# International Standardization of the Thyroid Markers Free T4 and TSH

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# La Standardisation Internationale des Paramètres Thyroïdiens T4 Libre et TSH

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# Disclosure

**Consultancy to:**

**Fujirebio Inc. (Europe – Japan)**
**Siemens Healthcare Diagnostics Inc. (USA)**
**Tosoh Corporation (Europe – Japan)**
**Imabiotech (France)**

# Economic impact of thyroid testing

## High burden on the healthcare system

**Yearly $180*10^6$ TSH & $60*10^6$ FT4 tests performed worldwide**

## Testing volume might even increase

**Among others, recent meta-analysis links subclinical thyroid dysfunction to coronary heart disease & "all-cause" mortality#**

**➔Underpins the indisputable value of thyroid function testing with fitness-to-the-purpose**

#Singh et al. Impact of subclinical thyroid disorders on coronary heart disease, cardiovascular and all-cause mortality: a meta-analysis. Int J Cardiol 2008;125:41–8.

# Benefits of using standardized assays

## Fit to address modern clinical & public health needs

- Common reference intervals/clinical decision limits
- Evidence-based clinical practice guidelines
- Application of consistent standards of medical care
- Translation of research into patient care & disease prevention activities
- Electronic patient records with inclusion of lab data

**➔Whether these needs can be met, depends on the availability of laboratory results that are comparable over time, location & across assays**

# IFCC Working Group/Committee for Standardization of Thyroid Function Tests (C-STFT)

## Chair: Prof. Dr. L. Thienpont

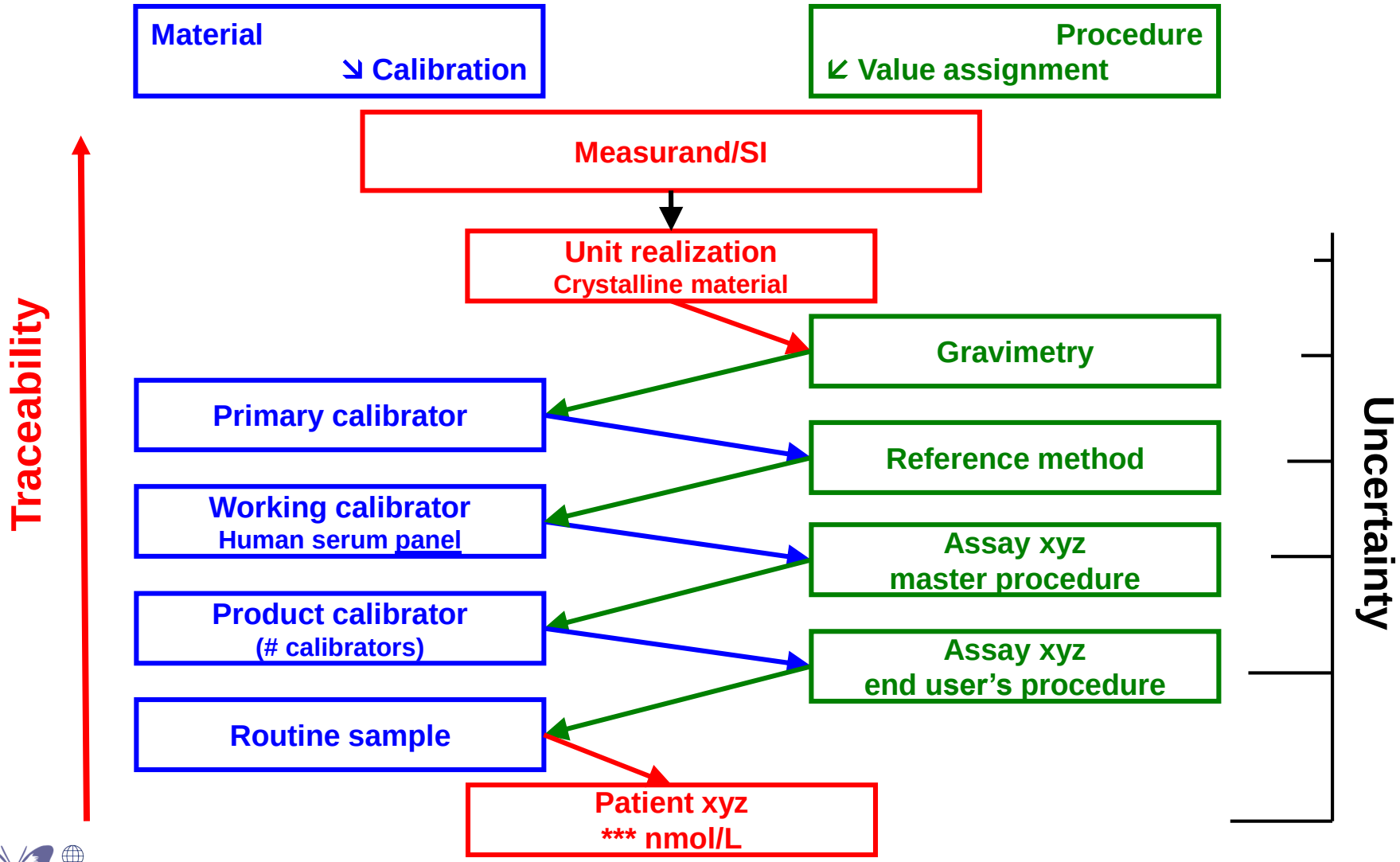

## Terms of reference

- Develop reference measurement systems for free thyroid hormones and TSH
- Establish a network of competent reference laboratories
- Liaise with key stakeholders to implement traceable methods in routine clinical practice

www.ifcc.org/ifcc-scientific-division/sd-committees/c-stft/

www.ifcc-cstft.org

# Reference measurement system#




#ISO 17511

# Development of a reference measurement system for FT4

# Definition of the measurand FT4

**Measurand#**

**"Quantity intended to be measured"**

**IUPAC/IFCC format***

**Component**

**Thyroxine(free)**

**Kind-of-Quantity (units)**

**Amount-of-substance concentration (pmol/L)**

**System**

**Plasma/Serum under physiological conditions (pH 7.4, 37°C)**

#Vocabulaire International des Termes Fondamentaux et Généraux de Métrologie (VIM) (ISO, 2007)

*Thienpont et al. Measurement of free thyroxine in laboratory medicine – Proposal of measurand definition. IFCC WG-STFT. Clin Chem Lab Med 2007;45:563–4.

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# FT4 reference measurement procedure

**International conventional reference measurement procedure (RMP)# based on**

- **Equilibrium dialysis (ED)**
- **Quantification of thyroxine in the dialysate with a "trueness-based" reference measurement procedure calibrated with the certified material IRMM-468**

**➔ED ID-LC/tandem MS**

**NOTE**

**The measurand is *operationally defined* as**

**"Thyroxine in the dialysate from ED of serum/ plasma prepared under defined conditions"**

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

# FT4 reference measurement procedure

## Development & validation

**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.**

# Development of a reference measurement system for TSH

# Definition of the measurand TSH

## TSH analysis is “mixture”

### Component#

**Human TSH – intact, total, glycosylation encountered in diagnostic applications which should be specified**

### Kind-of-quantity (units)#

**Amount-of-substance concentration (arbitrary, mIU/L)**

### System#

**Serum/Plasma**

**NOTE: Definition requires that TSH assays deliver a measure for “total TSH” & measure the specified TSH-glycoforms in an equimolar way**

#Thienpont et al. Traceability to a common standard for protein measurements by immunoassay for in-vitro diagnostic purposes Clin Chim Acta 2010;411:2058-61.

# TSH reference material & -procedure

## The problem

- **IU defined by a WHO standard**
- **WHO IRP 80/558 (also 81/565) from purified cadaver pituitary; mixture components differ from those in serum; dissolved in artificial matrix, it potentially does not give the same dose/response in immunoassays as TSH in its natural environment**

  **➔WHO IRP is not commutable and not suited for standardization**

- **Reference measurement procedure for TSH technically not to expect in the short- to midterm**

  **➔Harmonization instead of standardization#**

#Miller et al. Roadmap for Harmonization of clinical laboratory measurement procedures. Clin Chem 2011;57:1108-17.

# TSH harmonization approach

## Proposal by C-STFT

**Statistical "all-procedure trimmed mean" (APTM)**

- **From a method comparison with a clinically relevant panel**
- **With participation by (as many as possible) assays**

**➔Serves as "surrogate RMP"[1]**

**NOTE**

- **Statistical basis: robust factor analysis model[2]**
- **Requires excellent correlation of results by immunoassays to the APTM**

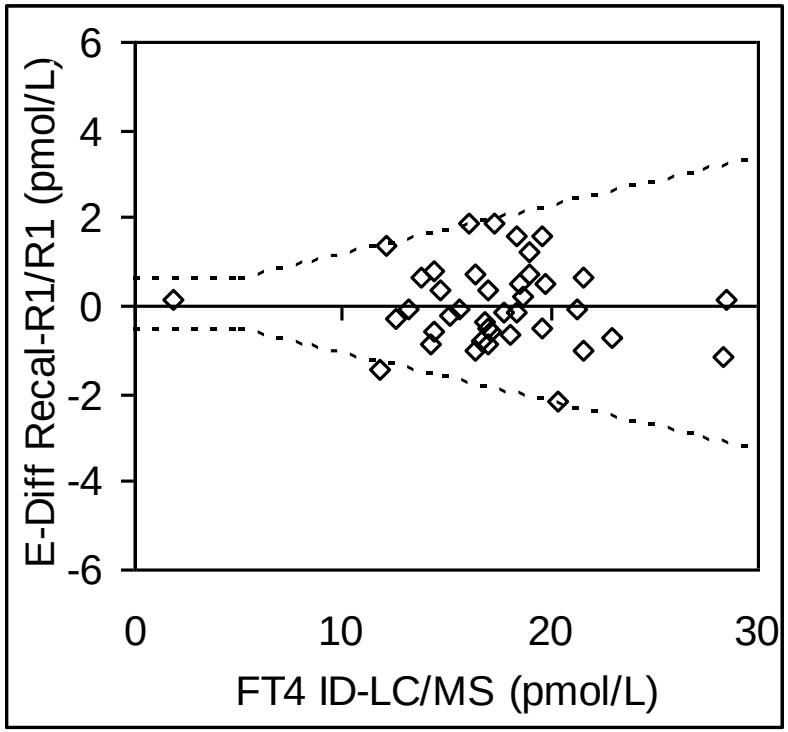
[1]Van Houcke et al. Harmonization of immunoassays to the all procedure trimmed mean - proof of concept by use of data from the insulin standardization project. Clin Chem Lab Med 2013;51:e103-5. doi: 10.1515/cclm-2012-0661.

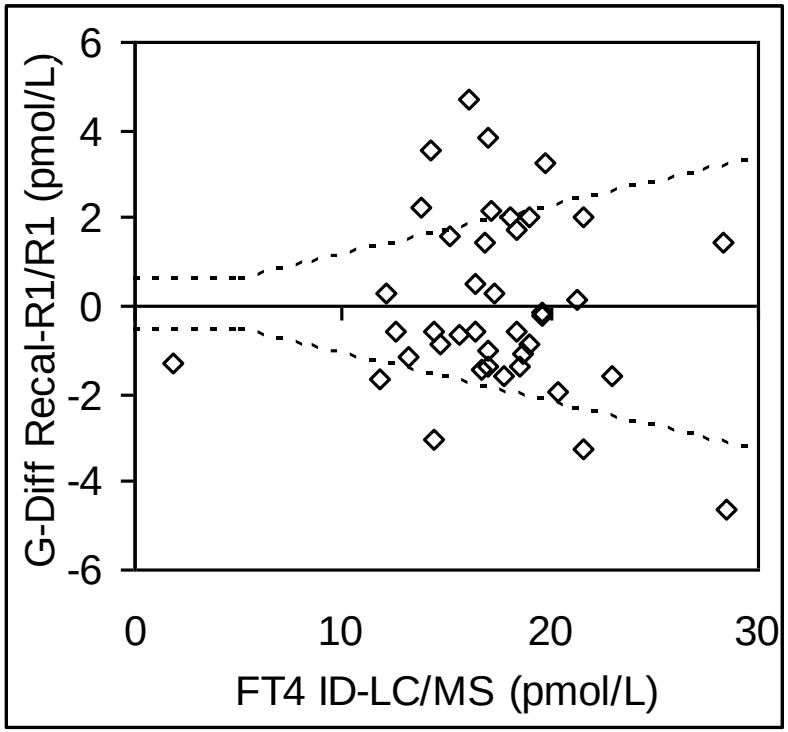
[2]Stöckl et al. A statistical basis for harmonization of thyroid stimulating hormone assays using a robust factor analysis model. Clin Chem Lab Med 2014;52:965-72.

# Requirements for successful standardization/harmonization

# Sufficient intrinsic quality of performance

## Total error *vs* biological limits to reflect sample-related effects (e.g., limit for FT4: 9.6%)#




**Other performance attributes: imprecision, correlation, stability (within-run, between-), IQC performance**

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

# "Step-up" approach#

## Familiarization phase[1]

- **First method comparison with high-volume sera from "apparently healthy" volunteers**
- **Assessment of assays' basic performance attributes**
- **Mathematical recalibration**

## First step-up[2]

- **Second method comparison with a clinically relevant panel and inclusion of master calibrators**
- **Verification that assays perform similarly on clinically relevant samples**
- **Recalibration by IVD manufacturers**

#Van Uytfanghe et al. Clin Chim Acta 2014;432:62-67.

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

[2]Thienpont et al. Eur Thyroid J 2014;3:109-16.

# “Step-up” approach

## Second step-up

- **Final method comparison with a new clinically relevant panel**
- **Technical recalibration of IVD assays**

## Phase after standardization/harmonization#

- **Procure a follow-up panel**
- **Assess sustainability of the status achieved after technical recalibration of IVD assays (= risk analysis implied by the FDA)#**
- **Prepare implementation of standardization/ harmonization**

#De Grande LA et al. Clin Chem Lab Med 2015 Jan 15 doi: 10.1515/cclm-2014-0959.

# Status of standardization of commercial FT4 and TSH assays

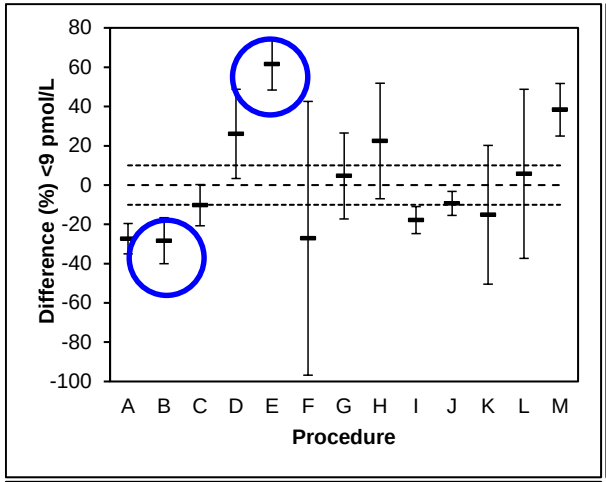
# Standardization status – FT4

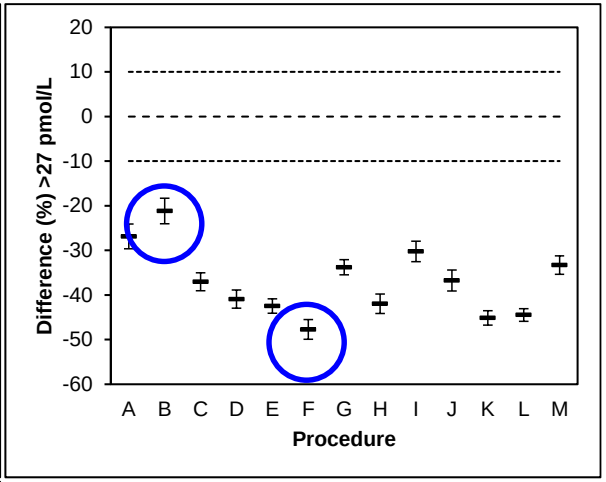
## Bias to ED ID-MS#

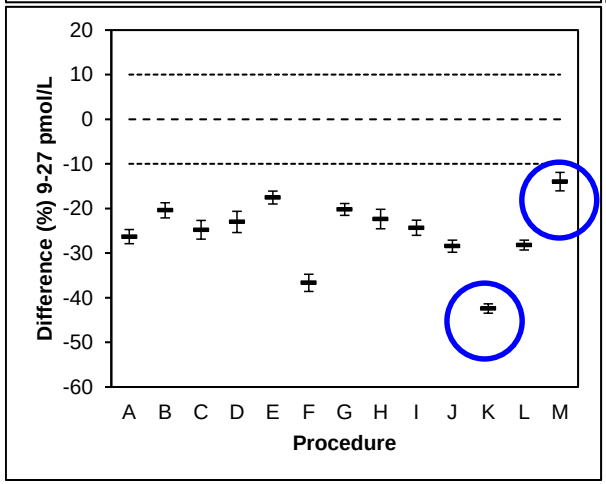
**9–27 pmol/L:**
**-25% (mean)**
**Range: -14% to -42%**

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

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



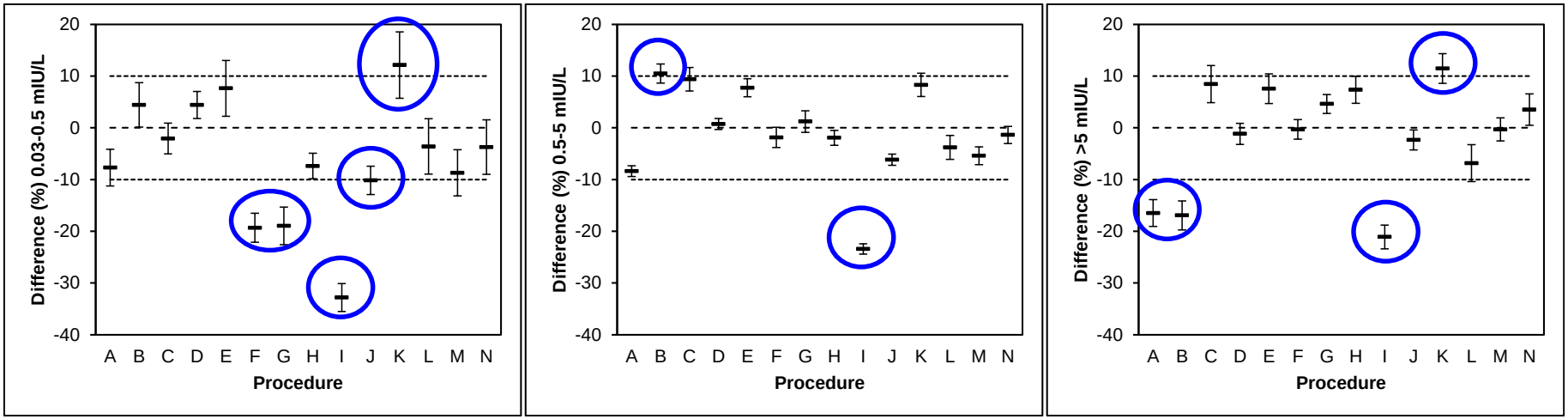

**FT4 concentration range of the panel: 3 to 77 pmol/L**

**➔ All assays strongly negatively biased**

#Thienpont et al. Eur Thyroid J 2014;3:109-16.

# Standardization status – TSH

## Bias to APTM#




**TSH concentration range of the panel: 0.04 – 80 mIU/L**

- **→0.5 – 5 mIU/L: comparability quite good; only 2 assays differ by >10% from APTM**
- **→ <0.5 mIU/L & >5 mIU/L: max 5 out of 14 assays outside the ±10% limit**
- **→ Max discrepancy between assays up to ~33% (whole range)**

#Thienpont et al. Eur Thyroid J 2014;3:109-16.

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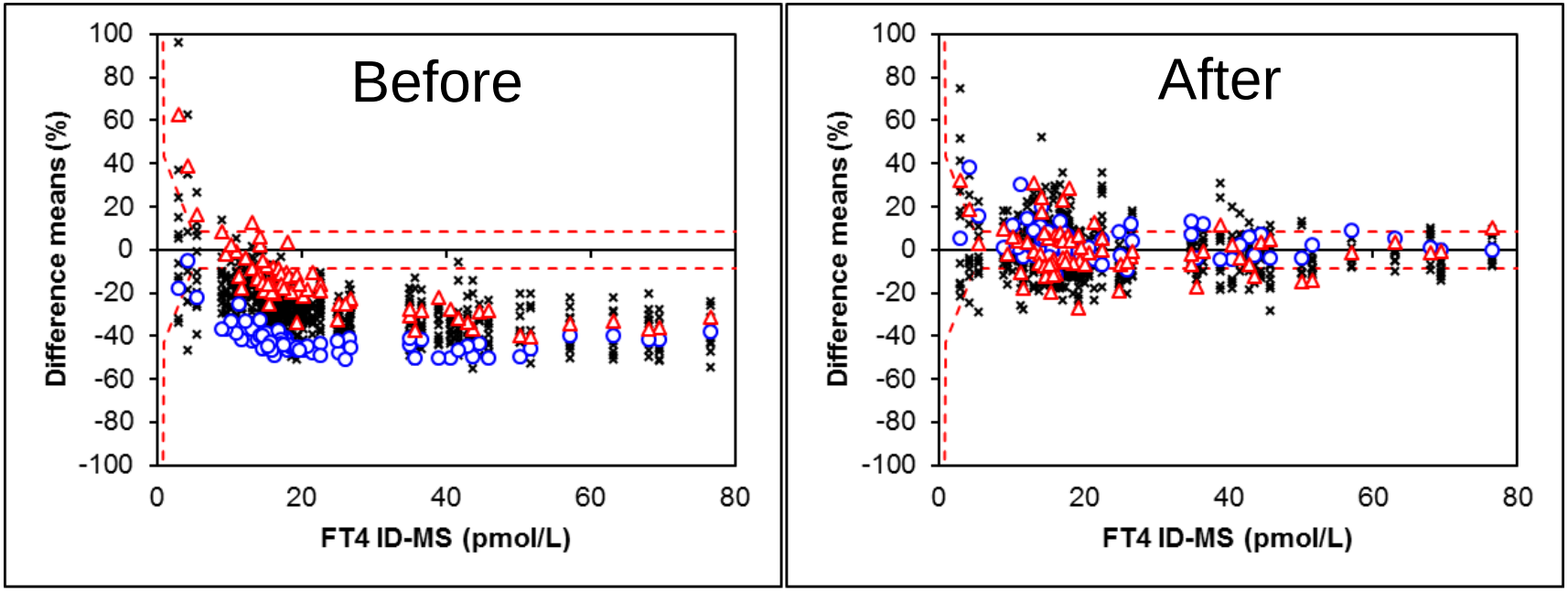
# Feasibility of standardization/harmonization

# Feasibility of standardization/recalibration

## FT4#



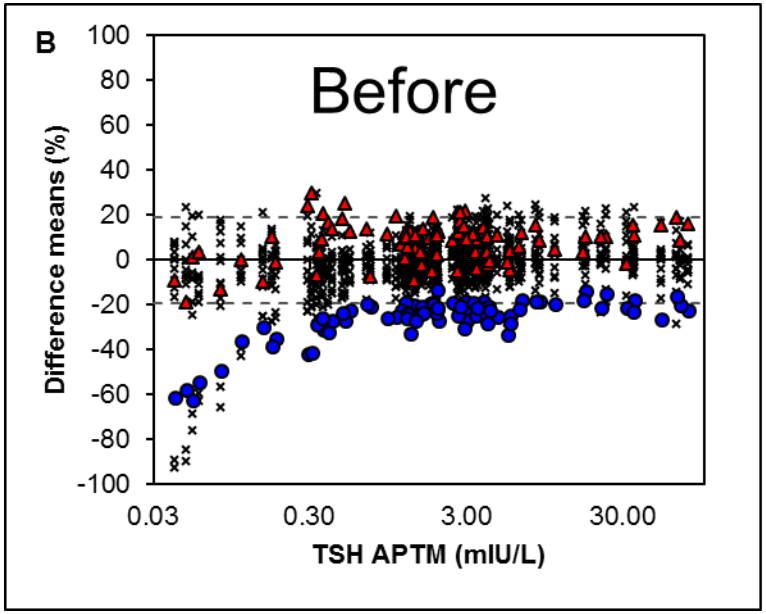

**➔Bias to ED ID-LC/tandem MS removed**

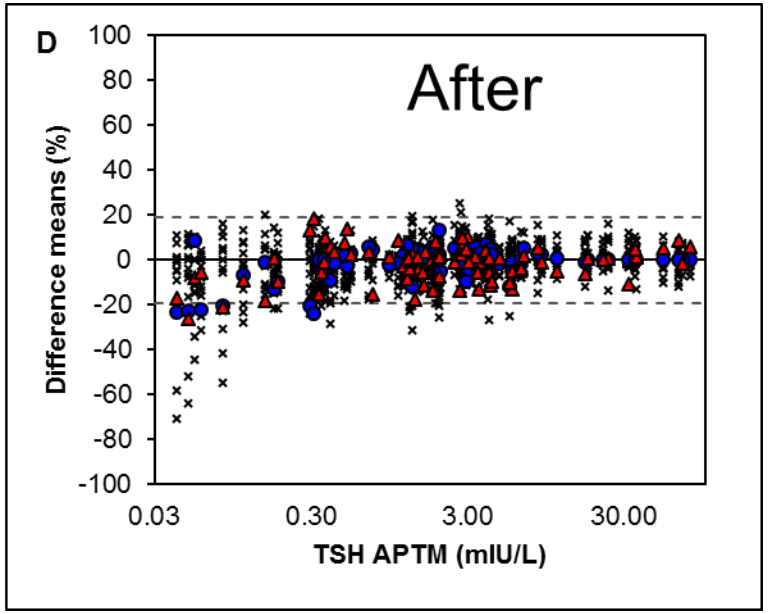
**➔Residual dispersion nearly entirely due to within-assay effects**

#Thienpont et al. Eur Thyroid J 2014;3:109-16.

# Feasibility of standardization/recalibration

## TSH#




**➔Recalibration nicely centers the distribution of the assay differences around zero**

**➔Remaining dispersion from within-assay effects**

#Thienpont et al. Eur Thyroid J 2014;3:109-16.

# Project status and way forward

# Step-up to standardization/harmonization

## Phase IV method comparison study

- Collection of new clinically relevant panels, to measure in parallel with master calibrators:
  - FT4: 4.5 – 164 pmol/L (by ED ID/MS), n = 91
  - TSH: ~0.002 to 75 mIU/L (APTM), n = 101
- Measurements were done last May
- Preliminary report is discussed with the IVD manufacturers
- Recalibration by manufacturers is currently on-going
- Final data treatment and manuscript will follow

## Preparation of follow-up panels

- TSH panel is ready (and targeted)
- FT4 panel is almost collected (will be targeted)

# Proof-of-concept

## Reference interval (RI)

- **Panel of 120 samples from apparently healthy Americans to be measured by recalibrated FT4 and TSH assays**
- **FT4 target values assigned by ED ID-LC/tandem MS at UGent**

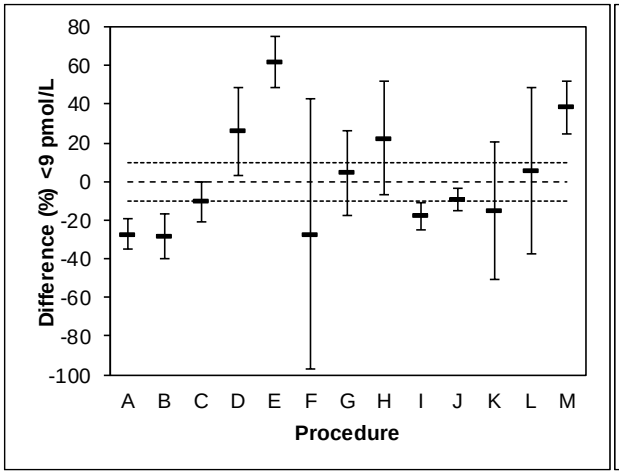
**➔Proof-of-concept for standardization/ harmonization and feasibility to use a common RI**

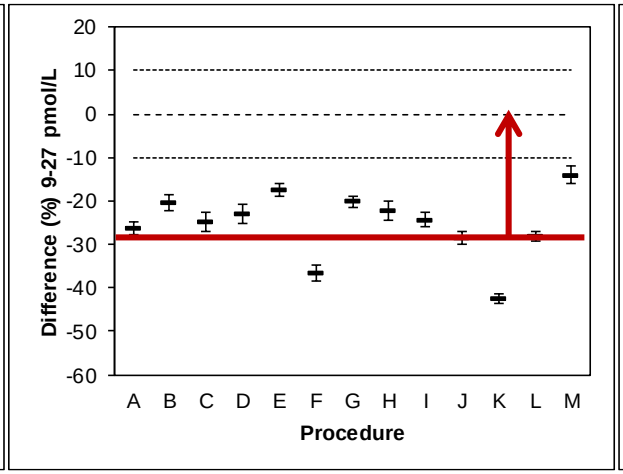
**➔Basis for further establishment by manufacturers of new RIs after standardization/harmonization**

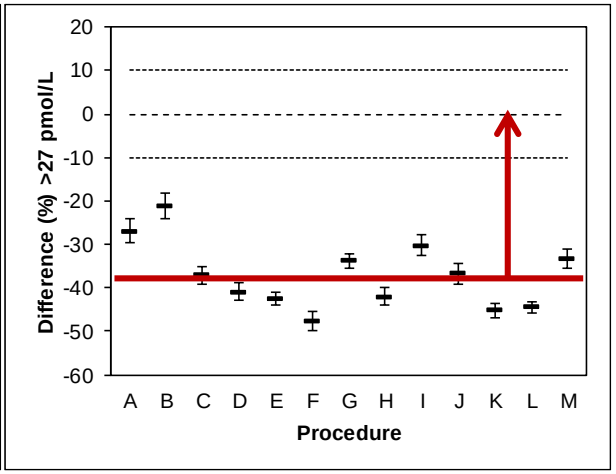
# Implementation

# Obstacles to implementation

## Impact of standardization




**➔Most pronounced for FT4 testing mainly in the eu- & hyperthyroid range**

**➔Measurement values will increase in general by 30 – 50%**

**➔Reference intervals will change**

**Risk?**

# Measures to waive obstacles

## Prior to implementation

- **Liaise with regulatory authorities (new FDA 510k clearance)**
- **Liaise with key stakeholders**
- **Do risk-benefit analysis at all levels of stakeholders**
- **Educate stakeholders about impact/changes**
- **Coordinate implementation of standardized/ harmonized assays by all manufacturers at the same point in time and worldwide**
- **Monitor sustainability of standardization status**

# Collaborating IVD manufacturers