

Move to harmonise TSH, FT4, FT3, Total T4 & T3 Assays

Symposium 4 – Thyroid Function Tests



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Overview

Introduction

- “History”
- IFCC WG for Standardisation of Thyroid Function Testing (WG-STFT)

Terms of reference

- Reference measurement systems

Current standardisation/harmonisation status and quality of performance

- Total T4/T3
- Free T4/T3
- TSH

Way forward

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History

European Commission – 5th Framework Programme. Competitive and Sustainable Growth Project G6RD-CT-2001-00587 (2001–2005).

Feasibility studies for the development of **reference measurement systems** for thyrotropin (TSH) and for free thyroxine (free T4), and validation of reference measurement systems (procedure and material) for thyroxine (T4) and triiodothyronine (T3) in human serum. Brussels: European Commission, 2001.

Selection of project topic

Worldwide survey conducted by the IFCC SD to identify “measurands of priority”

History



2005: Working Group for Standardisation of Thyroid Function Tests (WG-STFT) – Chair: LM Thienpont

Measurement paradigm

Measurement procedures that claim the same measurand should give “equivalent” results

Applies in particular to procedures that claim “traceability to the same measurement standard”

→ **Terms of reference: *Develop reference measurement systems for thyroid hormones, i.e., TT4 & TT3, FT4 & FT3, TSH***

WG-STFT – “To do” list

Metrological considerations

Measurands

Development

Reference materials

Reference measurement procedures

Reference laboratories

} Reference
measurement
system

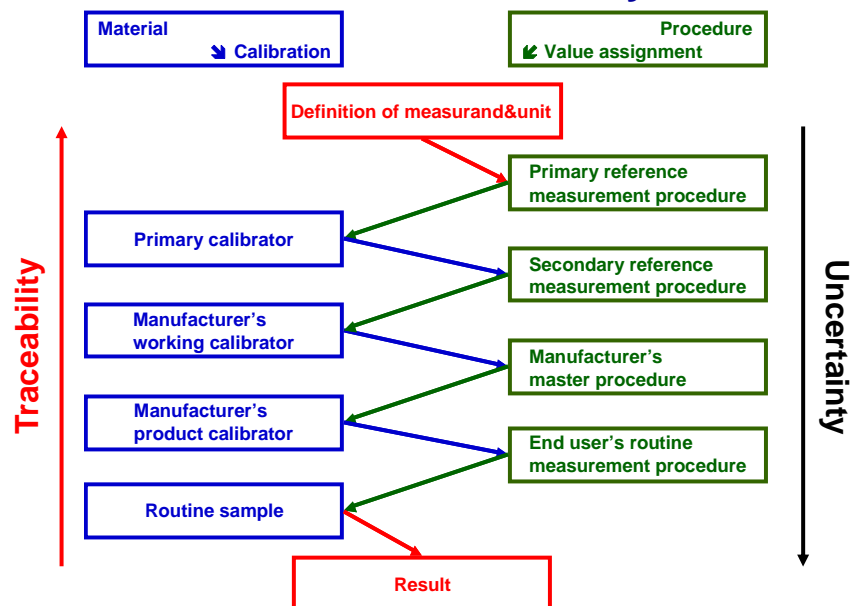
Assessment of the current standardisation/ harmonisation status and quality of performance

Method comparison with a panel of native sera

Use the method comparison to investigate the
feasibility of standardisation/harmonisation

Implementation of standardisation/harmonisation

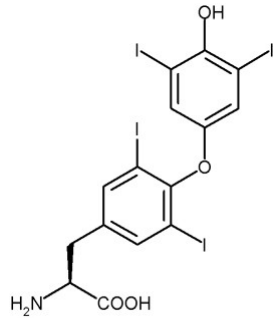
Reference measurement system



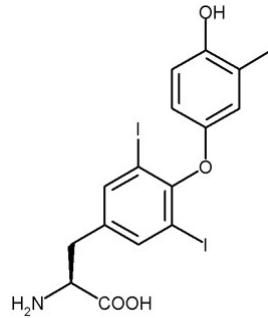
Total thyroxine/triiodothyronine

Measurand
Component (analyte)

T4



T3



Kind of quantity (units) – System

Amount-of-substance concentration (nmol/L) in
serum/plasma

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Total thyroxine/triiodothyronine

Complete and ISO conform reference
measurement system available

See database of the



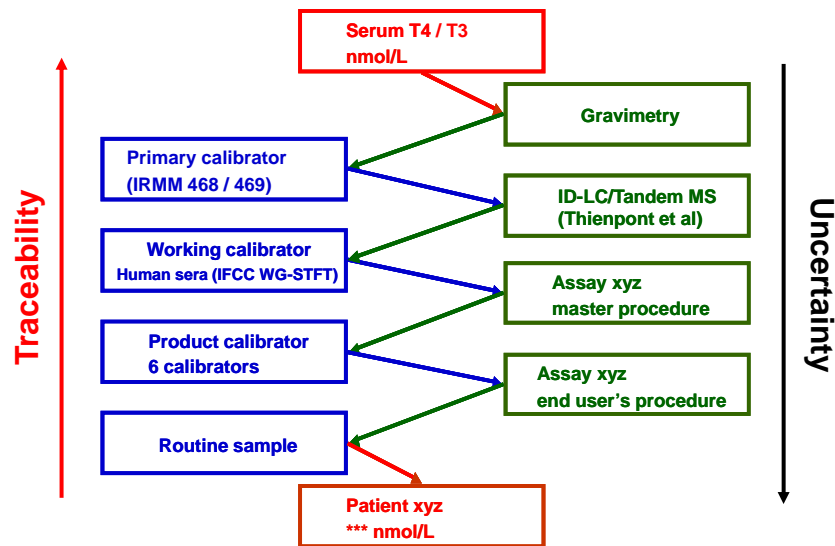
JCTLM Database
Laboratory medicine and *in vitro* diagnostics

→ www.bipm.org/jctlm/

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RMS for total thyroid hormones (T4/T3)



Note: SI-traceability = Trueness of measurement

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Free thyroxine/triiodothyronine

Measurand

Component (analyte)

Thyroxine/triiodothyronine that is not bound to proteins

Name: "Thyroxine/triiodothyronine(free)";
abbreviation: FT4/FT3

Kind of quantity (unit)

Amount-of-substance concentration (pmol/L)

System

Plasma or serum under physiological conditions (pH 7.4, temperature 37°C).

The WG-STFT does not require that specimens be collected under anaerobic conditions

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Free thyroxine/triiodothyronine

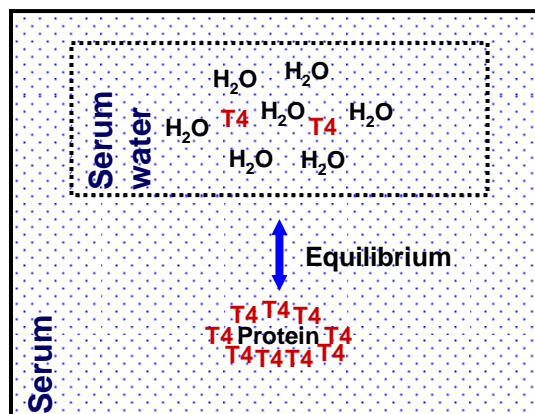
Thienpont et al. Measurement of free thyroxine in laboratory medicine – Proposal of measurand definition. IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT). Clin Chem Lab Med 2007;45:563–4.

IUPAC/IFCC format:

“Plasma/Serum – Thyroxine/triiodothyronine(free); amount-of-substance concentration” (pmol/L)

Free thyroxine/triiodothyronine

FT4/FT3 reference measurement procedure



Free thyroxine/triiodothyronine

Generation of serum water: Technical pitfalls?

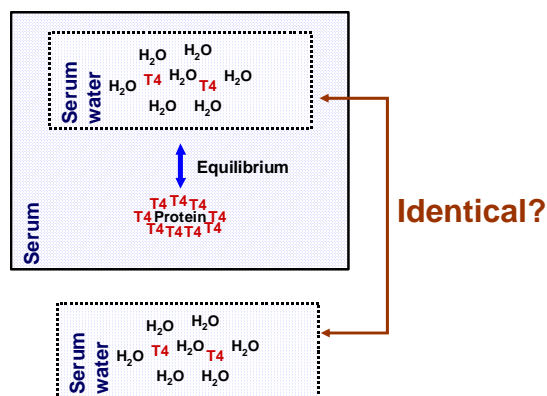
Sort	UF	ED
Membrane (type, cut-off) – Protein leakage – Adsorption	X X	[X] [X]
Buffer type	X	X
Dilution	[X]	X
Temperature	X	[X]
Donnan effect	X	
Osmotic effects		X
Time – FFA-generation – Protein deterioration		X? X?

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Free thyroxine/triiodothyronine

Generation of serum water: What about the equilibrium?



Unknown!

Conclusion: It might be that the separation step breaks the traceability chain!

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The whole truth and nothing but the truth?



Unfortunately NOT!

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Free thyroxine/triiodothyronine

Candidate international “conventional” reference measurement procedure#

- Equilibrium dialysis
- Combined with determination of T4/T3 in the dialysate with a trueness-based reference measurement procedure

#Van Uytvanghe 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.

Note

The measurand is thus *operationally defined* as
“Thyroxine/triiodothyronine in the dialysate from equilibrium dialysis of serum prepared under defined conditions”

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Free thyroxine/triiodothyronine

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.

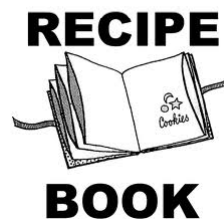
- Strictly adhere to the defined equilibrium dialysis procedure (cf. CLSI C45-A: Measurement of FTHs)
- Variants in the trueness-based RMP permissible
 - Isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/tandem MS) (JCTLM)
 - Calibration with IRMM-468/469 (pure T4/T3)
- Demonstrate transferability to a 2nd laboratory

Free thyroxine/triiodothyronine

Van Uytfanghe K...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.

Also consult: Supplemental data file



Thyroid stimulating hormone (TSH)

The molecule(s): 2 noncovalently linked subunits#

α -subunit (92 amino acids; common for other human glycoprotein hormones)

- 2 carbohydrate chains at Asn-52 and Asn-78

β -subunit (118 amino acids)

- 1 carbohydrate at Asn-23

Heterogeneity

- Sequence
- Glycosylation

#Szkudlinski MW, Fremont V, Ronin C, Weintraub BD. Thyroid-stimulating hormone and thyroid-stimulating hormone receptor structure-function relationships. *Physiol Rev* 2002;82:473-502.

Thyroid stimulating hormone

Measurand

All assays claim that they measure human “TSH”

Mixture analysis (<heterogeneity)

Component(s)?

Not yet formally defined; family of components

Kind of quantity (units) – System

Arbitrary amount of substance concentration (mIU/L) in serum#

Note

#Quantity value depends on the reference chosen

WHO International Reference Preparation (IRP) TSH 80/558; defines the IU

Ultimate aim: Expression of results in SI-units

Thyroid stimulating hormone

***“To standardise
or not to standardise –
That’s the question!”***



Thyroid stimulating hormone

WG-STFT opted for the Ekins’ pragmatic approach regarding measurement/harmonisation of heterogeneous measurands#



“It is nevertheless possible to **visualise** circumstances in which an assay system, though analytically invalid in the strictest sense, **responds** only to a particular atomic group common to the molecules of substances differing in overall **structure** (for example, the protein moiety in TSH).”

#Ekins R. Immunoassay standardization. Scand J Clin Lab Invest 1991;51 Suppl 205:33-46.

Thyroid stimulating hormone

WG-STFT proposal#

Definition of the component(s) in the measurand
“hTSH, intact, total, glycosylation encountered in diagnostic applications which should be specified”

Requires **equimolarity** of measurement to a diagnostic relevant extent

“Surrogate component-mixture” in the measurand

Epitopes at invariable peptide sequences that immunoassays should recognise

#Thienpont LM, Van Houcke SK. Traceability to a common standard for protein measurements by immunoassay for in-vitro diagnostic purposes. Clin Chim Acta 2010;411:2058-61.

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Thyroid stimulating hormone

WG-STFT proposal (continued)

“Surrogate” reference measurement procedure

“All-procedure trimmed mean” of a method comparison on a representative (for the diagnostic application) panel of native sera with several immunoassays.

Note: Sufficient correlation is required.

- Traceability to WHO standard remains
- Continuity is warranted by transfer of the IU from the first panel to the follow-up panels via consecutive method comparison studies
- Reference measurement system is dynamic, updatable according to scientific progress and technical possibilities

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Analytical & physiological requirements

“The immunoassays should measure the specified TSH-glycoforms equimolar to the diagnostic relevant extent”

To be demonstrated experimentally, because there exist different glycoforms in euthyroid subjects versus subclinical and overt hypothyroidism, and because there is evidence that immunoassays may be sensitive to the differences

→ There may be a need for exclusion of samples from patients with pituitary tumors

#Szkudlinski MW ... Weintraub BD. Thyroid-stimulating hormone and thyroid-stimulating hormone receptor structure-function relationships. *Physiol Rev* 2002;82:473-502.

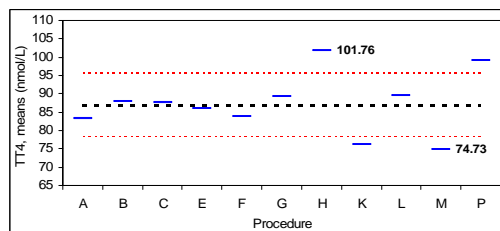
Donadio S ...Ronin C. Feasibility study of new calibrators for thyroid-stimulating hormone (TSH) immunoprocures based on remodeling of recombinant TSH to mimic glycoforms circulating in patients with thyroid disorders. *Clin Chem* 2006;52:286-97.

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Standardisation status

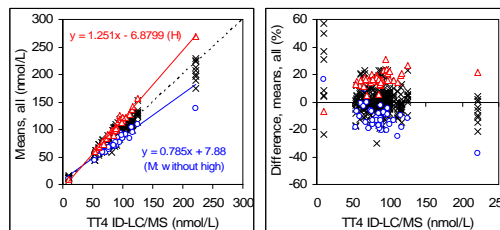
Total thyroxine IFCC WG-STFT – Project phase I (2008)



11 assays

Means varied from 75 to 102 nmol/L

Overall good agreement: 7 assays gave means that differed less than 10% from the RMP



→ Only 4 assays need standardisation

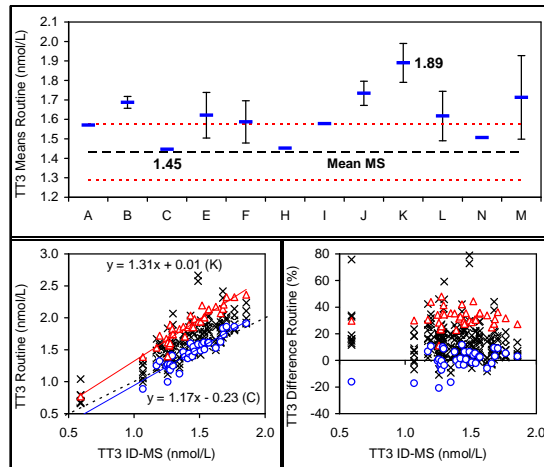
Note: Recalibration against the RMP

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Standardisation status

Total triiodothyronine IFCC WG-STFT – Project phase I (2008)



12 assays

Means varied from 1.45 to 1.89 pmol/L

Nearly all assays positively biased

7 assays deviated >10% from the RMP; 2 of them by >20%

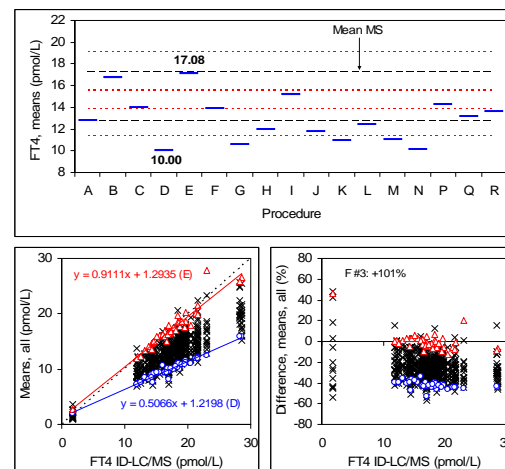
→ Standardisation is needed

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Standardisation status

Free thyroxine IFCC WG-STFT – Project phase I (2008)



15 assays

Means varied from 10 to 17 pmol/L

Except 2 assays, all measured FT4 much lower than the RMP

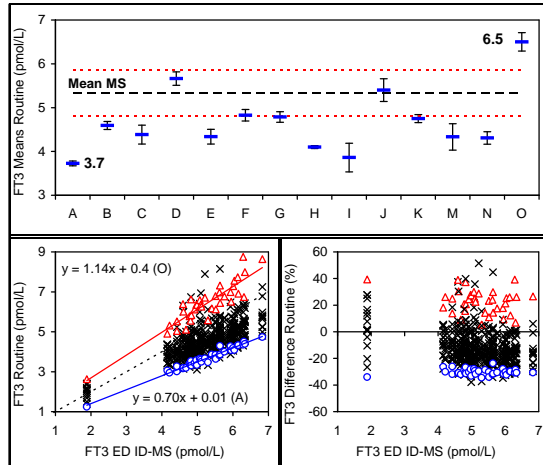
→ Standardisation is needed

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Standardisation status

Free triiodothyronine IFCC WG-STFT – Project phase I (2008)



14 assays

Means varied from 3.7 to 6.5 pmol/L

Most assays negatively biased

9 assays differed >10% from the RMP

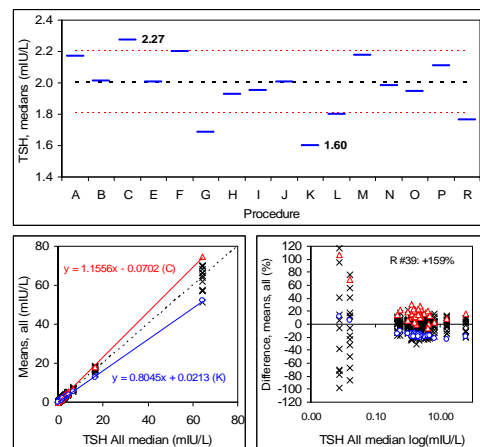
→ Standardisation is needed

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Harmonisation status

TSH IFCC WG-STFT – Project phase I (2008)



16 assays

Medians of 11 assays differed <10% from the overall median

Using the respective regression equations and a RI from 0.4 to 4 mIU/L, method K would give TSH values ranging from 0.34 to 3.24 mIU/L, method C from 0.39 to 4.55 mIU/L

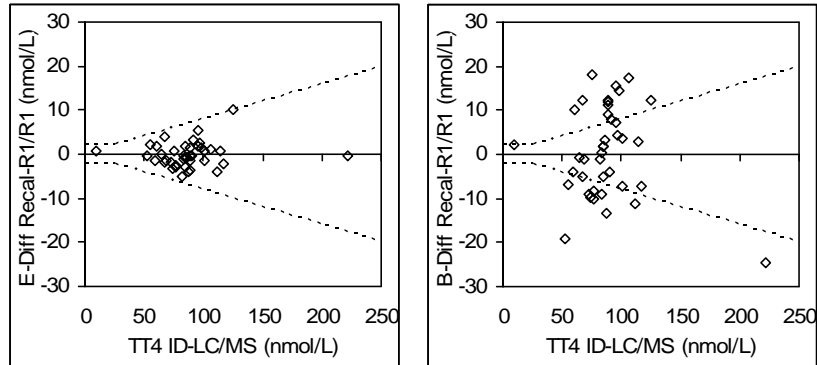
→ Harmonisation is desirable

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Quality of performance

No standardisation without assessment of quality
Difference plot (with TE limits) after recalculation of results with regression equation, e.g., TT4 best and worst quality of performance
IFCC WG-STFT – Project phase I (2008)



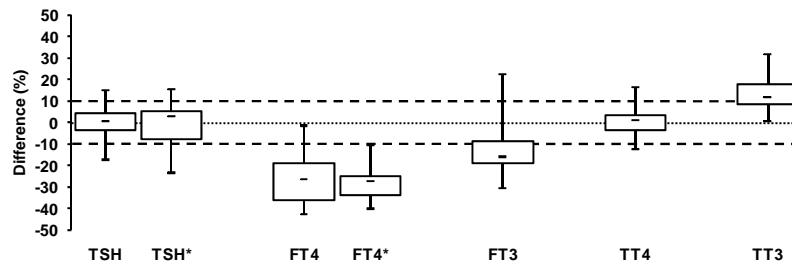
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Feasibility of standardisation/harmonisation

By recalibration
Phase I – Total and free T4/T3 and TSH (2008)
Phase II – Proof of concept for FT4 and TSH (2009)

Situation before recalibration



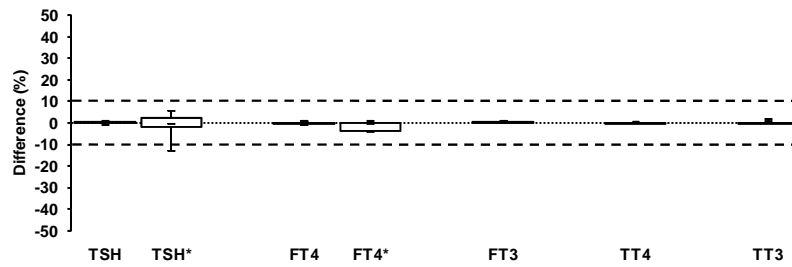
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Feasibility of standardisation/harmonisation

By recalibration (mathematical – master calibrators) Phase I and II

Situation after recalibration against the RMP or all-procedure trimmed mean



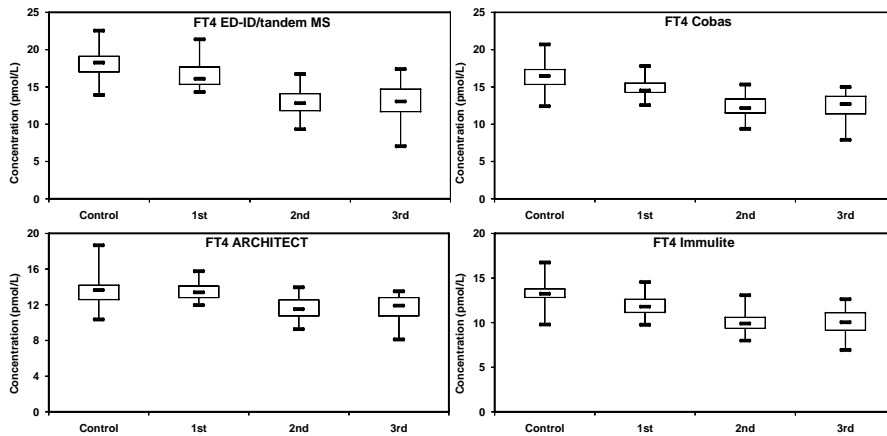
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Free T4 in pregnancy

Comparison of FT4 values by ED ID-MS and 4
immunoassays in non-pregnant controls (n = 26)
and pregnant (n = 107)

Trimester specific values



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References

Thienpont LM, Van Uytfanghe K, Beastall G, Faix JD, leiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B; for the IFCC Working Group on Standardization of Thyroid Function Tests. Report of the IFCC Working Group for Standardization of Thyroid Function Tests.

Part 1: Thyroid-Stimulating Hormone. Clin Chem 2010; 56:902-11.

Part 2: Free Thyroxine and Free Triiodothyronine. Clin Chem 2010;56:912-20.

Part 3: Total Thyroxine and Total Triiodothyronine. Clin Chem 2010;56:921-9.

Thienpont LM, Van Uytfanghe K, van Houcke S. Standardization activities in the field of thyroid function tests: a status report. Clin Chem Lab Med 2010;48:1577-83.

Anckaert E, ...Thienpont LM. FT4 immunoassays may display a pattern during pregnancy similar to the equilibrium dialysis ID-LC/tandem MS candidate reference measurement procedure in spite of susceptibility towards binding protein alterations. Clin Chim Acta 2010;411:1348-53.

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Way forward



Way forward

Method comparison on samples from hypo-, eu- and hyperthyroid patients – Project Phase III (2012)

Objective

Confirm feasibility of standardisation/harmonisation on different patient populations; for FT4 in relationship to the conventional reference measurement procedure; for TSH to the “all-procedure trimmed mean”

Way forward

Transformation of WG-STFT into Committee

Objective

Involve a broader forum of stakeholders and prepare the implementation of standardisation/harmonisation

Establish the physician/laboratory interface

Stakeholders

Laboratory directors

Regulatory agencies

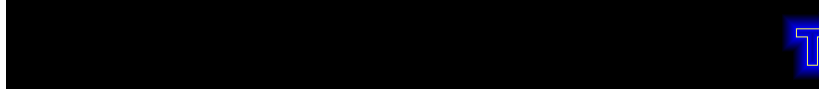
Professional societies

Pharmaceutical companies

Physicians and their patients

Additional vehicles

Medical journals and professional societies



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