**Minutes of the IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT) meeting at AACC 2006, July 24, 2006 (Dr. Linda Thienpont, August 14, 2006)**

The meeting was opened by Dr. Jocelyn Hicks, President of the IFCC. She emphasized the importance of the Working Group and wished the group good progress.

Dr. Linda Thienpont (chair of the IFCC Working Group), reviewed the background for the standardization of TT4 and FT4 measurements and presented the work that has been done within the EU-project G6RD-CT-2001-00587 (presentation attached). In summary (see also Table), she showed that the reference measurement system for TT4 is available and could be used by industry for the standardization of TT4 measurements. The reference measurement system for FT4 is near to completion (paper will be published in the September issue of Clin Chem 2006, 52), however, requires a clear definition of the measurand, the endorsement of the proposed standard measurement procedure, and its transfer to another laboratory.

| **Reference measurement systems for TT4 and FT4 measurement** | | |
|---|---|---|
| | **Total T4 (TT4)** | **Free T4 (FT4)** |
| **Measurand** | Amount of substance concentration (nmol/L) of T4 in serum/plasma | Amount of FT4 in serum or serum water (pmol/L serum or pmol/kg serum water) |
| **Primary reference material** | Pure T4, certified for its content (CRM IRMM-468; http://www/irmm.jrc.be) | Pure T4, certified for its content (CRM IRMM-468; http://www/irmm.jrc.be) |
| **Reference/standard measurement procedure** | ID-LC/MS reference measurement procedures (UGent; UBonn; LGC; NIST) | Equilibrium Dialysis ID-LC/MS standard ($) measurement procedure |
| **Standardization procedure** | Method comparison reference/routine with single donation sera manufactured by the CLSI C37A protocol | Method comparison standard/routine with single donation sera manufactured by the CLSI C37A protocol |
| ID-LC/MS: Isotope Dilution-Liquid Chromatography/Mass Spectrometry<br>CLSI: Clinical and Laboratory Standards Institute (formerly NCCLS)<br>$ Proposed as standard measurement procedure because it could not be proven that equilibrium dialysis represents the true serum water. | | |

After Dr. Thienpont's overview, other members of the Working Group took the opportunity to give a presentation.

- Dr. Graham Beastall presented on behalf of the British Thyroid Association (BTA). He welcomed the standardization efforts and supported the Working Group. He stressed that the public opinion in the UK has lost some faith in thyroid hormone measurements due to the discrepancy of results between assays.

- Dr. Jim Faix (Stanford University medical Center, CA, US) focused on FT4 measurements. He gave an overview of the currently available formats and emphasized the importance of standardization thereof.
- Dr. Alec Ross presented on behalf of the European Thyroid Association (ETA). He welcomed the IFCC initiative and expressed full support by the ETA. He reviewed the challenges for developing routine procedures for FT4 measurement and gave full support for the proposed equilibrium dialysis ID-LC/MS standard measurement procedure for FT4.

After the presentations, the discussion was opened of how to proceed with the project. There was general agreement of all parties present that standardization of FT4 & TT4 measurement would be desirable. It was also expressed that, strictly speaking, standardization of TSH measurements would have priority in view of the analyte's eminent diagnostic importance. Currently, however, standardization of TSH measurement is problematic because of the absence of a reference measurement procedure. Therefore, further discussions were restricted to FT4 and TT4 measurements. There was general agreement that the technical requirements for TT4 standardization are available. With regard to FT4, there was the opinion that the proposed standard procedure needed further validation (e.g., transfer to a second laboratory, which is already foreseen). There was an intense discussion of how to implement the standardization process. Industry expressed the opinion that clinical indications should be identified that show the need and the benefit of standardization of thyroid function tests. As example, FT4 testing in antenatal screening for thyroid failure was discussed. This application indeed requires harmonized trimester-specific FT4 reference intervals as a basis to decide on T4 supplementation. Industry also expressed the view that the standardization process should be a coordinated one, with a clear timeline, international implementation rules, and thoroughly discussed with all involved parties. The standardization of serum creatinine measurement was used as example, which is important for its use in calculating an estimated GFR. It was regretted that no representative of the American Thyroid Association was present at the meeting.

**Way forward**

**Technical issues to be addressed by the Working Group**

- The Working Group will propose a definition of the measurand FT4, which is then submitted to IFCC for formal endorsement.
- The Working Group will propose the FT4 procedure developed by Dr. Thienpont's group as standard measurement procedure, which is then submitted to IFCC for formal endorsement.
- The proposed FT4 standard procedure shall be transferred to another laboratory (Dr. Masao Umemoto has already been contacted and agreed to establish the procedure at HECTEF, Japan).

**Issues related to agreement on standardization and its implementation**

- Identification of clinical needs for standardization of TT4 and FT4 measurements.
- Integration of the American Thyroid Association in the project.
- Establishment of a consensus forum (thyroid associations, laboratory medicine associations, manufacturers, regulatory authorities) that plans and coordinates the standardization of thyroid measurements and its implementation in practice.
- Contact diagnostics industry worldwide, by publication in specific journals such as IVD-Technology

Inaugural meeting "IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)". AACC Annual Meeting 2006. Venue: Hyatt Regency Chicago, Monday July 24, 2006

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# IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)

Linda Thienpont
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## Introduction

**Need for standardization: Test results**

**Kit/Instrument dependency of FT4 measurement results**

| Kit/System | Euthyroid RI (pmol/L) | Interval (pmol/L) |
|---|---|---|
| A | 7.6 – 15.1 | 7.5 |
| B | 8.9 – 23.3 | 14.4 |
| C | 12.0 – 22.0 | 10.0 |
| D | 10.0 - 28.2 | 18.2 |

## Introduction

**Need for standardization: Legal requirements**

**Directive 98/79/EC**
**of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices.**

**EN/ISO 17511**
**In vitro diagnostic medical devices – Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials.**

**➔ Metrological traceability**

## Introduction

**Need for standardization: Scientific support**

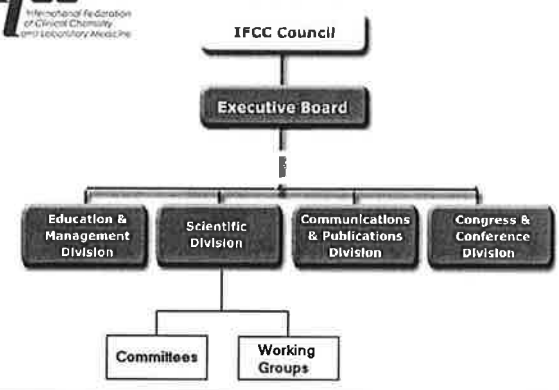
**National Academy of Clinical Biochemistry Guideline (2002): Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease.**

**Clinical and Laboratory Standards Institute C45-A (2004): Measurement of Free Thyroid Hormones; Approved guideline.**

**College of American Pathologists Analytic Bias of Thyroid Function Tests. Arch Pathol Labor Med 2005;129:310-7.**

**IFCC Working Group on STFT**

## Structure of the IFCC




## Scientific Division

**Mission statement**

**Instigate and promote developments in the field of standards and standardization in laboratory medicine**

**Committees & Working Groups**

- **Committees are "theme oriented"**
- **Working Groups are "task oriented"**
  - **Working Group on Standardization of Thyroid Function Tests: WG-STFT**

## WG-STFT members

- Prof. Dr. L.M. Thienpont, Chair (Gent, BE)
- Prof. Dr. J. Thijssen (Utrecht, NL)
- Prof. Dr. C. Ronin (Marseille, FR)
- Mr. R. Miller (Dade Behring, Newark, DE, US)
- Dr. M. Rottmann (Roche, Penzberg, DE)
- Dr. N. Christofides (Ortho-Clinical Diagn., Cardiff, UK)
- Dr. J. Faix (Stanford University Medical Center, CA, US)
- Dr. G. Miller (Virginia Commonwealth University, Richmond, US)
- Dr. B. Toussaint (JCR-IRMM, Geel, BE)
- Dr. G. Beastall (British Thyroid Association)
- Dr. H.A. Ross (European Thyroid Association)
- Prof. T. Ieiri (Japanese Thyroid Association)

## WG-STFT

"Terms of reference"
Develop reference measurement systems for thyroid function tests

Current project
Develop reference measurement systems for serum Total T4 (TT4) & Free T4 (FT4)

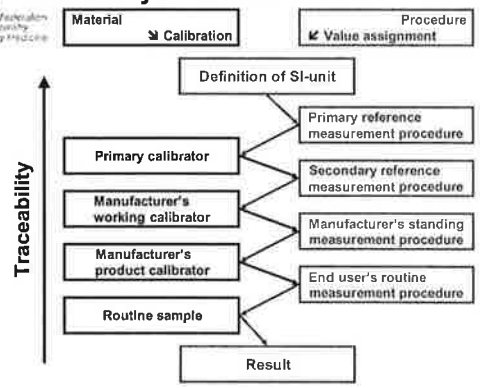
## Reference measurement system*

- Definition of the measurand
- Unit
- Primary calibrator
- Reference measurement procedure
- Reference materials
- Method comparison reference/routine

→Traceability chain

*See also:
Thienpont LM, Van Uytfanghe K, De Leenheer AP. Reference measurement systems in clinical chemistry [Review]. Clin Chim Acta 2002;323:73-87.

## Traceability chain – SI




## WG-STFT

Achievements

- TT4
- FT4

## Reference measurement system for TT4

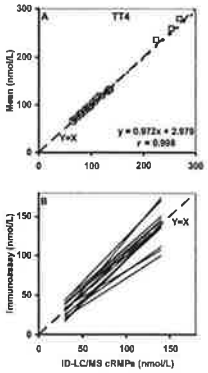
Available through EU-project G6RD-CT-2001-00587*

- T4 in serum/plasma
- nmol/L
- CRM IRMM-468 (http://www/irmm.jrc.be)
- ID-LC/MS reference measurement procedures performed within a Network of reference laboratories (UGent; UBonn; LGC; NIST)
- Serum reference materials

*See also:

- Thienpont LM et al. Metrologic traceability of total thyroxine measurements in human serum: efforts to establish a network of reference measurement laboratories. Clin Chem 2005;51:161-8.
- Thienpont LM et al. Feasibility study of the use of frozen human sera in split-sample comparison of immunoassays with candidate reference measurement procedures for total thyroxine and total triiodothyronine measurement. Clin Chem 2005;51:2303-11.

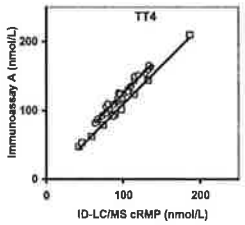
## Method comparison TT4*




*See also:
Thienpont LM et al. Feasibility study of the use of frozen human sera in split-sample comparison of immunoassays with candidate reference measurement procedures for total thyroxine and total triiodothyronine measurement. Clin Chem 2005;51:2303-11.

## Method comparison TT4

**Commutability problems**




○ EU panel
□ Fresh serum

- **Problems also observed with FT4 measurement**

## Commutability – Pilot study

**Participants**
- **Dade Behring (Newark, US)**
- **Roche (Penzberg, DE)**
- **Ortho Diagnostics (Cardiff, UK)**

**Single-donation sera**
- **Prepared by Solomon Park Research Inst. (Kirkland, US)**
- **CLSI C37-A***
- **Not filtered**

**Measurement of TT4 and FT4**
- **1 month**
- **3 months**
- **6 months**

*See also:
Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures (1999).

## CLSI C37-A

**Production – Some details**
- **Blood collection with plastic blood bag (ice water) (avoid contact with plastic, lipoprotein alteration)**
- **Centrifugation of cold blood bags (➔ plasma with adequate platelets for clotting)**
- **Aseptic transfer of plasma into sterile glass bottles**
- **Clotting**
- **Aseptic separation of serum**
- **[Filtration (critical analytes measured before/after)]**
- **Aliquoting (in borosilicate glass vials with Teflon®-lined caps) and freezing at –70°C**

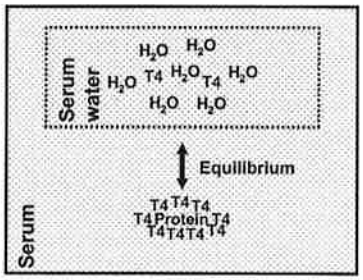
**All manipulations in one center within 48h**

## Reference measurement system for FT4

- **Definition of the measurand**
- **Unit**
- **Primary calibrator**
- **Reference measurement procedure**
- **Reference materials**
- **Method comparison reference/routine**

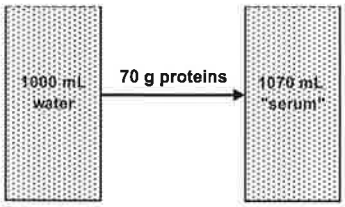
## Definition of the measurand

**T4 in serum water (= FT4)**



## The measurement system

**Serum water *vs* serum**




**Concentration** 100 units/L (water)

**Concentration** 93.5 units/L (serum)

➔ "Protein displacement effect"

## Unit

- pmol/kg serum water?
- pmol/L serum?

➔ Needs to be defined!

## Primary calibrator

**T4 CRM IRMM-468 (http://www/irmm.jrc.be)**

Institute for Reference Materials and Measurements

CERTIFIED REFERENCE MATERIAL
IRMM-468

CERTIFICATE OF ANALYSIS

| THYROXINE ($T_4$) | | |
|---|---|---|
| Substance | Mass fraction | |
| | Certified value [a] [%] | Uncertainty [b] [%] |
| Thyroxine ($T_4$) | 98.6 | 0.7 |

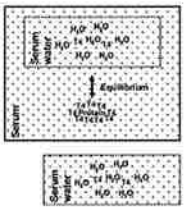
a) The certified value is the purity after taking into consideration inorganic residues, water, ethanol and organic impurities detectable by HPLC-UV and HPLC-MS. The certified value is traceable to the International System of Units (SI).

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

## Reference measurement procedure

**Are we able to measure FT4 trueness-based?**

**Current situation**




**Directly: NO**

**In <u>separated</u> serum water: YES (ID-MS)**

## Generation of serum water

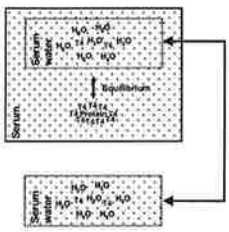
**Technically feasible**

**Ultrafiltation (UF)**

**Equilibrium dialysis (ED)**

## Generation of serum water

**What about the equilibrium?**




**Identical?**

**Unknown!**
**Separation may break the SI-traceability chain!**

## Generation of serum water

**EU-project: Investigate comparability between UF & ED**

**Work hypothesis**
**If it can be shown that UF and ED produce "serum water" with identical T4 concentrations, one can infer with sufficient probability that the serum water they generated is the "true" water fraction present in serum**

**Model sample**
- **T4 spiked albumin/buffer solution (~5 µM) with a FT4 fraction of ~1% (nM concentrations of FT4)**
- **Buffer: HEPES-buffer (pH = 7.4)**

## Comparability between UF and ED

**Results for model sample (n = 38)**
- **UF 10 K : highest results**
- **ED : -1.9%**
- **UF 3 K : -5%**

**Conclusion**
**Currently, there is no evidence that "true" serum water can be generated**

**➔ No trueness-based reference measurement procedure!**

## SI-traceability of FT4 measurements

**The whole truth and nothing but the truth?**

**Unfortunately NOT!**

## Standardization alternative

**Standard measurement procedure**

**Proposal by the WG-STFT***
- **Standard ED procedure**
- **ID-LC/tandem MS measurement of T4 in dialysate**

**➔ Needs to be agreed upon!**

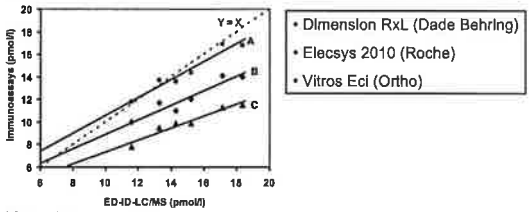
*See also:
Van Uytfanghe K, Stöckl D, Ross HA, Thienpont LM. Use of frozen sera for the purpose of FT4 standardization - Investigation by equilibrium dialysis combined with isotope dilution-mass spectrometry and immunoassay. Clin Chem (in press)

## Reference materials

- **Pilot study**

## Method comparison FT4

**Pilot study with C37-A prepared sera (without filtration)***




*See also:
Van Uytfanghe K, Stöckl D, Ross HA, Thienpont LM. Use of frozen sera for the purpose of FT4 standardization - Investigation by equilibrium dialysis combined with isotope dilution-mass spectrometry and immunoassay. Clin Chem (in press)

## Conclusion

Reference measurement systems

- TT4 : available
- FT4 : available
  - If there is agreement on the units and standard measurement procedure
  - If a 2nd reference laboratory can be involved

## Conclusion

Possible immediate action

- Preparation of a TT4 reference panel and method comparison

Reference panel

- ± 30 Selected single-donation sera
- 3 Sera supplemented with T4
- Prepared according to the CLSI C37-A protocol (without filtration)
- Aliquoted in 1-mL portions
- Certified by 2 RMLs
- Price quotation (n = 20): ± 5 000 USD ( 3x1-mL per serum)
- Shipment on dry ice

## Points of discussion

- Is the time ripe for standardization?
- Include TSH?
- Structure?
- Finance?
- Timeline?