Leids Universitair
Medisch Centrum

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

C.M. Cobbaert, EuSpLM, PhD, Prof.

Department of Clinical Chemistry and Laboratory Medicine
Coagulation Reference Laboratory
Leiden University Medical Centre, Leiden, the Netherlands

Disclosures

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.

Content

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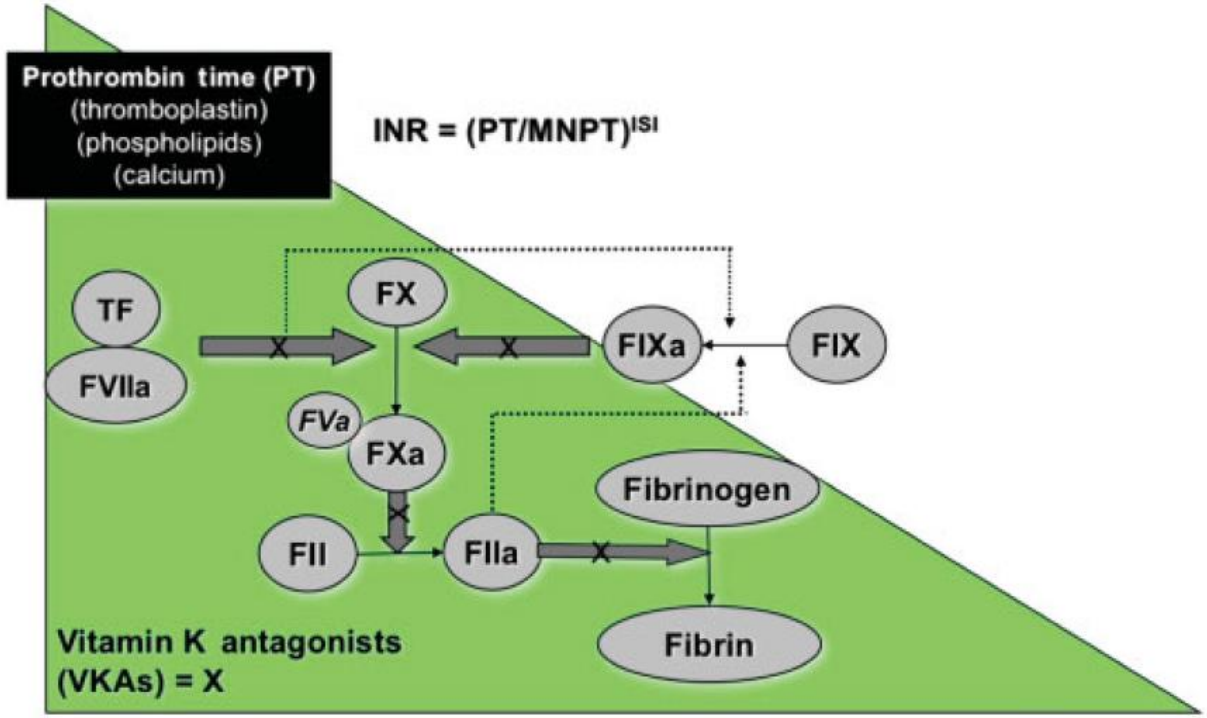
II. Clinical Case - real world experience

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

IV. Conclusions

I. Introduction: PT/ INR Testing & VKAs

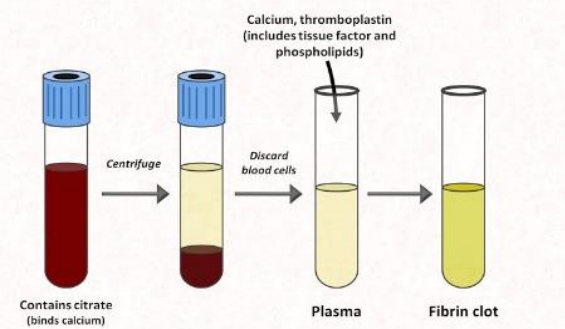


Prothrombin Time or PT:

i. invented by Armand Quick in 1935

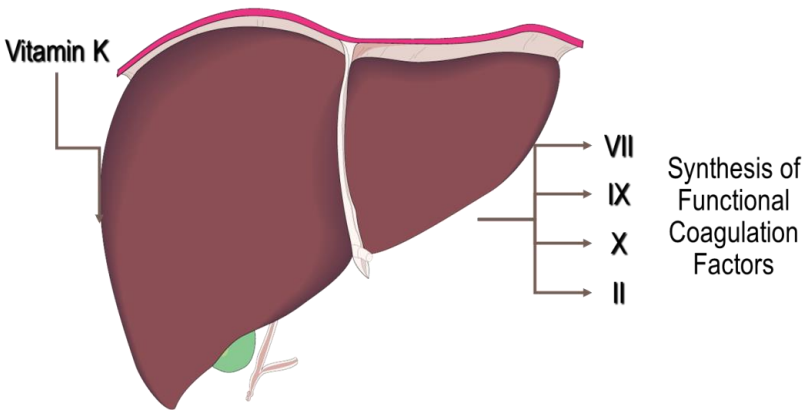
ii. misnomer!

iii. scientific name: tissue factor-induced coagulation time

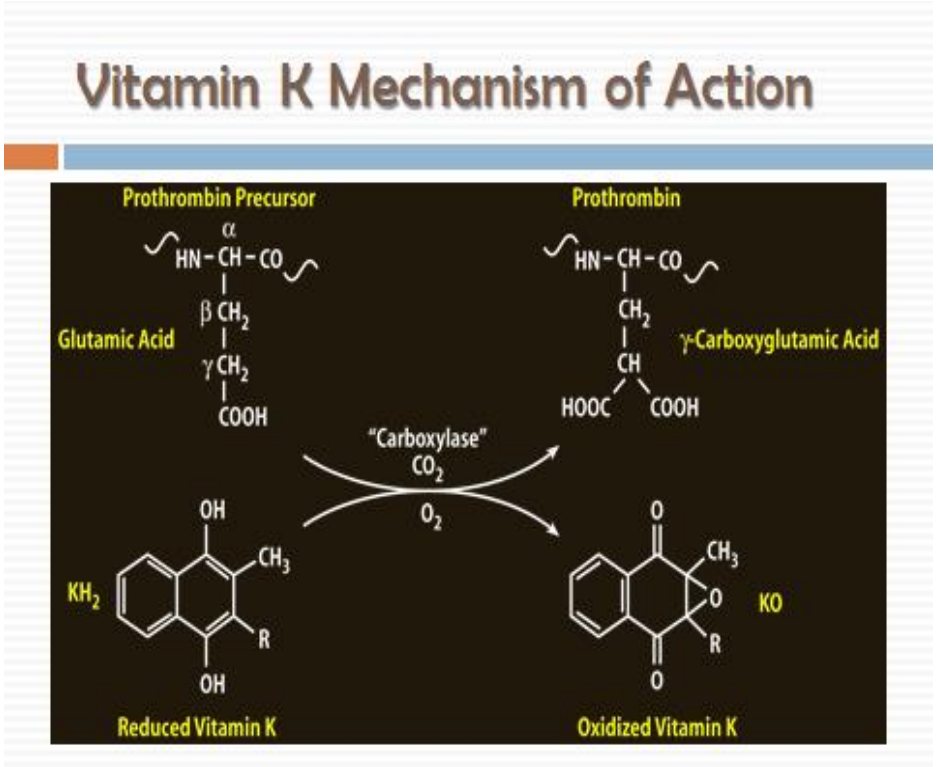


- PT is the time in seconds for the fibrin clot to form.
- Measures function of the tissue factor (extrinsic) and common pathways.

Vitamin K-Dependent Clotting Factors



Synthesis of Functional Coagulation Factors



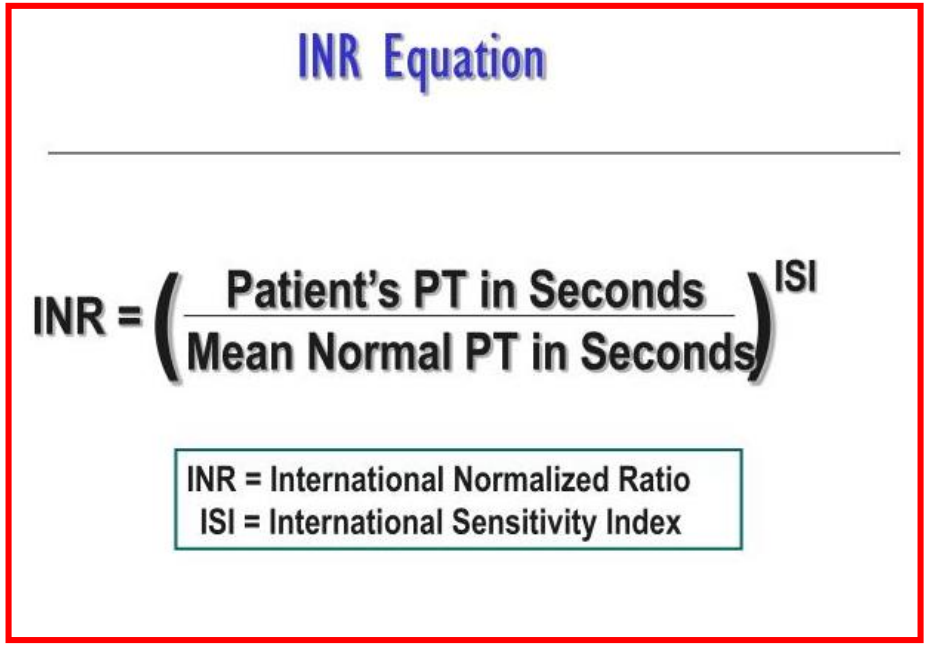
Recycling of Vitamin K is blocked by Vitamin K Antagonists

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.

INR: International Normalized Ratio since 1985

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.



Optimal oral anticoagulant therapy

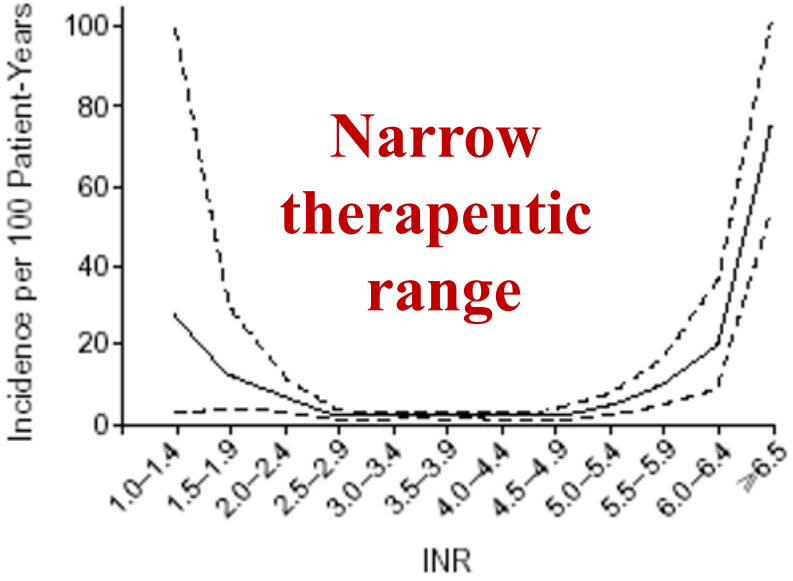


Figure 3. INR-Specific Incidence of All Adverse Events (All Episodes of Thromboembolism, All Major Bleeding Episodes, and Unclassified Stroke).
The dotted lines indicate the 95 percent confidence interval.

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.**

Cannegieter SC, Rosendaal FR, Wintzen AR, van der Meer FJ, Vandenbroucke JP, Briet E. Optimal oral anticoagulant therapy in patients with mechanical heart valves. NEJM 1995; 333:11-7

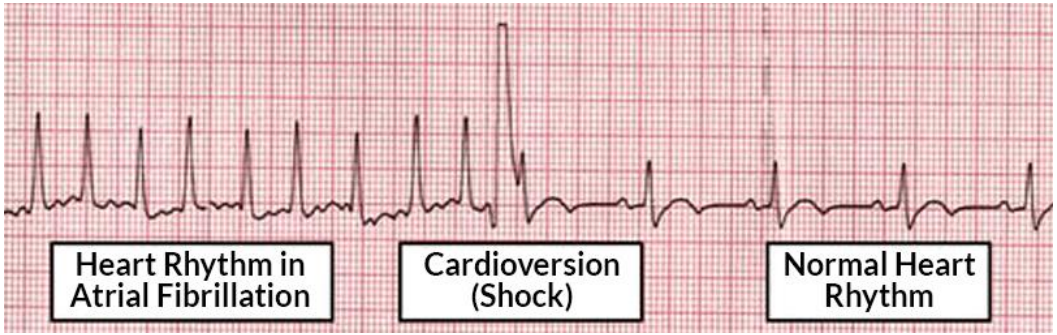
II. Clinical Case Scenario – INR discrepancies

- 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.

Van den Besselaar A, Am J Clin Pathol., 2012

Clinical Case Scenario in Leiden

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.



Hypotheses & Research Questions

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.

PT systems

Laboratories	Tromboplastin reagent	Analyzer
Lab 1	Hepato Quick	STA-R Evolution
Lab 2	Innovin	Sysmex CA-1500
Lab 3	Recombiplastin 2G	ACL-9000

Van den Besselaar A, Am J Clin Pathol., 2012

All VKA patients (n = 165)

Tromboplastin reagent	Analyzer	Average INR (reported)	Average INR (after local calibration)
Hepato Quick	STA-R Evolution	3,24	3,24
Innovin	Sysmex CA-1500	2,82 (- 13,0 %)	2,94 (- 9,3 %)
Recombiplastin 2G	ACL-9000	2,78 (-14,2 %)	2,96 (- 8,6 %)

Maximum bias for all patients amounted to 0.46 INR (14%)

Van den Besselaar A, Am J Clin Pathol., 2012

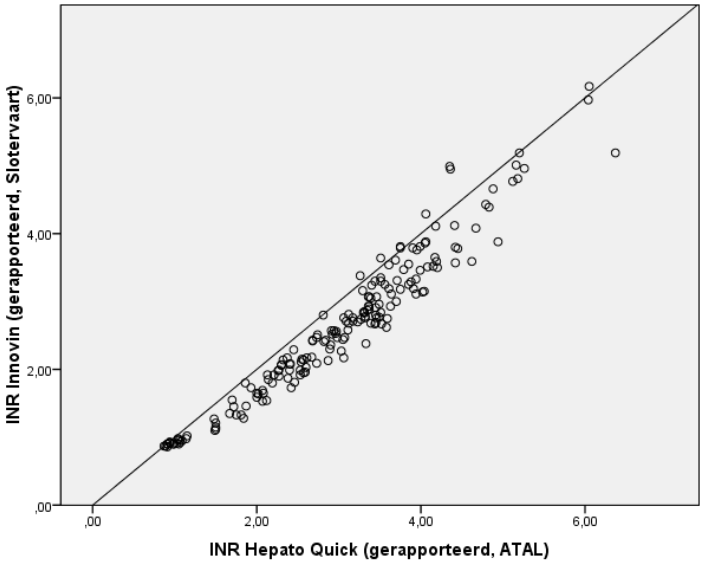
Stable VKA patients (n = 84)

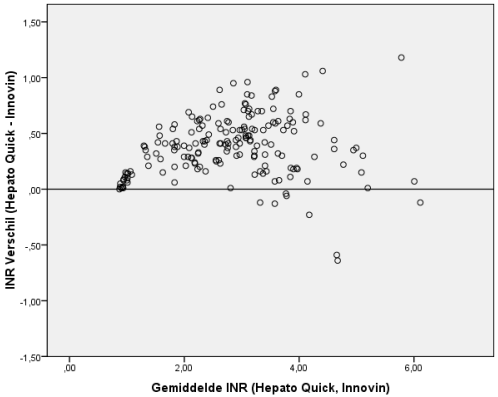
Tromboplastin reagent	Analyzer	Average INR (reported)	Average INR (after local calibration)
Hepato Quick	STA-R Evolution	3,33	3,33
Innovin	Sysmex CA-1500	2,87 (- 13,8 %)	2,98 (- 10,5 %)
Recombiplastin 2G	ACL-9000	2,82 (-15,3 %)	3,01 (- 9,6 %)

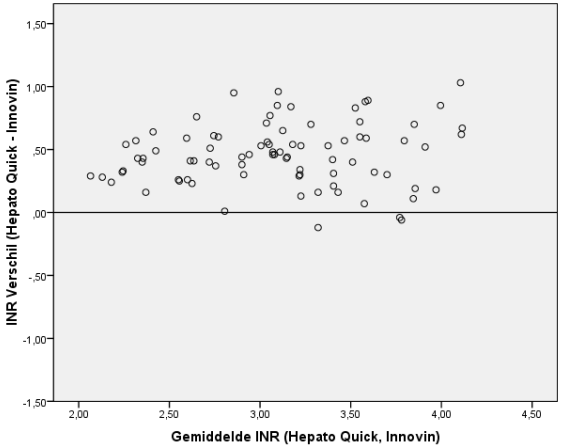
Maximum bias for stable VKA patients amounted to 0.51 INR (15%)

Van den Besselaar A, Am J Clin Pathol., 2012

Reported INR in VKA patients





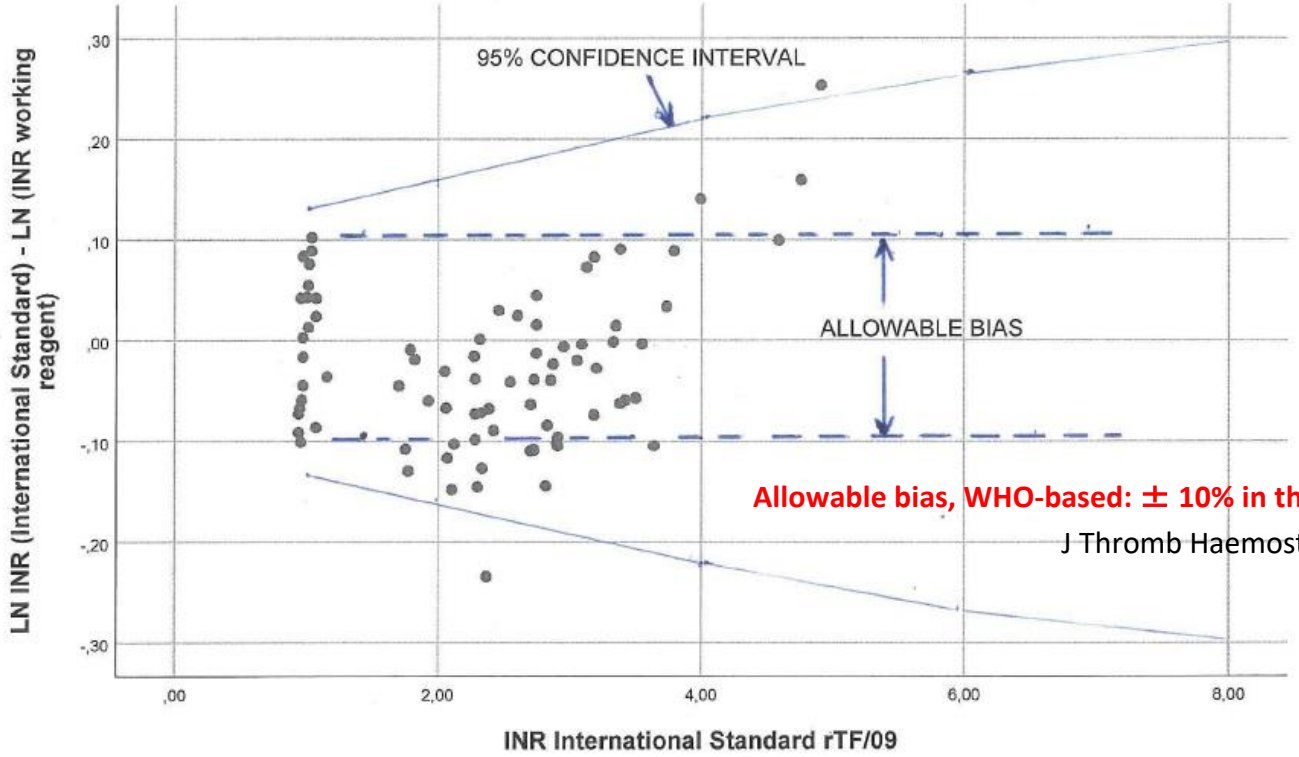


Stable VKA patients (n = 84)

All VKA patients (n = 165)

Van den Besselaar A, Am J Clin Pathol., 2012

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



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

J Thromb Haemostas 2004; 2: 1946-53.

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.

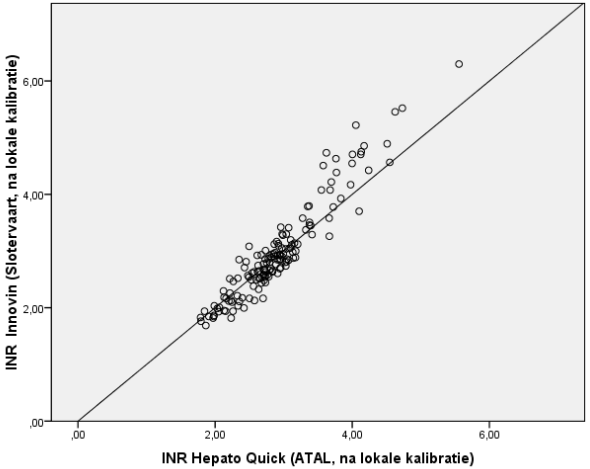
Van den Besselaar A, Am J Clin Pathol., 2012

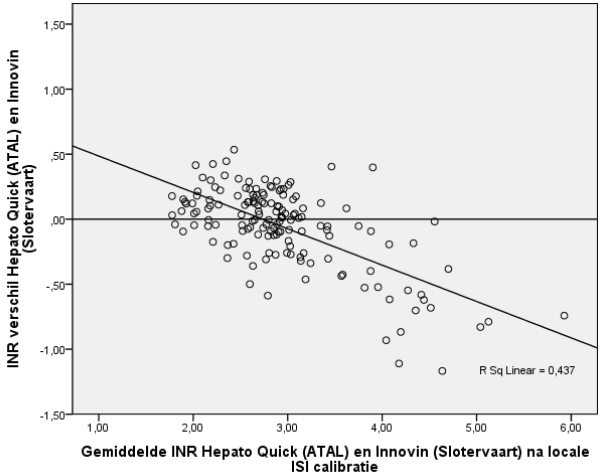
Workplan II: 153 patients

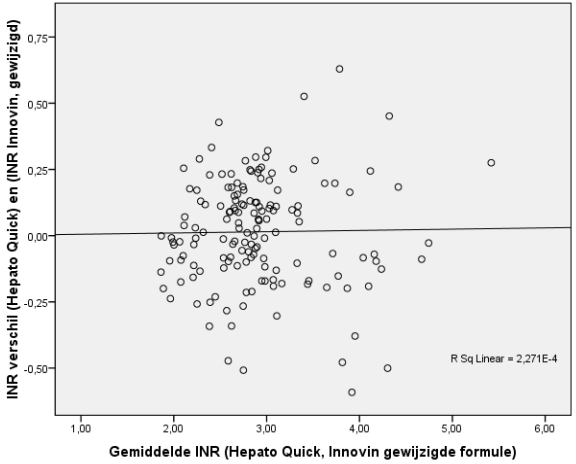
Tromboplastine	Analyzer	Average INR (reported)	Average INR (after local calibration)
Hepato Quick	STA-R Evolution	2,87	2,91
Hepato Quick	Sysmex CA-1500	2,96	3,07
Innovin	Sysmex CA-1500	2,86 (- 0,3 %)	2,97 (+ 2,1 %)
Recombiplastin 2G	ACL-9000	2,73 (- 4,9 %)	2,91 (+ 0.3%)

Van den Besselaar A, Am J Clin Pathol., 2012

Part II: 153 VKA-patients







$$INR = (PT/MNPT)^{ISI}$$

Innovin: $INR = Exp(-1{,}66 + 0{,}79 \times \ln PT)$

Model of Tomenson

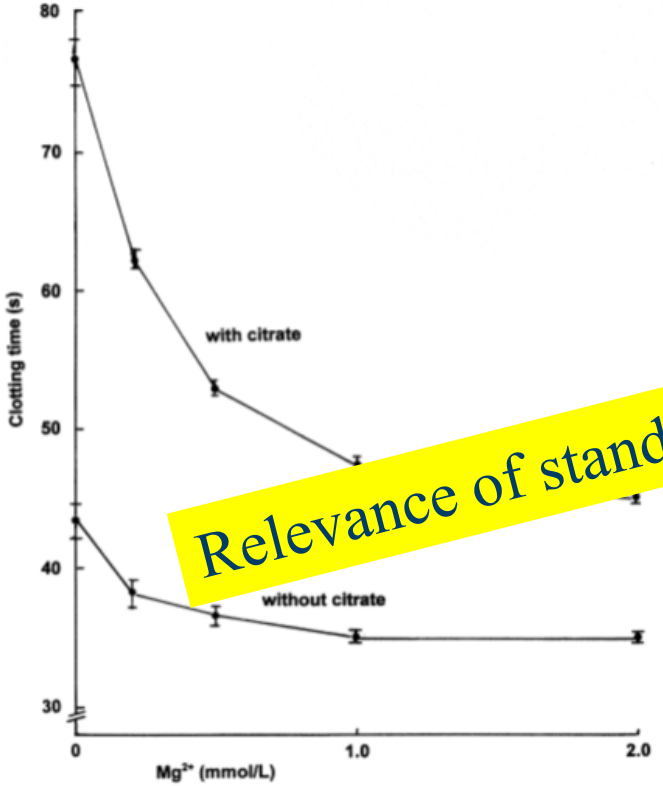
Van den Besselaar A, Am J Clin Pathol., 2012

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

Mg^{2+} concentration in citrate solution	**BD Vacutainer**	**Sarstedt Monovette**
Colorimetric method	2,76 -2,53 mmol/L	0,27 – 0,25 mmol/L
Atomic Absorption Spectrometry	2,99 – 2,67 mmol/L	0,30 – 0,26 mmol/L

Van den Besselaar A, Am J Clin Pathol., 2012

Effect of $MgCl_2$ on PT time



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.

Relevance of standardized preanalytics

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

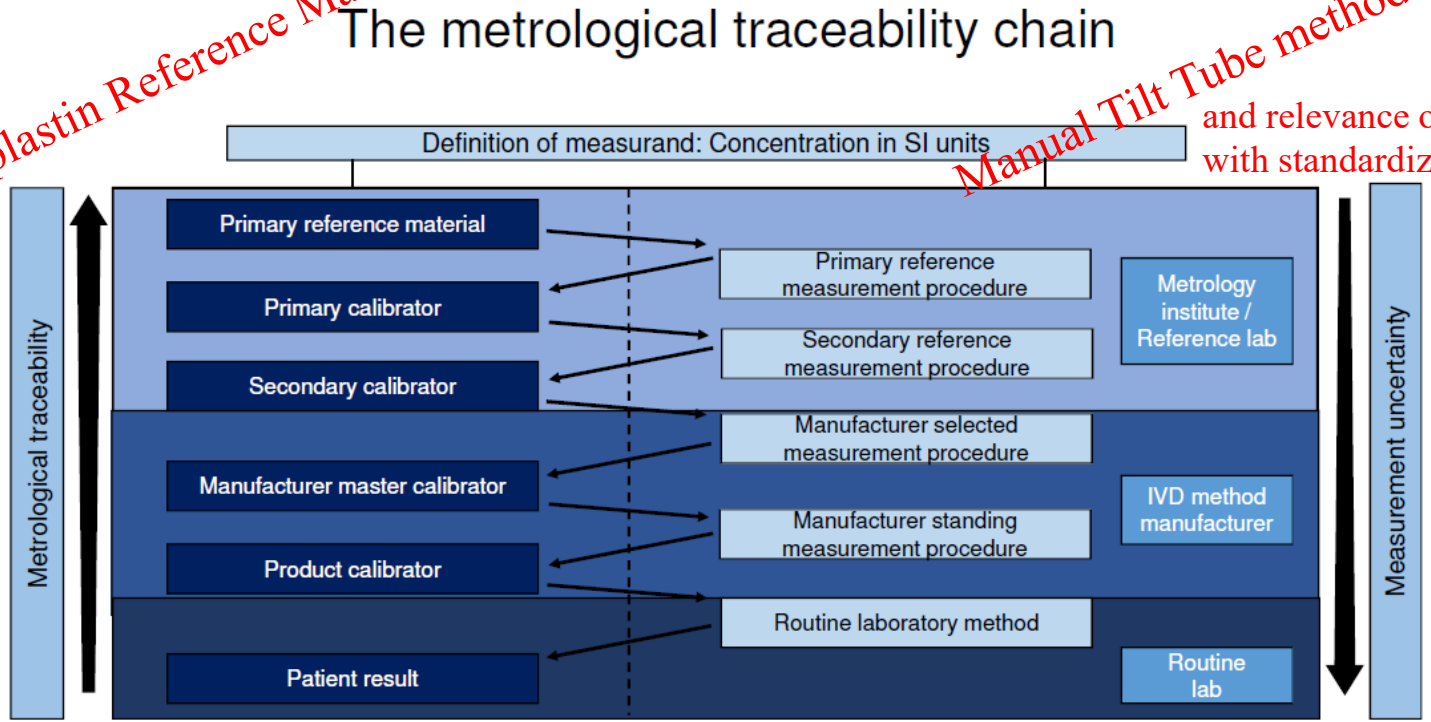
III. Metrological Traceability of PT/INR results

for PT-INR

Thromboplastin Reference Materials

Manual Tilt Tube method

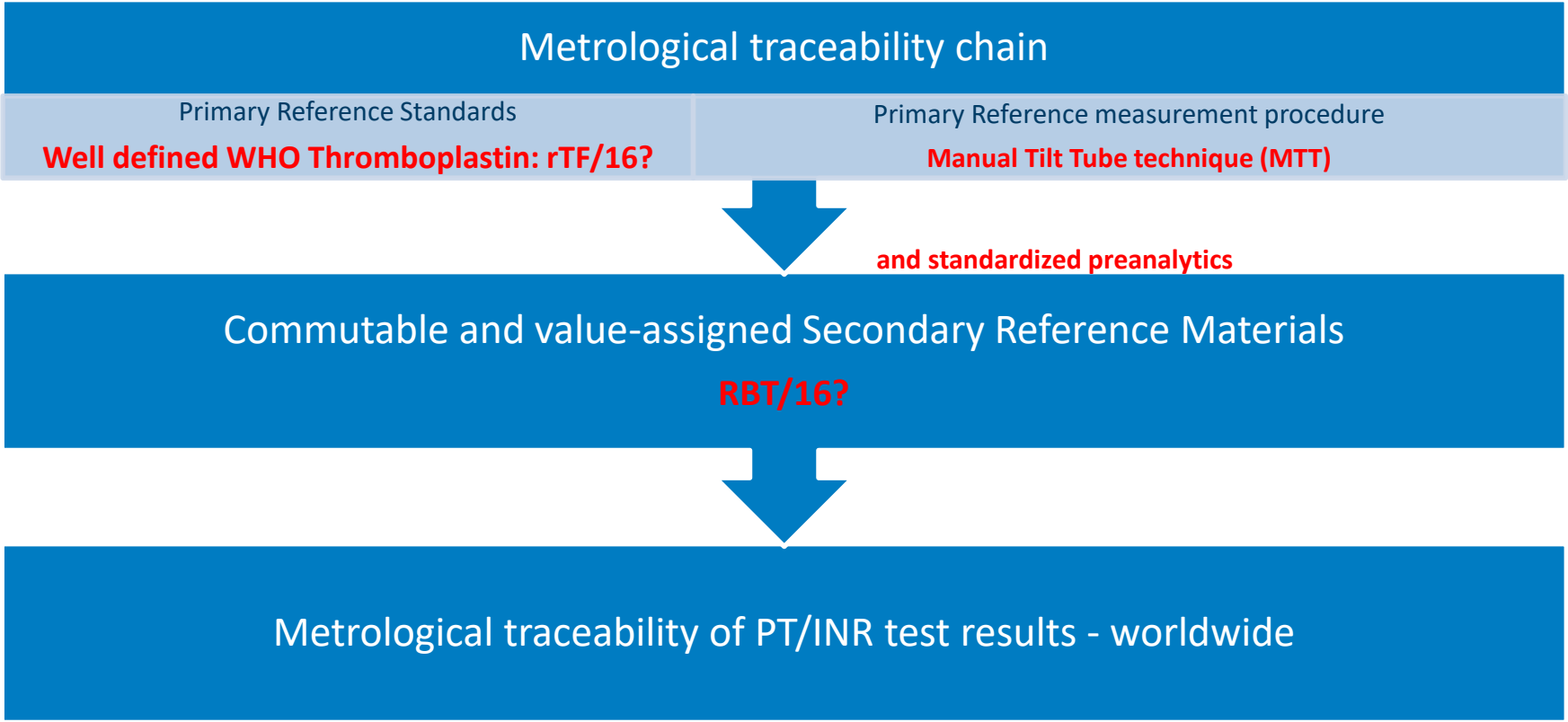
and relevance of citrated samples with standardized preanalytics!!!



Ensures that the measurements will be equivalent to those made using different reagents/instruments from different suppliers.

Adapted from EN ISO 17511 2003

Urgent Need of a PT/INR Reference Measurement System



IV. Conclusions

- **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.

Conclusions (continued)

- 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

Clin Chem Lab Med. 2019 Jun 26;57(7):e169-e172. doi: 10.1515/cclm-2018-1194.

Requirement of a reference measurement system for the tissue factor-induced coagulation time and the international normalized ratio.

van den Besselaar AMHP[1], van Rijn CJJ[2], Hubbard AR[3], Kitchen S[4], Tripodi A[5], Cobbaert CM[2].

Author information

1 Coagulation Reference Laboratory, Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, P.O. Box 9600, Leiden, 2300 RC, The Netherlands.

2 Coagulation Reference Laboratory, Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands.

3 National Institute for Biological Standards and Control, Potters Bar, UK.

4 Sheffield Thrombosis and Haemostasis Centre, Royal Hallamshire Hospital, Sheffield, UK.

5 IRCCS Cà Granda Maggiore Hospital Foundation, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milano, Italy.

Dr. T. van den Besselaar, consulent CRL

All **ambassadors** of the CRL team, especially Claudia van Rijn

Email: c.m.cobbaert@lumc.nl