

**IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)
Open meeting at Euromedlab 2007. Monday June 4 (9:00-13:00)**

PARTICIPANTS

The meeting was attended by 12 persons from academia, industry and clinical societies (see attached list).

REPORTS

Status report of L. Thienpont (see also attached powerpoint presentation)

FT4

The working group recently published a proposal for the definition of the measurand FT4 (1) and a proposal for a candidate international conventional reference measurement procedure for FT4 in serum (2). Candidate reference laboratories for the FT4 procedure are at the HECTEF Standard Reference Center Foundation (Japan; contact person: M. Umemoto), at ARUP Laboratories (USA; contact person: W.L. Roberts), and at the University of Ghent (Belgium; contact person: L.M. Thienpont). This group plans an interlaboratory comparison in the fall of 2007.

TSH

The group undertook brainstorming to investigate the possibilities of standardizing the TSH assay. Note that this topic was also intensively discussed at the meeting (see below).

Report of T. Ieiri on the Japanese efforts for standardizing thyroid function tests

In Japan, there is a broad consensus that thyroid function tests should be standardized. Support comes, for example, from the Japanese Committee for Clinical Laboratory Standards (JCCLS) and the Japan Thyroid Association (JTA). Recently, a reference measurement procedure for free thyroxine (FT4) has been implemented at the HECTEF Standard Reference Center Foundation (contact: M. Umemoto, President & Chairman of the Board).

Report of A. Ross on initiatives to get support from clinical societies

The European Thyroid Association (ETA) considers to establish a working group on the standardization of the FT4 assay.

DISCUSSION

Future meetings

In view of the small number of participants, it was proposed to intensify the announcement of future meetings. For the AACC 2007 meeting in San Diego (Monday, July 16; 3:00pm - 5:00pm, San Diego Marriott Hotel and Marina - Manchester Room), information to manufacturers shall be disseminated through the industry division of the AACC. It was also proposed to consider dedicated manufacturer workshops. Further, a more active participation of the clinical societies/thyroid associations shall be pursued, including Asian-Ocean and Latin American societies.

FT4

Manufacturers are interested in a pilot method comparison study with the candidate international conventional reference measurement procedure. This would give the manufacturers the opportunity to assess where "their assays stand versus equilibrium

dialysis – mass spectrometry”. L. Thienpont will make a proposal for such a study based on 40 single donations.

There was also general interest to look into the quality of current FT4 assays. The working group could serve as a platform for developing tools for manufacturers to address current measurement issues and to deliver up-to-date assays for the laboratory medicine community. For example, to make clinically important samples available to manufacturers.

TSH

For the standardization of the TSH assay 2 strategies were proposed. The first strategy was based on the development of reference materials (e.g., recombinant TSH; specific glycosylation forms of TSH) based on the work of C. Ronin (3, 4). This would allow a basic calibration of the assay and an investigation of recognized epitope, specificity, and cross-reactivities. The second strategy was based on the development of a reference measurement procedure, preferably based on peptide mapping. Such a procedure, however, may need a development time of 3-5 years. In any case, standardization efforts should be preceded by the definition of the measurand. Such a definition could follow the hemoglobin A1c example. Further discussion is needed before moving forward. Nevertheless, the opinion was expressed that the variation between TSH assays is not that big, in practice, and that standardization may be feasible. The only problem is, on which “numbers” to standardize. No short term actions were planned for this topic.

Similar to FT4, it was considered important to make clinically important samples available to manufacturers (for example, as defined in the NACB guideline, 5).

Besides the standardization issues, also measurement issues were considered important, in particular, the lower limit of quantitation of the assays.

A part of the discussion was devoted to the reference interval for TSH. However, this discussion relates to the activities of the group only if a common reference interval would be proposed. The decision about which reference individuals constitute the reference interval (and hence about defining the upper and lower limit of the interval) is beyond the scope of the group.

Structure of the group

There was a general discussion whether the platform of the group should be expanded to include thyroid associations, endocrinologists, and other parties interested in standardization of thyroid function tests. Models such as the US National Kidney Education Program were discussed, however, no concrete actions were taken.

REFERENCES

1. 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.
2. 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:in press.
3. 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.
4. Donadio S, Pascual A, Thijssen JH, 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.
5. The National Academy of Clinical Biochemistry: 2002. Laboratory Medicine Practice Guidelines. Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. Laurence M. Demers and Carole A. Spencer.

Open meeting "IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)".
 Euromedlab 2007. Venue: Rai congress Centre, Monday June 04, 2007

List of attendees

Name	Affiliation	Address
Linda Thienpont	Ghent University, Laboratory for Analytical Chemistry	Gent, Belgium
Greg Miller	Dept. of Pathology, Virginia Commonwealth University	Richmond, VA, USA
Michael Rottmann	Roche Diagnostics	Penzberg, Germany
Mike Minihan	Olympus	Lismeehan O'Callaghan's Mills Co. Clare, Ireland
Véronique Jarrige	Beckman Coulter Eurocenter,	Nyon, Switzerland
Gérard Baudino	BioMérieux	Lyon, France
Brigitte Toussaint	IRMM, Joint Research Centre, European Commission	Geel, Belgium
Alec Ross	Radboud University, Medical Centre, Nijmegen	Nijmegen, Netherlands

Name	Affiliation	Address
Uremoto Masao	Hectef, Standard Reference Center Foundation	Kawasaki, Japan
Tamio Ieiri	Dokkyo University School of Medicine, Japanese Thyroid Association	Tochigi 321-0293, Japan
Jos Thijssen	Laboratory of Endocrinology, University Medical Centre Utrecht	Utrecht, The Netherlands
Dietmar Stöckl	Ghent University, Laboratory for Analytical Chemistry	Gent, Belgium

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



Monday June 4 (9:00-13:00)

Linda Thienpont
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Inaugural meeting: AACC 2006 – July 24

Introduction of the members of the WG-STFT

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

Standardization of total & free thyroxine

- TT4 reference measurement system is available
- FT4 near completion

Way forward

- Integration of the American Thyroid Association
- Technical issues to be addressed by the WG

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Agenda

Status free thyroxine

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

Status TSH

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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 – Measurand

Proposal of measurand definition (IUPAC/IFCC format)
“System – component; kind-of-quantity”

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.

Component

Thyroxine that is not bound to proteins

Name: “Thyroxine(free)”; abbreviation: FT4

Kind-of quantity and unit

- “Amount-of-substance concentration”
- pmol/L

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Free thyroxine – Measurand

Full measurand

“Plasma/Serum – Thyroxine(free); substance
concentration”

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Free thyroxine – Procedure

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:xxx.

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Free thyroxine – Procedure

Candidate international conventional reference measurement procedure (FT4 in serum)

- Equilibrium dialysis
- Combined with determination of thyroxine in the dialysate with a trueness-based reference measurement procedure.
 - Isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/tandem MS)
 - Calibration with IRMM-468 (pure thyroxine)

Note

The measurand is thus operationally defined as “thyroxine in the dialysate from equilibrium dialysis of serum prepared under defined conditions”.


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Free thyroxine – Procedure

Candidate international conventional reference measurement procedure (FT4 in serum)

The WG-STFT stresses that according to this recommendation it is a prerequisite to strictly adhere to the defined equilibrium dialysis procedure,

whereas it is permissible to introduce variants in the ID-LC/tandem MS procedure.

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Free thyroxine – Laboratories

Candidate reference laboratories

- Ghent University (Belgium)
 (see: 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)
- HECTEF Standard Reference Center Foundation, Kawasaki (Japan) (Contact: M. Umemoto)
- ARUP Laboratories, Salt Lake City, UT (USA) (Contact: W.L. Roberts)

Foreseen activities

- Interlaboratory comparison in Sept.-Oct. 2007
- Pilot study

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Free thyroxine

Clinical requirement for standardization



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Free thyroxine

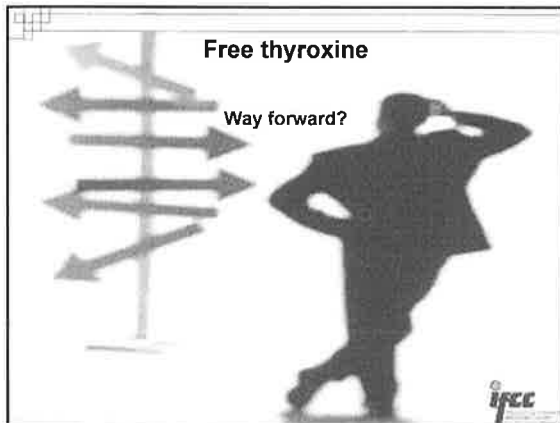
Clinical requirement for standardization



Endocrine Society Position Statement Calls for Standardized Testosterone Assays

Why not for FT4?

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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 Uytendaele 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.

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 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?

