The Free Thyroxine Reference Measurement System

Session 1: "Impact of Reference Measurement Systems on Clinical Evidence"

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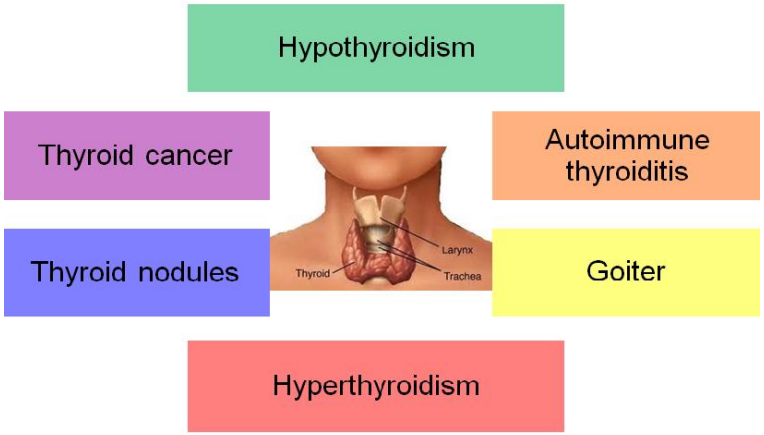
Background

Thyroid disease spectrum

Subclinical to overt



High prevalence#

Overt (0.8%); subclinical (5%)

Silent epidemic

High morbidity; chronic course

Diagnosis

Clinical diagnosis by symptoms

Laboratory data

➔ Worldwide, 60 million of FT4 tests yearly performed

#Hollowell et al. J Clin Endocrinol Metab 2002;87:489-99.

Background

FT4 testing

High burden on the healthcare system

Diagnosed patients need follow-up, and/or move from one hospital/clinician to another

Repeat measurements

Patients expect that measurement results are comparable over time, location and laboratory assay

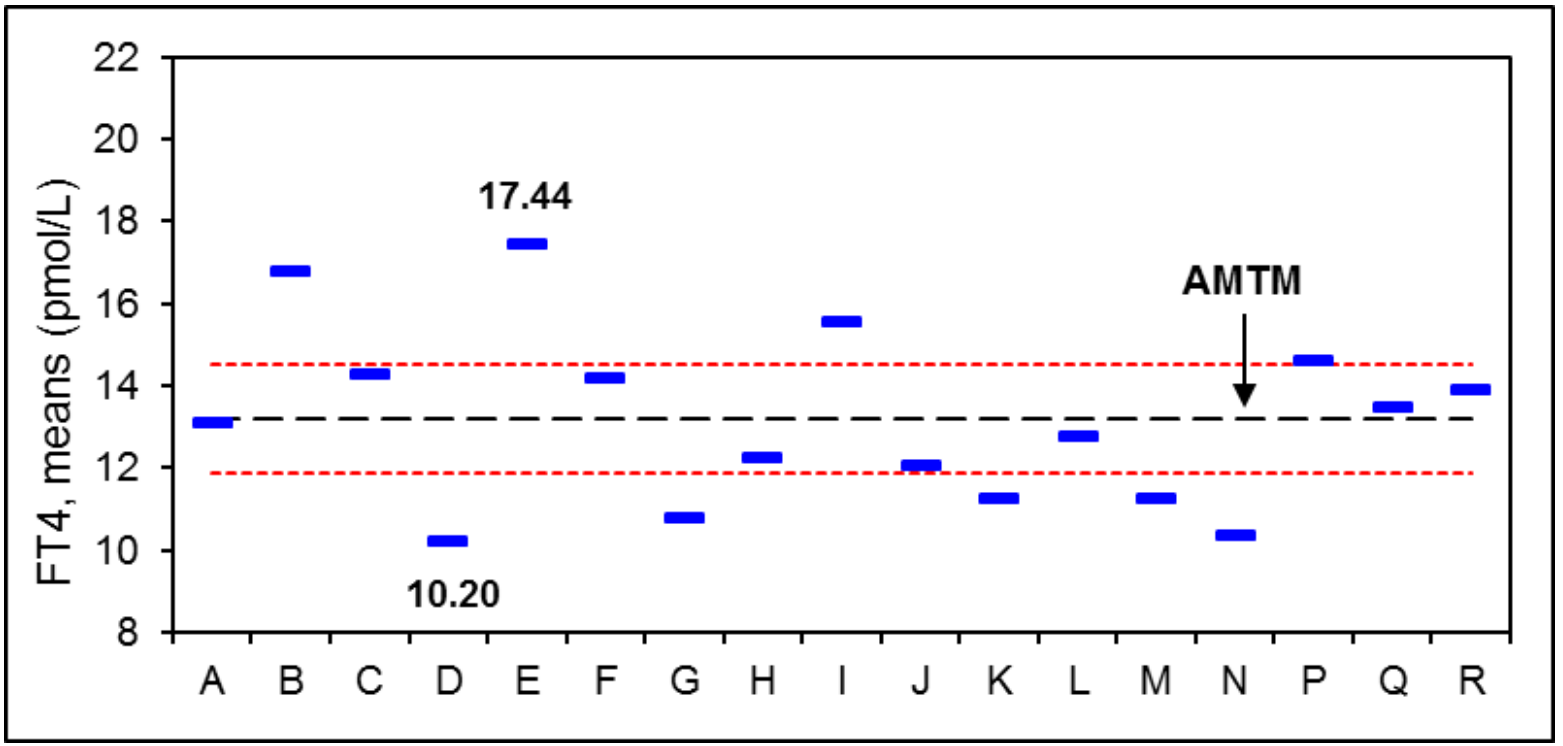
Commercial immunoassays from different IVD-manufacturers; differently designed

➔ Standardization

Where does FT4 testing stand in terms of standardization?

FT4 Standardization Status

Thienpont et al. Clin Chem 2010;56:912-20.



Considerable variation of FT4 results generated by immunoassays: means range from 10 to 17 pmol/L

➔ Use of assay-specific reference intervals/decision limits (+ in publications)

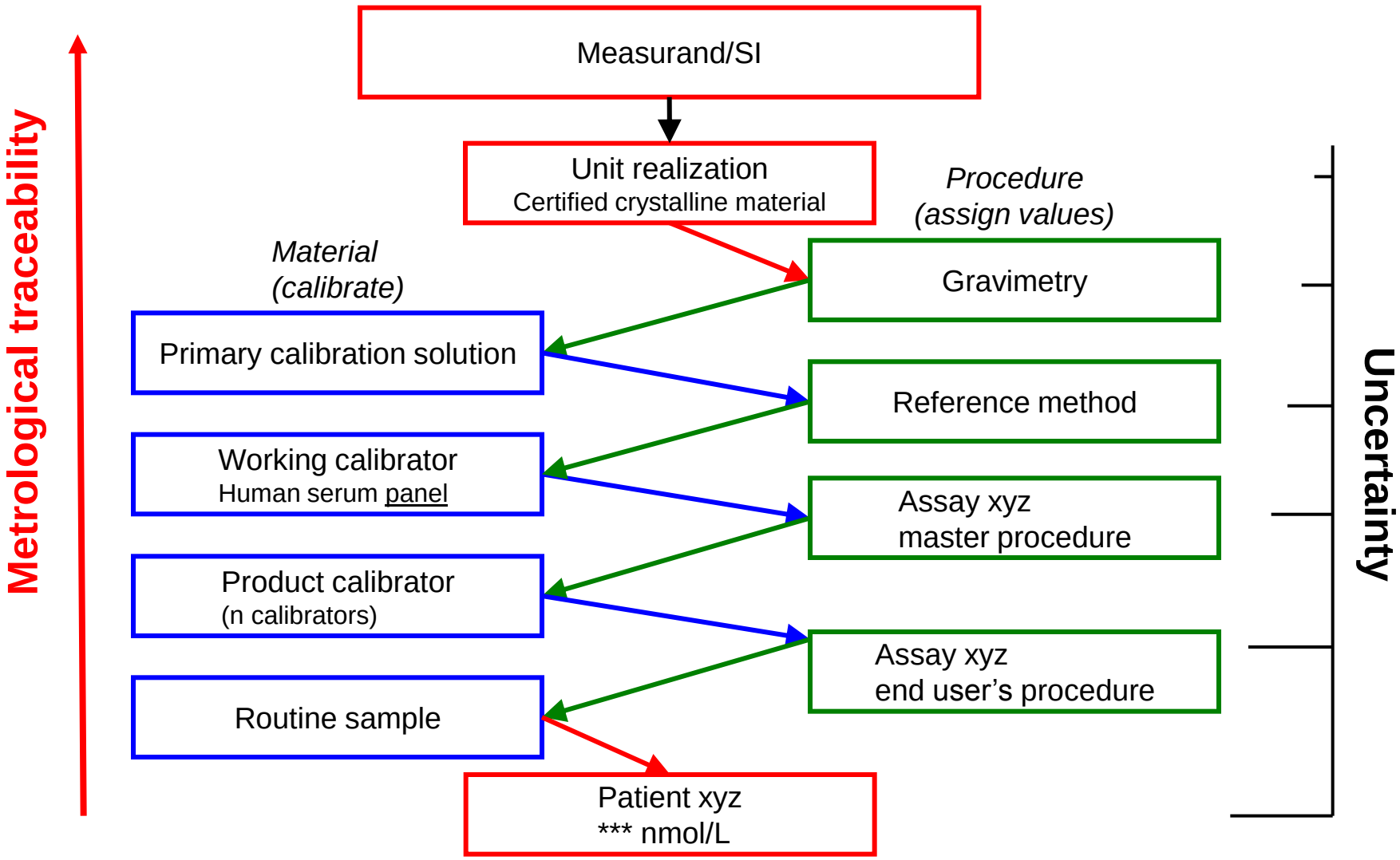
Impact on Clinical Evidence

Laboratory data from non-standardized assays fail to address clinical and public health needs

- **Definition of generally accepted clinical decision limits**
- **Development of evidence-based clinical practice guidelines for application of consistent standards of medical care**
- **Research translation into patient care and disease prevention activities (comparison of laboratory data across studies)**
- **Introduction of electronic patient records**

➔ Standardization urgently needed

Reference Measurement System*



*ISO 17511:2003

IFCC – Mandate

2005: Working Group for Standardization of Thyroid Function Tests (WG-STFT) – Chair: L. Thienpont

2012: Committee (C-STFT) (same chair)

Terms of reference

"Develop reference measurement systems for thyroid hormones, i.e., TT4 & TT3, FT4 & FT3, TSH"

➔ FT4 Reference Measurement System

Development of the FT4 RMS

Measurand

Quantity intended to be measured#

#Vocabulaire International de Métrologie – Concepts Fondamentaux et Généraux et Termes Associés (VIM) (3rd Ed.).

Measurand, full description

System – component (= analyte); kind-of-quantity

FT4 measurand, not really defined

Thienpont et al. Measurement of free thyroxine in laboratory medicine – Proposal of measurand definition. IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT). Clin Chem Lab Med 2007;45:563–4.

FT4 Measurand

Component

Thyroxine that is not bound to proteins
Name: ‘‘Thyroxine(free)’’; abbreviation: FT4

Kind-of quantity; unit

Amount-of-substance concentration; pmol/L

System

Plasma or serum under physiological conditions (pH 7.4, temperature 37°C).
Note: no demand to collect specimens under anaerobic conditions

IUPAC/IFCC format:
‘‘Plasma/Serum – Thyroxine(free); amount-of-substance concentration’’ (pmol/L)

Development of the FT4 RMS

Thyroxine Primary Calibrator – IRMM-468#

Substance	*Certified mass fraction* [1)] *[%]*	*Uncertainty* [2)] *[%]*
thyroxine	98.6	0.7

1) The certified value is the purity after taking into consideration inorganic residues, water, ethanol and organic impurities detectable by HPLC-UV and HPLC-MS. The certified value is traceable to the International System of Units (SI).

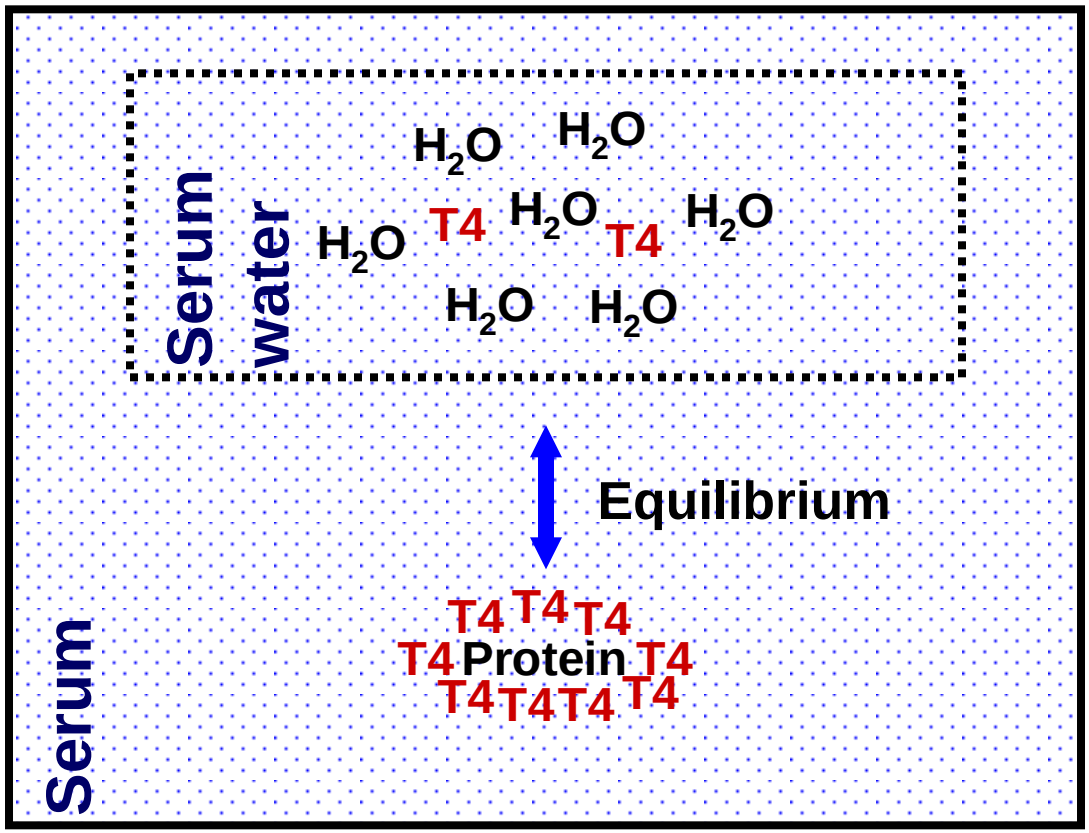
2) The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1]. It is expressed with a coverage factor $k = 2$, corresponding to a level of confidence of about 95%.

#Toussaint B, Klein CL, Wiergowski M. The certification of the mass fraction of thyroxine in a CRM intended for calibration. EUR 21763 EN (2005). ISBN 92-894-9919-2.

Development of the FT4 RMS

Reference measurement procedure

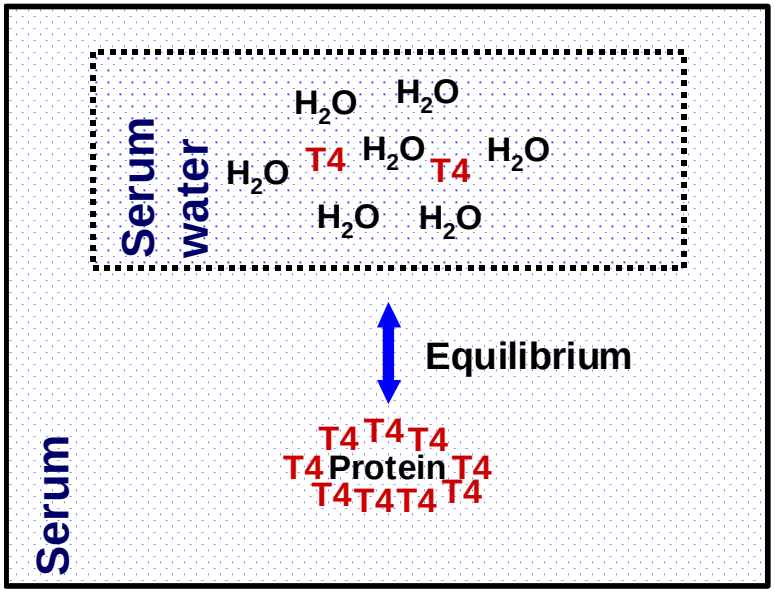
To measure the measurand as defined; should ideally be trueness-based



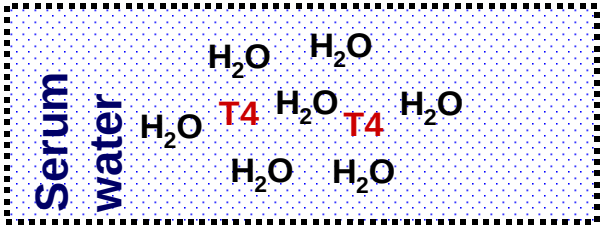
Possible for FT4?

FT4 Reference Measurement Procedure

Direct measurement in the system? NO



Indirect measurement in separated serum water? YES

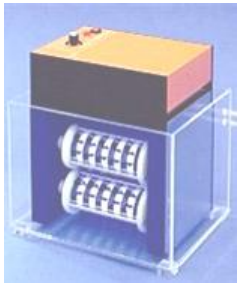


Generation of Serum Water

Technically feasible

Ultrafiltration

Equilibrium dialysis



Potential pitfalls

Membrane type and cut-off (risk for protein leakage, adsorption)

Buffer type, pH, control

Dilution

Temperature, control

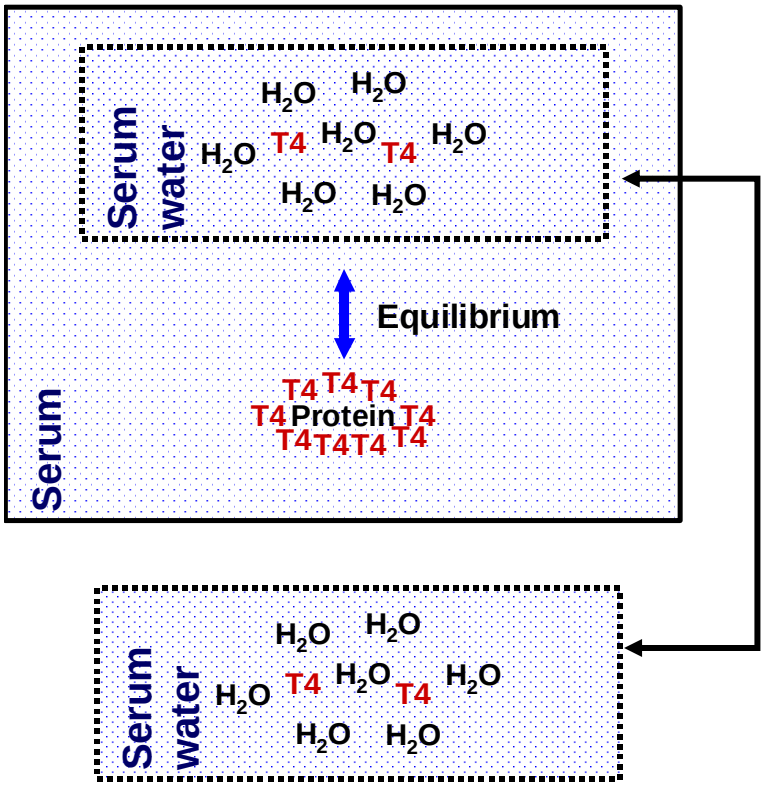
Donnan- and osmotic effects

Time of process (risk for free fatty acid generation, protein degeneration, etc.)

…

Generation of Serum Water

T4 in water separated from the serum sample *vs* FT4 in serum (in equilibrium with protein bound T4)



Identical?

Difficult/impossible to prove!

➔ **ED/UF separation may break the traceability chain. Trueness?**

Reference Measurement Procedure

C-STFT opted for an international conventional reference measurement procedure based on

– Equilibrium dialysis

– Quantification of thyroxine in the dialysate with a trueness-based reference measurement procedure

➔ ED ID-LC/tandem MS

Note

The measurand is *operationally defined* as

“Thyroxine in the dialysate from equilibrium dialysis of serum prepared under defined conditions”

Reference Measurement Procedure

Equilibrium dialysis

Strictly adhere to the defined equilibrium dialysis procedure (cf. CLSI C45-A#)

#Measurement of Free Thyroid Hormones; approved guideline. CLSI document C45-A (ISBN 1-56238-548-8). CLSI, Wayne, PA, 2004.

ID-LC/tandem MS RMP

Variants permissible
Calibration with IRMM-468

Thienpont et al. Proposal of a candidate international conventional reference measurement procedure for free thyroxine in serum. IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT). Clin Chem Lab Med 2007;45:934-6.

Reference Measurement Procedure

Van Uytfanghe K, Stöckl D, Ross HA, Thienpont LM. Use of frozen sera for FT4 standardization: investigation by equilibrium dialysis combined with isotope dilution-mass spectrometry and immunoassay. Clin Chem 2006;52:1817-21.

Van Houcke SK, Van Uytfanghe K, Shimizu E, Tani W, Umemoto M, Thienpont LM. IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT). IFCC international conventional reference procedure for the measurement of free thyroxine in serum. Clin Chem Lab Med 2011;49:1275-81.

Validation of the ED part of the RMP

Performance of ED

Compliance with the predefined requirements

Sufficient robustness against relevant variables during dialysis

pH

Temperature

Dialysis time necessary to reach equilibrium

Membrane cut-off and brand

Generation of non-esterified fatty acids during dialysis

…

Validation of Performance Attributes

	Imprecision (%)		
	Within-run	Between-run	Total
Inclusive ED [1]	2.8	2.4	3.7
MS-measurement [2]	1.7	1.0	2.0

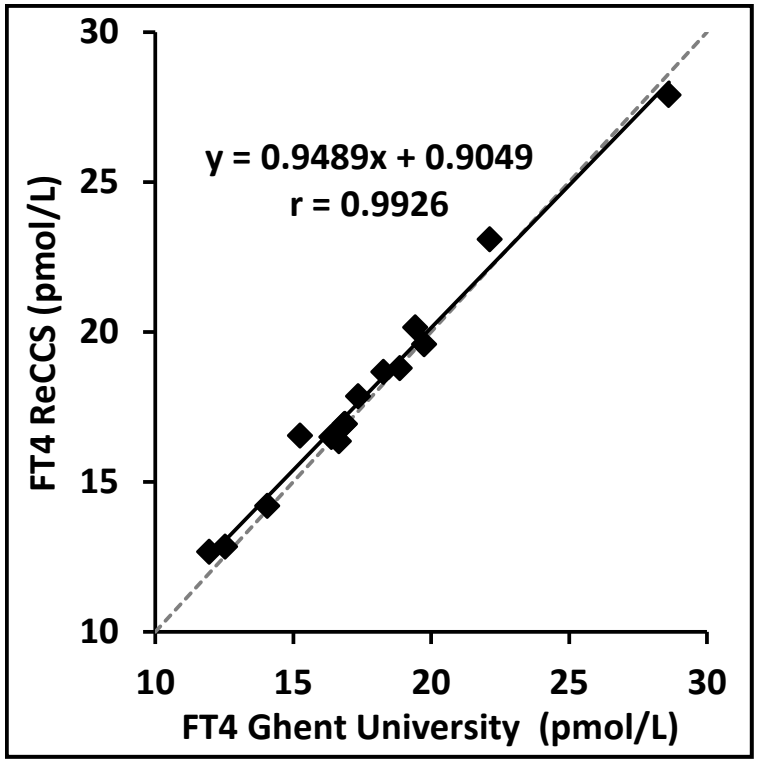
	Trueness (Deviation from target, %)
Inclusive ED [1]	-0.2
MS-measurement [2]	+0.03

	Expanded (k = 2) uncertainty (%) for measurement protocol n = 3
Incl. ED [1]	7.6 [3]

[1] n = 61
[2] n = 66
[3] Constant over the measurement range 1.8 – 79.8 pmol/L

Transferability of the RMP to a 2nd lab

Reference Material Institute for Clinical Chemistry Standards (ReCCS, Kawasaki, Japan)



15 samples; 4 replicates

Mean total CVs:

– **UGent: 3.1% (1.3 - 4.5%)**

– **ReCCS: 4.2% (1.0 - 8.9%)**

➔ **Clin Chem Lab Med 2011;49:1275-81**

➔ **Listed in the JCTLM Database**

FT4 RMS – Milestones

Three method comparisons

Phase I – III (2008 - 2012)

Objectives

Assess/investigate the standardization status of current FT4 assays

Assays' quality of performance

Feasibility of standardization by method comparison with the FT4 conventional RMP on a clinical panel

Impact of standardization/recalibration

FT4 RMS – Milestones

Step-up approach

Phase I: Method comparison with high-volume sera from volunteers; mathematical recalibration[1]

Phase II: Proof-of-concept but with inclusion of master calibrators and recalibration by IVD-manufacturers[2]

Phase III: Method comparison with a clinically relevant panel (again with inclusion of master calibrators and and recalibration by IVD-manufacturers)[3]

[1]Thienpont et al. Clin Chem 2010;56:912-20.

[2] Thienpont et al. Clin Chem Lab Med 2010;48:1577-83.

[3]Submitted to Eur Thyroid J (revision)

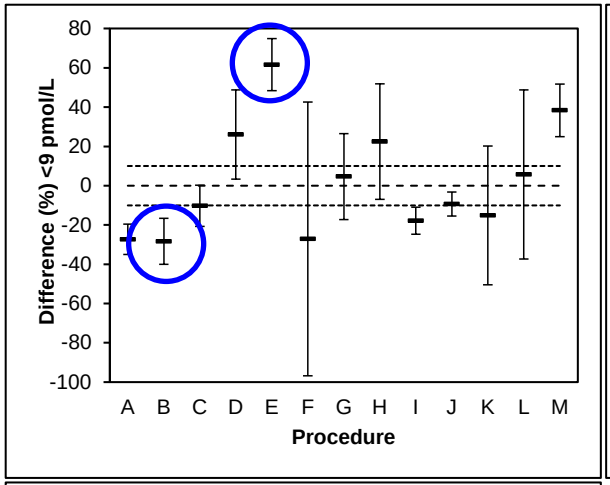
Assessment of Standardization Status

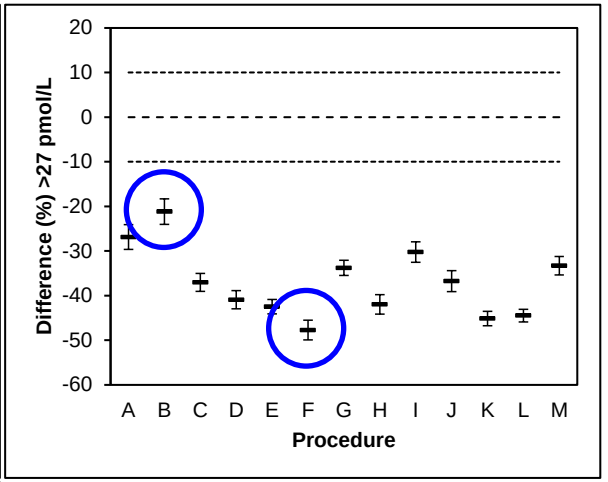
Phase III
Biases to ED ID-MS

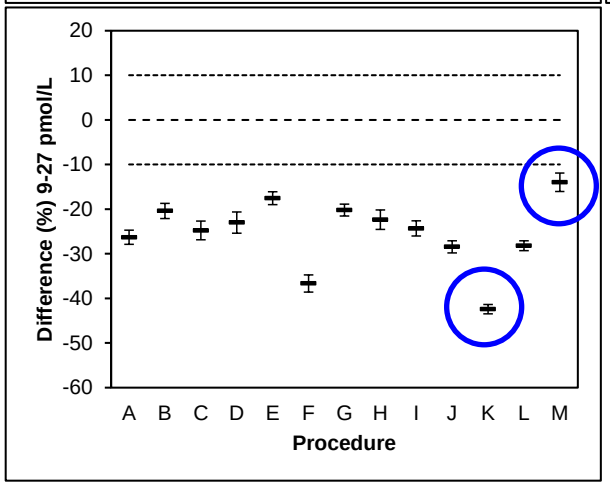
9–27 pmol/L:
-25% (mean)
Range: -14% to -42%

>27 pmol/L:
-37% (mean)
Range: -21% to -48%

<9 pmol/L:
2% (mean)
Range: -28% to 62%







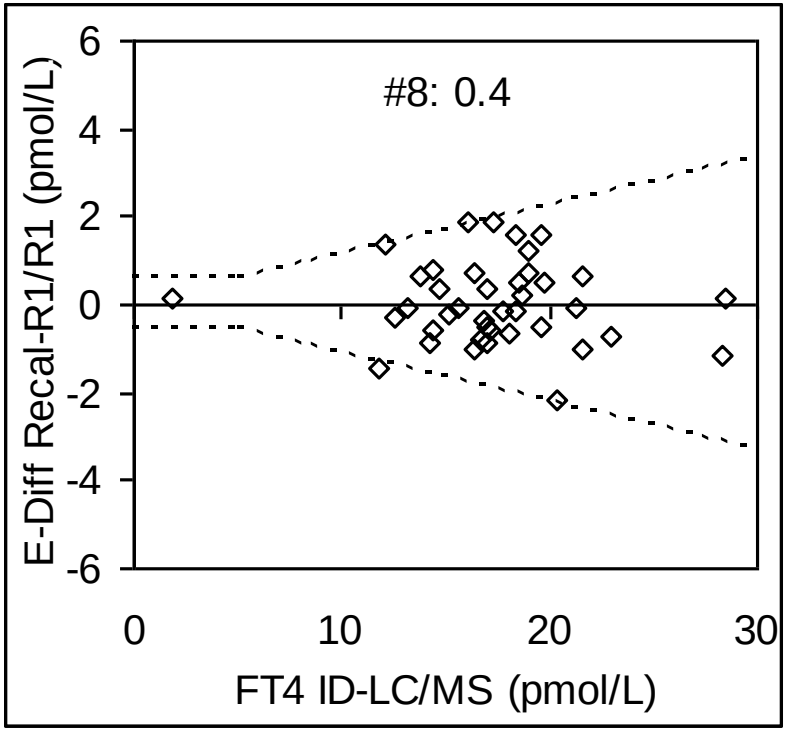
Concentration range of panel: 3 to 77 pmol/L

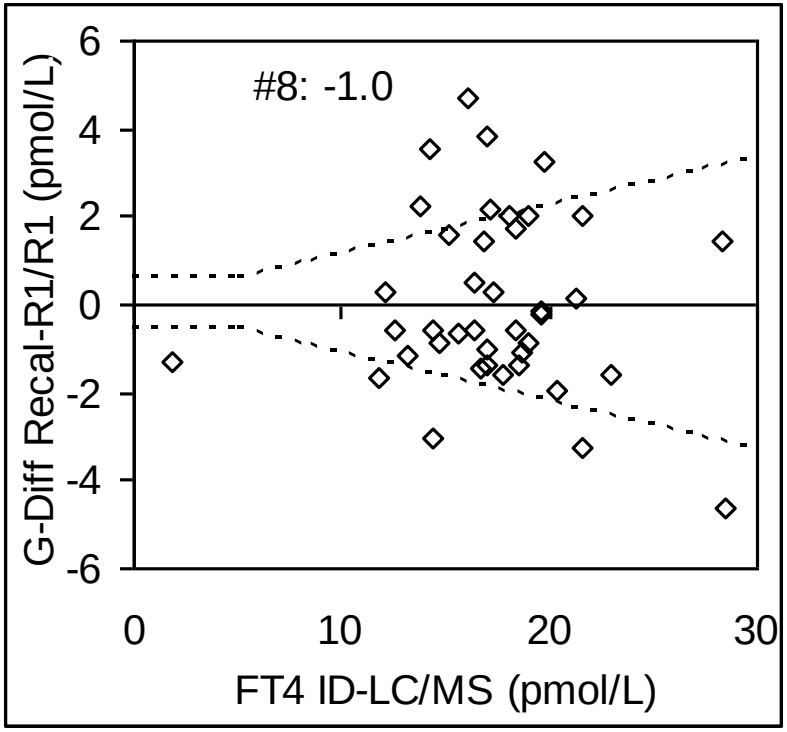
➔ **All assays strongly negatively biased**
➔ **Physiological studies flawed**

Assessment of Quality of Performance

No standardization without assessment of quality

Total error: difference plot after recalculation of data with regression equation: best and worst (Phase I)

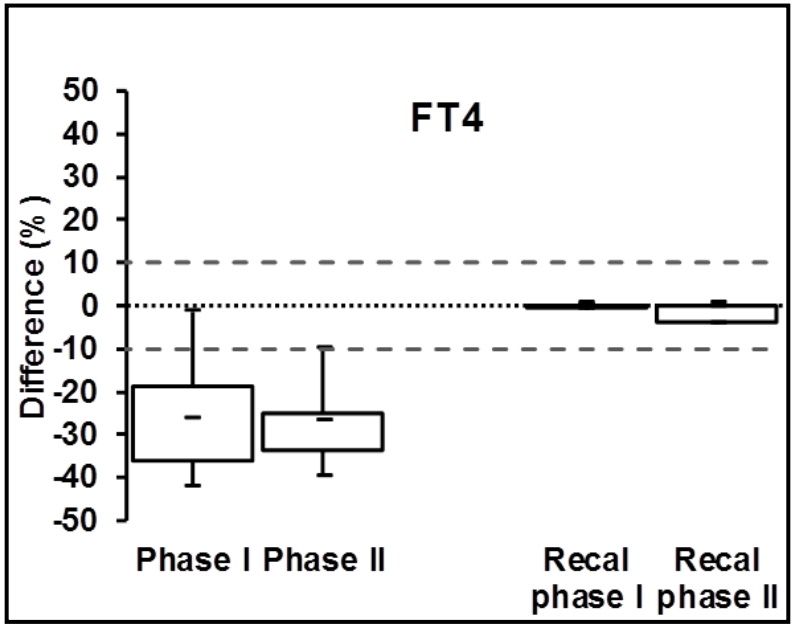




Other performance attributes: imprecision, correlation, stability (within-run, between-), deviation from IQC target

Proof-of-Concept

Stability of relationship RMP – Routine immunoassays#



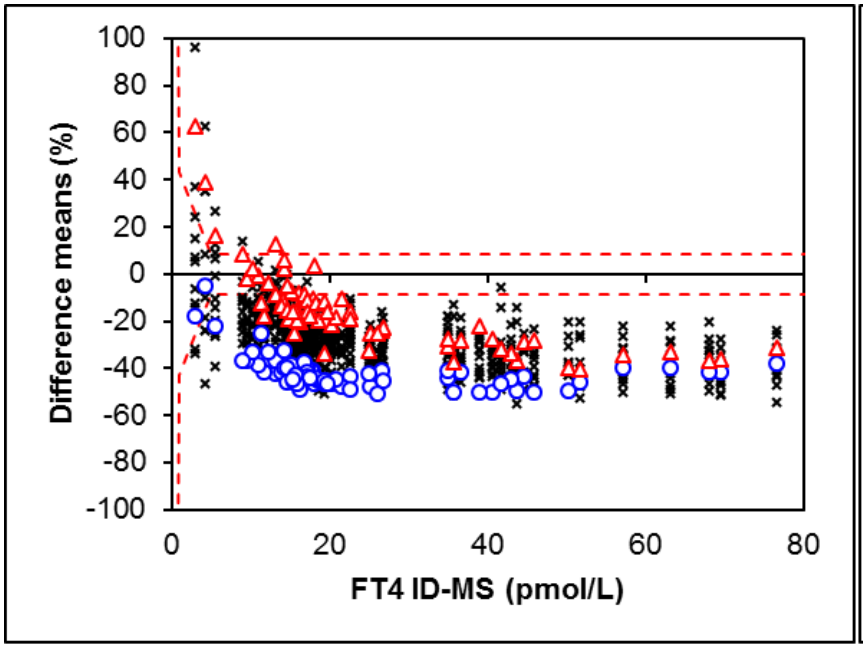
Relationship stable within the typical batch to batch variation of current assays

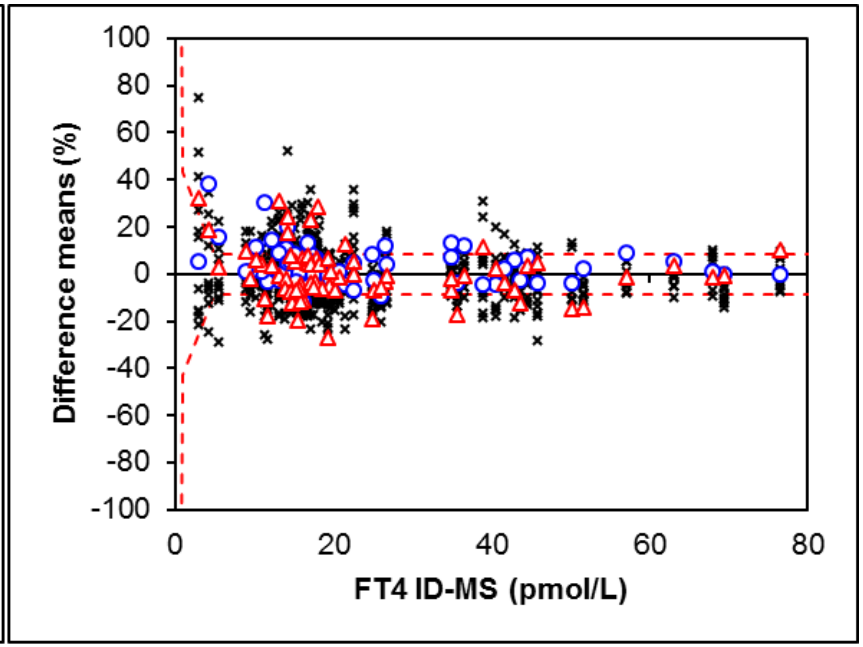
Recalibration# removes assay-specific biases

#Phase I: mathematical
#Phase II: master calibrator-based

Effect of Standardization/Recalibration

Phase III



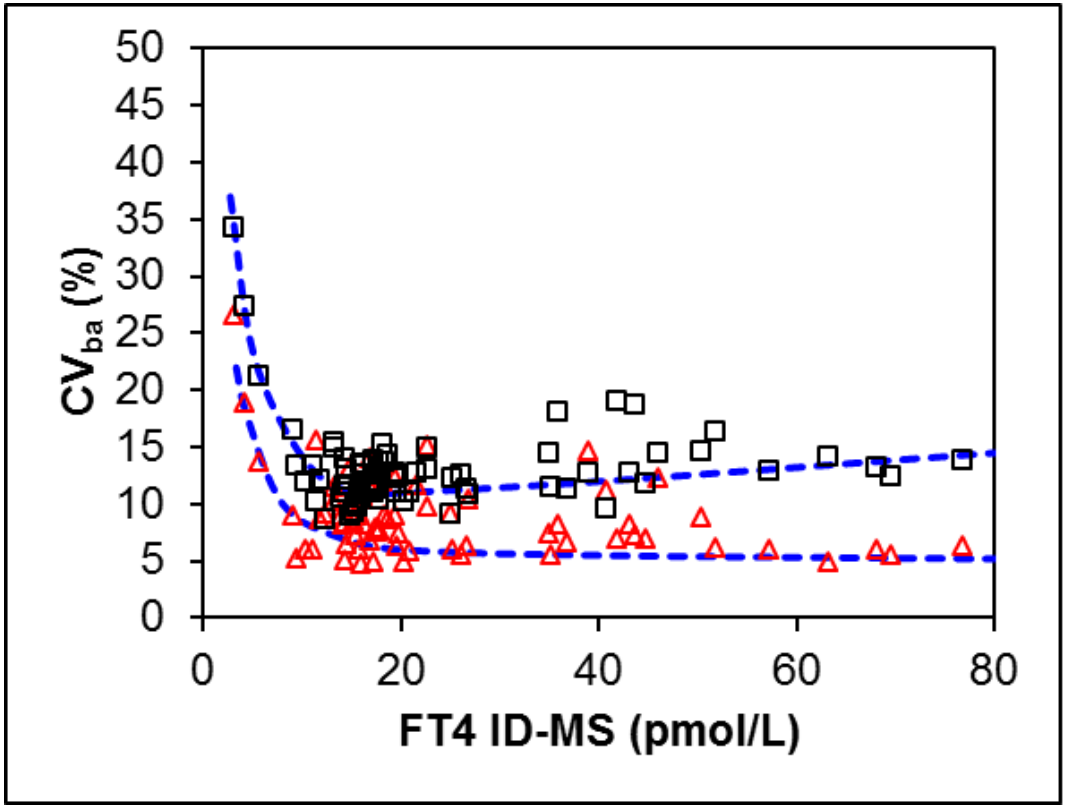


➔ Bias to the ED ID-MS RMP removed.

➔ Residual dispersion nearly entirely due to within-assay effects

Effect of Standardization/Recalibration

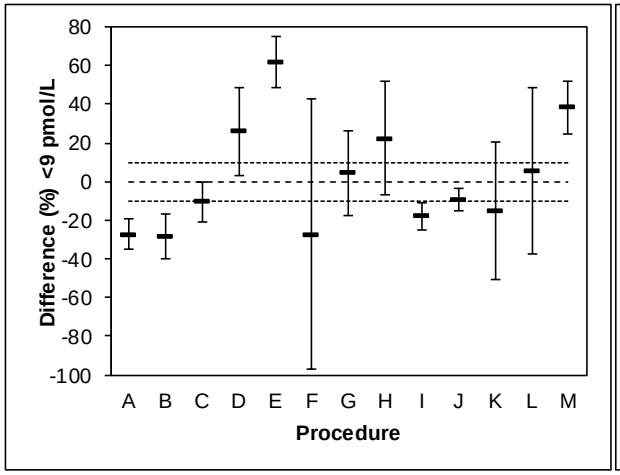
Phase III

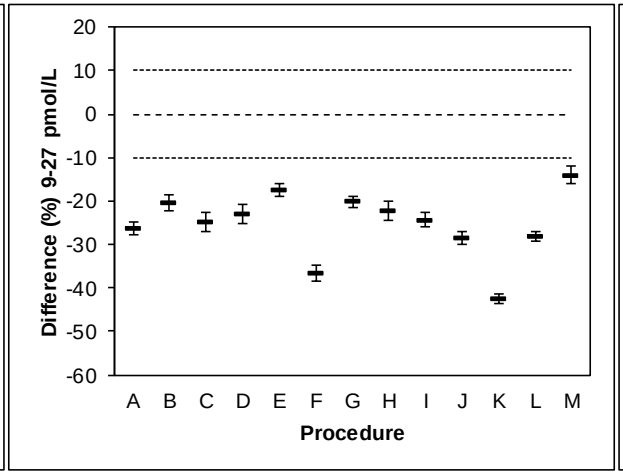


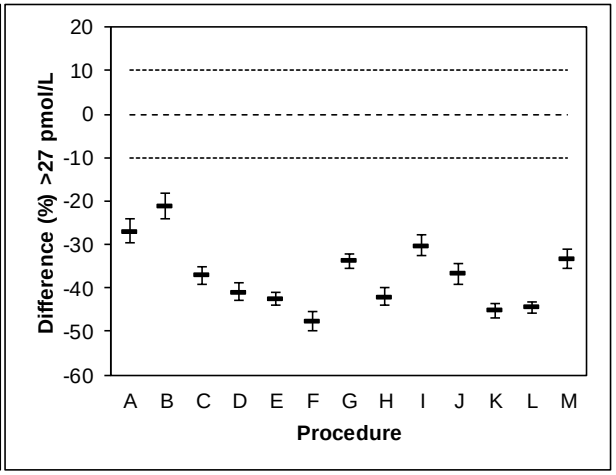
➔ **Between-assay CV decreases from 9.7% (mid concentration range, before recalibration) to 3.4% (after –)**

Impact of Standardization

Phase III







➔ Most pronounced effect in the eu- & hyperthyroid range

➔ FT4 concentrations will increase in general by 30 – 50%

➔ Δ Reference intervals

Way Forward?

Way Forward

Phase IV (timeline 2014-'15)

Technically prepare standardization (without direct implementation though); use a clinically relevant panel

Establish an infrastructure for procurement of serum panels#

Set-up a network of reference laboratories
Currently: UGent (L. Thienpont) & ReCCS (M. Umemoto); potential other candidates: Stanford University (J. Faix) & CDC (H. Vesper)

Liaise with regulatory authorities

#Van Houcke SK, Thienpont LM. "Good samples make good assays" - The torturous way to sourcing clinical samples for the thyroid standardization project. Clin Chem Lab Med 2013;51:967-72.

Way Forward

Liaise with key stakeholders

Establish reference intervals with standardized assays

In cooperation with IFCC Committee on Reference Intervals and Decision Limits (C-RIDL)

Educate manufacturers, clinicians and patients

In collaboration with IFCC Education and Management Committees

Coordinate implementation of standardization

All manufacturers/assays at the same time

Timeline: 2018?

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