

**IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)
Open meeting at AACC 2007, Monday July 16 (3:00 - 5:00 pm)**

PARTICIPANTS

The meeting attendance list is attached in annex.

STATUS REPORT (see also attached powerpoint presentation)

▪ *TT4/TT3*

Dr. Thienpont presented that the reference measurement system (RMS) for total T4/T3 is available as a result of the EU-project G6RD-CT-2001-00587 (2001-'05). The primary reference materials (1,2) and reference measurement procedures (RMPs) (3,4) are listed in the database of the Joint Committee for Traceability in Laboratory Medicine (JCTLM) (accessible at www.bipm.org/jctlm/BIPM). She concluded that in principle the working group (WG) can start the standardization process. She reported that in Euromedlab 2007 Prof. Ieiri (member of the WG) had reported that in Japan there is a general consensus, supported by the Japanese Committee for Clinical Laboratory Standards (JCCLS) and the Japan Thyroid Association (JTA), that thyroid function tests should be standardized.

▪ *FT4*

Dr. Thienpont reported that the WG recently had published 2 proposals (i) for the definition of the measurand FT4 (5) & (ii) for a candidate international conventional RMP for FT4 in serum (6) (pdf.files added in attachment). Candidate reference laboratories for the FT4 procedure are at the HECTEF Standard Reference Center Foundation (Japan; contact person (c.p.): M. Umemoto), at ARUP Laboratories (USA; c.p.: W.L. Roberts), and at Ghent University (Belgium; c.p.: L.M. Thienpont). They plan an interlaboratory comparison in the fall of 2007. If successful, a pilot study for standardization of FT4 measurements could in principle be initiated in 2008. Dr. Thienpont proposed that 40 single-donation sera would be measured in quadruplicate by Ghent University (UGent) with equilibrium dialysis (ED) isotope dilution-liquid chromatography/tandem mass spectrometry (ED-ID-LC/tandem MS) (7). Backup measurement would be done by a 2nd laboratory. Solomon Park Research Laboratories (Kirkland, WA) would be approached for blood collection and processing of the 40 sera according to the Clinical and Laboratory Standards Institute (CLSI) C37-A protocol (8). She also presented the budget needed for the pilot study. A summary of this part of the status report is given in the table below.

Reference measurement systems for TT4/TT3 and FT4 measurement		
	Total T4/T3 (TT4/TT3)	Free T4 (FT4)
Measurand	Plasma/Serum – Thyroxine/Triiodothyronine; substance concentration; (nmol/L)	Plasma/Serum – Thyroxine(free); substance concentration; pmol/L
Prim. reference material	Pure T4, T3 CRMs IRMM-468/469	Pure T4, CRM IRMM-468
Reference measurement procedure (RMP) /International conventional RMP	ID-LC/MS RMPs	Equilibrium Dialysis ID-LC/MS conventional RMP
Standardization procedure	Method comparison RMP/routine with single donation sera processed according to CLSI C37A I	Method comparison conventional RMP/routine with single donation sera processed according to CLSI C37A

- *TSH*

Dr. Thienpont reported on the brainstorming that the WG had undertaken to investigate the possibilities of standardizing the TSH assay. On the basis of the further discussions in the open meeting at Euromedlab 2007, 2 strategies were identified: (i) development of reference materials (e.g., recombinant TSH; specific glycosylation forms of TSH) based on the work of C. Ronin (9, 10), (ii) development of a RMP, either of the most representative intact TSH-form or based on peptide mapping. Any strategy would require that the standardization efforts be preceded by the definition of the measurand. Such a definition could follow the hemoglobin A1c example.

- *Implementation of standardization*

Dr. Thienpont reported on the status of the WG's intention to acquire statements from endocrine, thyroid, obstetricians & gynaecologists societies/associations regarding the need and the benefit of standardization of thyroid function tests. She recalled that indeed the WG had agreed to do so in response to an explicit demand from the IVD industry in the inaugural meeting. Apart from the fact that Dr. A. Ross (member of the WG) had reported in Euromedlab that the European Thyroid Association considers to establish a WG in this context, no progress had been made.

DISCUSSION

After the presentations, the discussion was opened on how to proceed.

- *TT4/TT3/FT4*: as in the past, there was again general agreement of all parties present that standardization would be desirable. In answer to the proposal by Dr. Thienpont to conduct a pilot standardization study for FT4, the representatives from IVD industry declared not to be able to make the decision on the spot, in particular because it supposed a financial commitment. Therefore, the chair agreed with them upon a strategy to acquire a declaration of interest (see below 'path forward'). It was suggested to also invite the routine laboratories, who recently introduced ED-ID-LC/tandem MS to substitute the FT4 immunoassay technology.

Again there was a general discussion on the fact that the standardization process should be a coordinated one, with a clear timeline, international implementation rules, and thoroughly discussed with all involved parties. The chair expressed the hope that, whenever possible, the members of the WG would do efforts to expand the platform to include endocrinologists, obstetricians/gynaecologists and other parties interested in standardization with the perspective to release guidelines for use/interpretation of thyroid function tests. The 'Endocrine Society Position Statement Calls for Standardized Testosterone Assays' was referred to as a model.

- *TSH*: It was expressed that, strictly speaking, standardization of TSH measurements would have priority in view of the analyte's eminent diagnostic importance. However, considering that development of the proposed strategies may be problematic and need a development time of several years, no short term actions were planned.

WAY FORWARD

Technical issues to be addressed by the WG

- Interlaboratory (UGent-HECTEF-ARUP) comparison of FT4 measurements in fall 2007.
- Dr. Thienpont will write a proposal for the pilot standardization study and make it available to her contact persons in the different IVD companies.

Issues related to agreement on standardization and its implementation

- Pursue efforts to identify clinical needs for standardization of thyroid function tests and to establish 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.

Dr. Linda Thienpont, UGent, August 06, 2007
Tel. +32/9/264 81 04; e-mail: linda.thienpont@ugent.be

References

1. Toussaint B, Klein CL, Wiergowski M. The certification of the mass fraction of thyroxine in a CRM intended for calibration: Certified Reference Material IRMM-468. Report EUR 21872 EN. Luxembourg: Office for Official Publications of the European Communities, 2006.
2. Toussaint B, Klein CL, Wiergowski M. The certification of the mass fraction of 3,3',5-triiodothyronine in a CRM intended for calibration: Certified Reference Material IRMM-469. Report EUR 21893 EN. Luxembourg: Office for Official Publications of the European Communities, 2006
3. Thienpont LM, Van Uytfanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, Bunk D, Tai S. Metrologic traceability of total thyroxine measurements in human serum: efforts to establish a network of reference measurement laboratories. *Clin Chem* 2005;51:161-8.
4. Thienpont LM, Van Uytfanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, Bunk D, Tai S. 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 measurements. *Clin Chem* 2005;51:2303-11.
5. Measurement of free thyroxine in laboratory medicine – proposal of measurand definition. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) IFCC Scientific Division. Working Group for Standardization of Thyroid Function Tests (WG-STFT). Linda M. Thienpont, Graham Beastall, Nicholas D. Christofides, James D. Faix, Tamio Ieiri, W. Greg Miller, Richard Miller, Jerald C. Nelson, H. Alec Ross, Cathérine Ronin, Michael Rottmann, Jos H. Thijssen and Brigitte Toussaint. *Clin Chem Lab Med* 2007;45:563–4.
6. Proposal of a candidate international conventional reference measurement procedure for free thyroxine in serum. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). IFCC Scientific Division Working Group for Standardization of Thyroid Function Tests (WG-STFT). Linda M. Thienpont, Graham Beastall, Nicholas D. Christofides, James D. Faix, Tamio Ieiri, Véronique Jarrige, W. Greg Miller, Richard Miller, Jerald C. Nelson, Cathérine Ronin, H. Alec Ross, Michael Rottmann, Jos H. Thijssen and Brigitte Toussaint. *Clin Chem Lab Med* 2007;45: 934-6.
7. 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
8. Clinical Laboratory and Standards Institute (CLSI). Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline. CLSI document C37-A [ISBN 1-56238-392-2]. CLSI, Wayne, PA, 2004.
9. Morelle W, Donadio S, Ronin C, Michalski JC. Characterization of N-glycans of recombinant human thyrotropin using mass spectrometry. *Rapid Commun Mass Spectrom* 2006;20:331-45.
10. Donadio S, Pascual A, Thijssen JH, Ronin C. Feasibility study of new calibrators for thyroid-stimulating hormone (TSH) immunoprocudures based on remodeling of recombinant TSH to mimic glycoforms circulating in patients with thyroid disorders. *Clin Chem*. 2006;52:286-97.
11. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardization. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004;50:166-74.
12. The Endocrine Society. Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes. An Endocrine Society Clinical Practice Guideline.

Attachments (4)

1. Meeting attendance list
2. pdf.file of slides
3. pdf.file of *Clin Chem Lab Med* 2007;45:563–4
4. pdf.file of *Clin Chem Lab Med* 2007;45:934-6.

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



AACC Annual Meeting
& Clinical Lab Expo
JULY 15-19, 2007
SAN DIEGO, CA

3:00pm - San Diego Marriott Hotel and
5:00pm Marina - Manchester Room

Linda Thienpont
Linda.thienpont@ugent.be



Agenda

Introduction

Total thyroxine/triiodothyronine

Free thyroxine

- Measurand
- Reference measurement procedure
- Reference laboratories
- Clinical requirement for standardization

TSH

Way forward

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Introduction

WG-STFT "origin"

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 (FT4), and validation of reference measurement systems (procedure and material) for thyroxine (T4) and triiodothyronine (T3) in human serum. Brussels: European Commission, 2001.

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Linda M Thienpont - AACC 2007



Introduction

Working Group Standardization of Thyroid Function Tests (WG-STFT) "**terms of reference**"

- Development of new reference materials and reference measurement systems for thyroid hormones (total T4 and T3; free T4 and T3) and TSH
- Investigation of the use of synthetic or recombinant materials for mass calibration of TSH immunoassays

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Introduction

Inaugural meeting: AACC 2006 – July 24

Introduction of the members of the WG-STFT

- Academics; IRMM (JRC, EC)
- Thyroid Associations
- IVD-manufacturers

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WG-STFT members

Dr. L.M. Thienpont, Chair (Gent, BE)
J. Thijssen (Utrecht, NL)
Dr. C. Ronin (Marseille, FR)
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)
Dr. T. Ieiri (Japan Thyroid Association)
Dr. J. Nelson (American Thyroid Association)
Mr. R. Miller (Dade Behring, Newark, DE, US)
Dr. M. Rottmann (Roche, Penzberg, DE)
Dr. N. Christofides (Ortho-Clinical Diagn., Cardiff, UK)
Dr. V. Jarrige (Beckman-Coulter Eurocenter, Nyon, Switzerland)
Dr. F. Quinn (Abbott Diagnostics, Abbott Park, IL, USA)

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Introduction

Aims

- Defining the measurands
- Approving/developing the technical means for a complete reference measurement system (primary calibrator; reference measurement procedure; reference laboratory)
- Implementing the reference measurement system in a concerted action together with all parties involved in patient care (for example: laboratory and clinical profession; manufacturers; patient organisations; regulatory authorities).

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Total thyroxine/triiodothyronine

Measurand: compound (analyte)

T4

T3

Amount-of-substance concentration in serum/plasma

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Total thyroxine/triiodothyronine

Reference materials

- Toussaint B, Klein CL, Wiergowski M. The certification of the mass fraction of thyroxine in a CRM intended for calibration: Certified Reference Material IRMM-468. Report EUR 21872 EN. Luxembourg: Office for Official Publications of the European Communities, 2006.
- Toussaint B, Klein CL, Wiergowski M. The certification of the mass fraction of 3,3',5-triiodothyronine in a CRM intended for calibration: Certified Reference Material IRMM-469. Report EUR 21893 EN. Luxembourg: Office for Official Publications of the European Communities, 2006.

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Total thyroxine/triiodothyronine

Reference measurement procedures/Network of reference laboratories

- Thienpont LM, Van Uytvanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, Bunk D, Tai S. 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, Van Uytvanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, Bunk D, Tai S. 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 measurements. *Clin Chem* 2005;51:2303-11.

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Total thyroxine/triiodothyronine

Complete reference system available

See database of the
Joint Committee for Traceability in Laboratory
Medicine
→ www.bipm.org/jctlm/

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Free thyroxine – Measurand

Measurement of free thyroxine in laboratory medicine – proposal of measurand definition International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

IFCC Scientific Division
Working Group for Standardization of Thyroid Function Tests (WG-STFT)

Linda M. Thienpont, Graham Beastall, Nicholas D. Christofides, James D. Faix, Tamio Ieiri, W. Greg Miller, Richard Miller, Jerald C. Nelson, H. Alec Ross, Cathérine Ronin, Michael Rottmann, Jos H. Thijssen and Brigitte Toussaint

Clin Chem Lab Med 2007;45:563-4.

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Free thyroxine

Clinical requirement for standardization

one
silence
for thyroxine

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Free thyroxine

Clinical requirement for standardization

THE ENDOCRINE SOCIETY
Endocrine Society Position Statement Calls for Standardized Testosterone Assays

Why not for FT4?

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Free thyroxine

Way forward?

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Thyroid stimulating hormone

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.

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Thyroid stimulating hormone

POINT
"Insofar as the antigenic substances present in standards or test samples are dissimilar and/or molecularly heterogeneous, an immunoassay is invalid, and the results it yields have no universal significance. Attempts to standardize "analytically-invalid" immunoassays inevitably fail. It is thus impossible to "measure TSH". The only long-term solution to this problem is the development of assay systems measuring individual components of such heterogeneous mixtures".

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

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Thyroid stimulating hormone


COUNTERPOINT
Standardizing immunoassays for heterogeneous analytes: opt for a practical solution. Define a surrogate compound (analyte) that is present in all members of the heterogeneous mixture. This has been done, for example, for the triacylglycerides (also a heterogeneous mixture of mono-, di- and triglycerides, in addition esterified with different fatty acids): the surrogate analyte is glycerol. This example seems to work for the intended clinical purpose.


Thienpont LM, Van Uytanghe K, De Leenheer AP. Reference measurement systems in clinical chemistry. *Clin Chim Acta* 2002;323:73-87.

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**Thyroid stimulating hormone
Brainstorm**

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




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**Thyroid stimulating hormone
Outcome brainstorm**

Standardize

- Preferably with reference measurement procedure
 - Option 1: Most representative intact TSH-form
 - Option 2: Surrogate from “peptide mapping”
- Note: may take 3 to 5 years
- [Consensus-based on “mean of current values”]


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**Thyroid stimulating hormone
EU-project G6RD-CT-2001-00587**


Morelle W, Donadio S, Ronin C, Michalski JC.
Characterization of N-glycans of recombinant human thyrotropin using mass spectrometry. *Rapid Commun Mass Spectrom* 2006;20:331-45.

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


Donadio S, Morelle W, Pascual A, Romi-Lebrun R, Michalski JC, Ronin C. Both core and terminal glycosylation alter epitope expression in thyrotropin and introduce discordances in hormone measurements. *Clin Chem Lab Med* 2005;43:519-30.

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**Thyroid stimulating hormone
Clinical requirement for standardization**

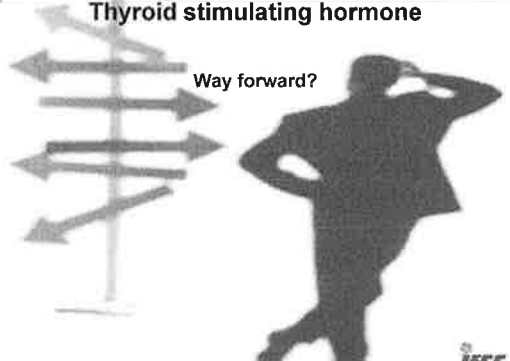



Known clinical guidelines/recommendations?

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Thyroid stimulating hormone

Way forward?




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Way forward

Clinical requirements for standardization

- The Japan Thyroid Association (JTA) will support standardization of thyroid function tests.
- The European Thyroid Association (ETA) considers to establish a working group on the standardization of FT4 assays.

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Way forward – Free T4

Free T4 panel

- 40 Single-donation sera (3 mL of each serum to provide per manufacturer)
- Prepared according to CLSI C37-A protocol (Solomon Park Research Laboratories, Kirkland, WA)
- Measurement of sera in quadruplicate by ED-ID-LC/tandem MS at UGent
- Backup measurement by a 2nd laboratory
- Estimated cost: ~95 000 USD
- 15 Participants: ~6 350 USD/participant