastin 2G was small if citrated solutions in blood tubes had < 1 mmol/L Mg^{2+}.

• Manufacturing process of the blood collection systems had to be changed so that contamination with magnesium is reduced/maintained to a level which does not interfere with prothrombin time testing.
• Systematic differences in INR between Hepato Quick and Innovin/Recombiplastin 2G were partially explained by a deviation of the ISI model.

• The systematic INR differences could be further reduced by using a modified formula (Tomenson’s model) for the calculation of INR.
Acknowledgements

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