70th AACC
ANNUAL SCIENTIFIC MEETING
& CLINICAL LAB EXPO
JULY 29-August 2, 2018 · Chicago, IL

Accurate Measurement of Thyroid Hormones in Disease and Pregnancy

Moderator: Ron Whitley, PhD

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

PATH
PARTNERSHIP for the ACCURATE TESTING of HORMONES

ENDOCRINE SOCIETY

AMERICAN THYROID ASSOCIATION
FOUNDED 1923

ENDOCRINOLOGY DIVISION | AACC
Better health through laboratory medicine

Diagnosis and treatment of thyroid disorders depend on accurate and reliable laboratory tests. This scientific session will explore the applications, short falls and future of thyroid hormone testing.

SPEAKERS

- Ron Whitley, Ph.D., University of Kentucky Medical Center, Lexington, KY
  - ***Facilitating the Transition to Accurate Measurement of Thyroid Hormones***
- Gregory Brent, M.D., UCLA and VA Greater LA Healthcare, Los Angeles, CA
  - ***Clinical Challenges of Thyroid Hormone Testing***
- Katleen Van Uytfanghe, Ph.D., AZ Sint-Jin Hospital , Brugge, Belgium
  - ***Standardization and Harmonization of Free Thyroxine (FT4) and Thyrotropin (TSH) Measurements***

Learning Objectives

After attending this session, you should be able to:

1. Understand the role of thyroid function biomarkers in clinical decision making.
2. Summarize the current state of thyroid hormone testing including analytical performance and its impact on patient care, research translation and public health.
3. Describe activities of organizations such as the IFCC to standardize and harmonize thyroid function tests.
4. Outline efforts to assess thyroid hormone test performance using certification and accuracy-based proficiency testing.
5. Appreciate the activities of national and international groups to improve the quality of thyroid hormone tests.

Which best describes your facility?

A Community hospital

B Reference or private laboratory

C University or children's hospital

D Public health agency

E Manufacturer or LIS vendor

Facilitating the Transition to Accurate Measurement of Thyroid Hormones

Ronald J. Whitley, PhD, DABCC, FAACC

Professor Emeritus, Pathology and Pediatrics

University of Kentucky Medical Center

Lexington, KY

HealthCare.

BEST HOSPITALS U.S.News & WORLD REPORT NATIONAL RANKED IN 4 SPECIALTIES 2017-18

Disclosure

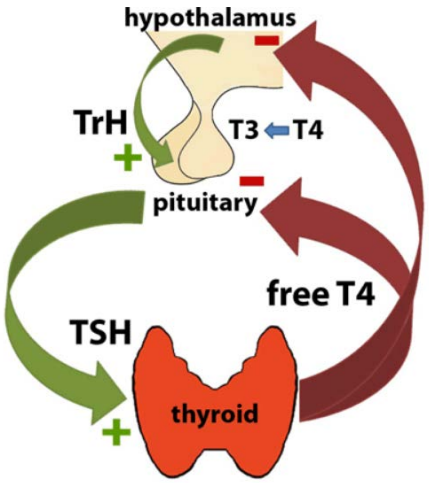
I have no commercial/financial relationships that relate to the topic of this presentation.

Outline

I. Thyroid function tests - principles and problems
II. Improving accuracy and reliability
III. Establishing metrological traceability
    A. Standardization vs. Harmonization
    B. Commutability of reference materials
IV. National/international efforts to improve accuracy
    A. IFCC/AACC
    B. JCTLM
    C. ICHCLR
    D. CDC
    E. PATH
V. Stakeholders in improving accuracy
VI. Why Can I do?

Thyroid Function Tests

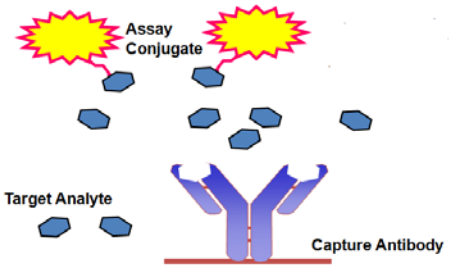
- Thyrotropin (TSH)
- Free Thyroxine (FT4)
- Total Thyroxine (TT4)
- Total Triiodothyronine (TT3)
- Thyroid Peroxidase Antibody (TPOAb)
- Thyroglobulin (Tg)
- Tg Autoantibody (TgAb)

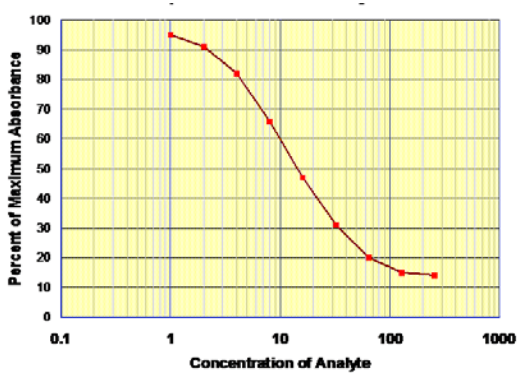


Assay Principles

- Immunoassay
  - Competitive:

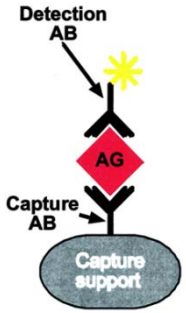
    TT4, TT3, FT4, FT3

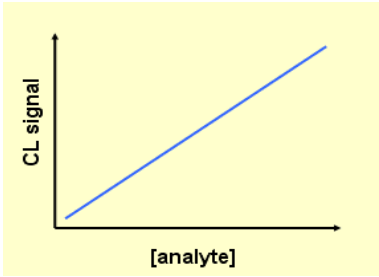




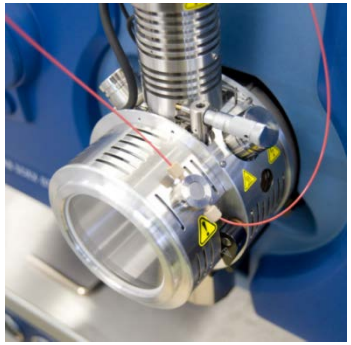
  - Immunometric (sandwich):

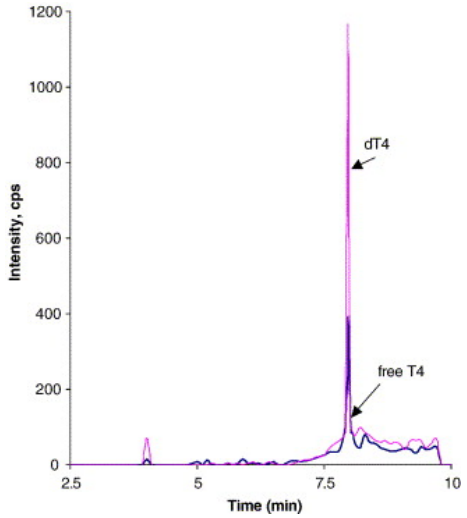
    TSH, Tg, TgAb,TPOAb





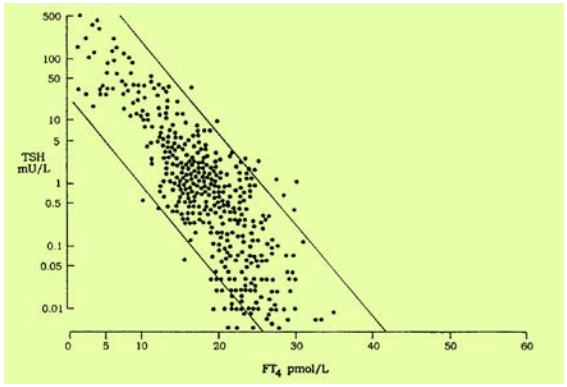
- Mass spectrometry
  - TT4, TT3, FT4, FT3





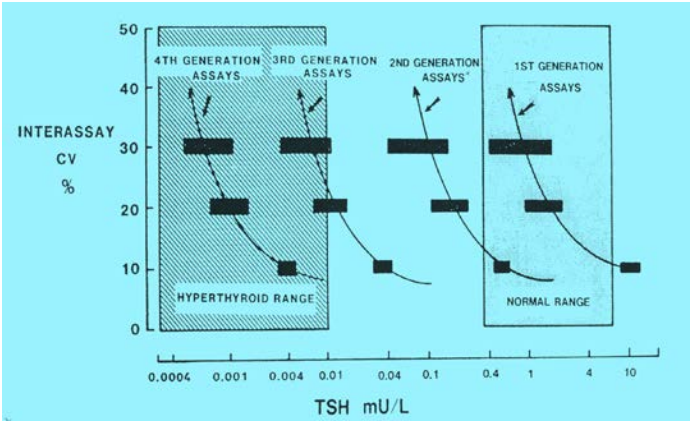
TSH

- Dramatic improvements in TSH assay sensitivity and specificity over last 4 decades
  - first-line test
    - superior to FT4
    - Inverse log/linear relationship



  - therapeutic target: replacement therapy for hypothyroidism, suppressive therapy for DTC

- Assays
  - automated ICMAs
  - 3rd generation sensitivity
    - Most labs don't monitor performance at 0.01 mIU/L
    - EQA/PT challenges not available at this limit

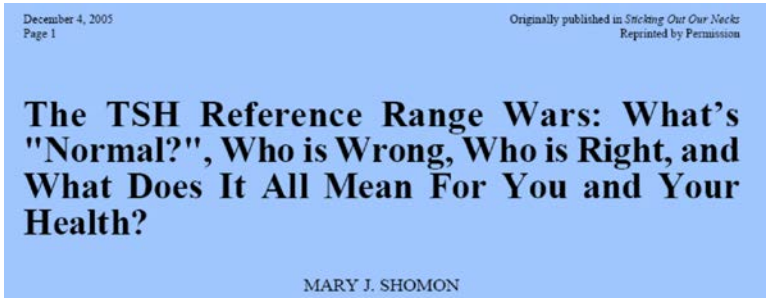


TSH

- Biological Variability
  - Relatively narrow intra-individual variability
- Reference Intervals
  - No universal population or trimester specific RI
    - Different methods report different ranges for the same population
    - Large numbers of rigorously screened participants needed to establish RI (e.g., exclude thyroid antibody-positive individuals); costly & inconvenient
    - Age, ethnicity, iodine intake, geography, lack of assay standardization may impact universal ranges
  - Consensus on lower limit: 0.2 to 0.4 mIU/L
  - Debate about upper limit: 2.5 to 6 mIU/L

The Evidence for a Narrower Thyrotropin Reference Range Is Compelling

Leonard Wartofsky and Richard A Dickey


December 4, 2005
Page 1

Originally published in *Sticking Out Our Necks*
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The TSH Reference Range Wars: What's "Normal?", Who is Wrong, Who is Right, and What Does It All Mean For You and Your Health?

MARY J. SHOMON

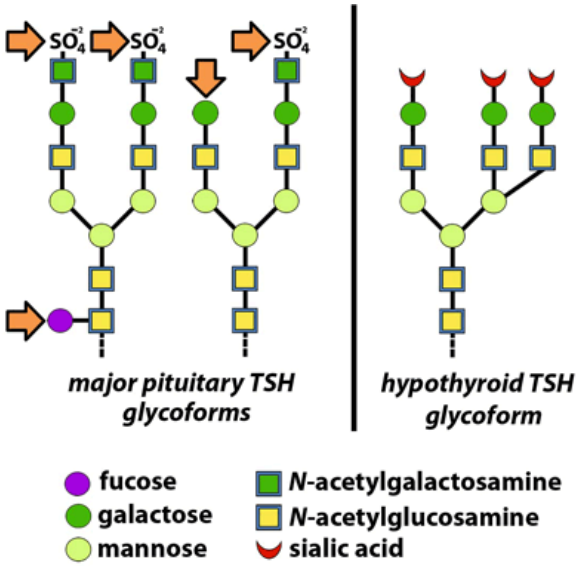
The Thyrotropin Reference Range Should Remain Unchanged

Martin I. Surks, Gayotri Goswami and Gilbert H. Daniels

  - Special cases
    - Pregnant women: trimester-specific RIs required
    - Newborns or children: adult RIs do not apply
    - Neonatal screening: many labs have switched to initial TSH screen using program-specific cutoffs

TSH Glycoforms

- TSH is a glycoprotein hormone
  - Normal pituitary contains primarily branched glycans terminating with sulfated residues

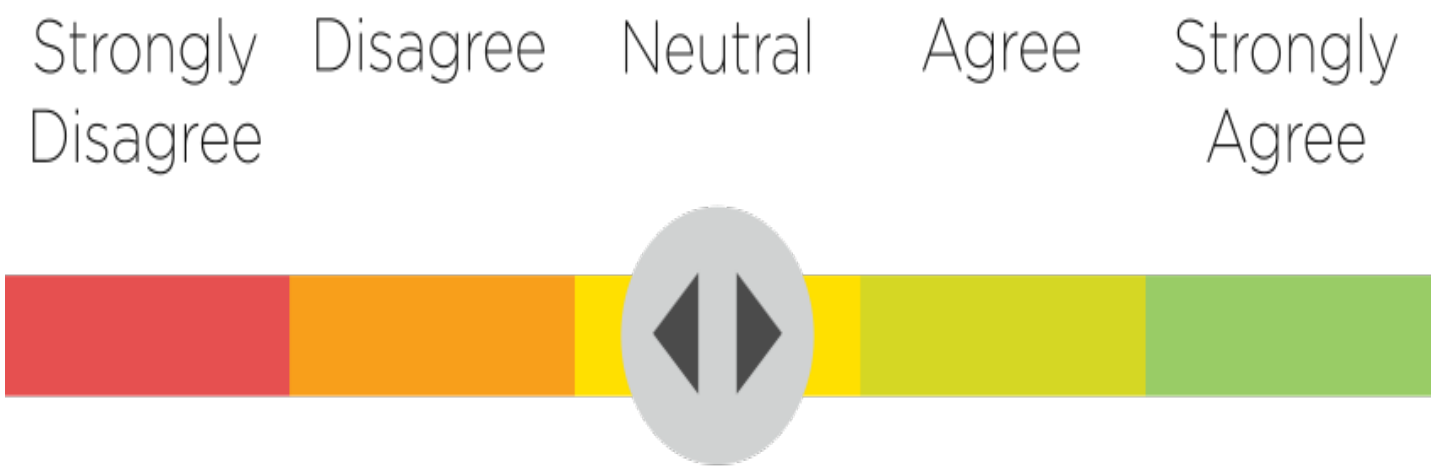


  - Hypothyroid patients have altered glycoforms that circulate longer in blood
    - Various monoclonal assays may capture different isoforms

Some Causes of Misleading TSH Measurements

- Biological factors
  - Acute illness
  - Pituitary/hypothalamic dysfunction
    - central hypothyroidism
    - TSH-secreting adenomas
  - Resistance to thyroid hormone
- Technical factors
  - Heterophile antibodies
  - TSH autoantibodies
  - Tests employing streptavidin-biotin

TSH assays performed by different laboratories have comparable results



TSH Between-Method Variability: Non-Commutable EQA/PT Samples

Test	Number Labs/Methods/ Mean	Lowest Result	Highest Result	Ratio H/L
TSH (0.4-4.2 mIU/L)	2851/19/0.472	0.390	0.604	1.5
TSH	2735/19/7.971	5.292	13.007	2.5
Free T4 (0.8-1.7 ng/dL)	2297/13, 1.07	0.73	3.25	4.5
Free T4	2101/13, 3.92	2.97	6.94	2.3
Total T4 (µg/dL)	1309/12, 2.73	4.03	1.74	2.3
Total T4	1292/12, 16.72	13.90	20.83	1.5
Total T3 (87-187 ng/dL)	744/9, 50.89	40.39	57.40	1.4
Total T3	773, 8	172.68	567.12	3.3

Data from: CAP Proficiency Testing Program, 2017 C-survey

TSH Between-Method Variability: Commutable EQA/PT Samples

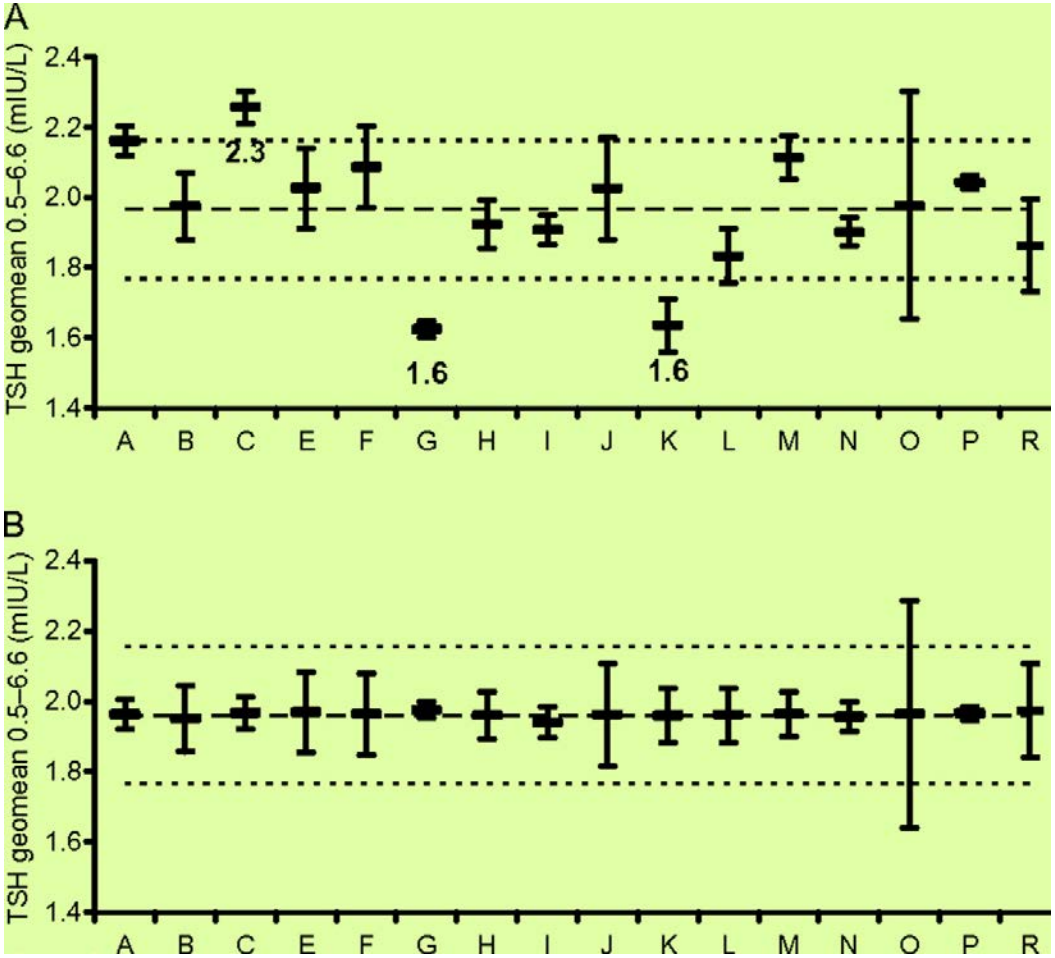
Table 1. Bias from the target value (Mt or the concentrations measured by the reference method[a]) for the concentrations of serum X.

	Serum X	Quality goal[b]	Abbott Architect	Beckman Coulter Unicel	Roche Cobas	Roche Modular	Siemens ADVIA Centaur
Cobalamin, pmol/L	329	±58	-0.15	**−108**	15	15	−21
Folate, nmol/L	14.0	±2.7	−1.4	−1.5	2.1	0.6	0.1
Ferritin, µg/L	62.4	±3.2	−1.5	**−13.8**	**9.4**	**11.1**	**−8.7**
TSH, mU/L	1.69	±0.13	−0.15	0.01	**0.16**	**0.20**	−0.03
Free T_4, pmol/L	14.3	±0.5	**−1.2**	**−3.1**	**0.8**	**0.7**	0.4
Free T_4, pmol/L[a]	19.7	±0.7	**−6.5**	**−8.4**	**−4.5**	**−4.6**	**−4.9**

[a] Reference method values [IFCC *(6)*].
[b] The quality goal (±absolute values) for desirable bias is based on biological variation data: cobalamin 17.7%, folate 19.2%, ferritin 5.2%, free T_4 3.3%, and TSH 7.8% *(11)*. Biases exceeding the quality goal are shown in bold.

Source: Kristensen Clin Chem 2016; 62:1255-63

Method Comparison Study of 16 TSH Immunoassays



Geometric means of the results before (A) and after (B) recalibration.
Thirteen assays gave results within 10% of the overall mean (dotted lines).

Source: Thienpont et al. Clin Chem 56:901-911 (2010)

Total Thyroid Hormones: TT4 and TT3

- Diagnostically unreliable due to numerous thyroid hormone binding protein abnormalities; rarely used as stand-alone tests
- Easier to measure than free
- TT3 concentrations are 10x lower than TT4

Immunoassays

- **Used by most labs; performed on automated platforms**
- **Use enzyme, fluorescence and chemiluminescent signal molecules**

ID-LC Tandem Mass Spectrometry

- **Reference method procedures (RMPs) available globally**
- **Primary reference standards available for calibration**

Between method variability → **Matrix differences between calibrators & patient sera, efficiency of blocking agents, lot-to-lot variability** → **Recalibrating methods against RMPs would be beneficial**

TT4 and TT3 Between-Method Variability: Non-Commutable EQA/PT Samples

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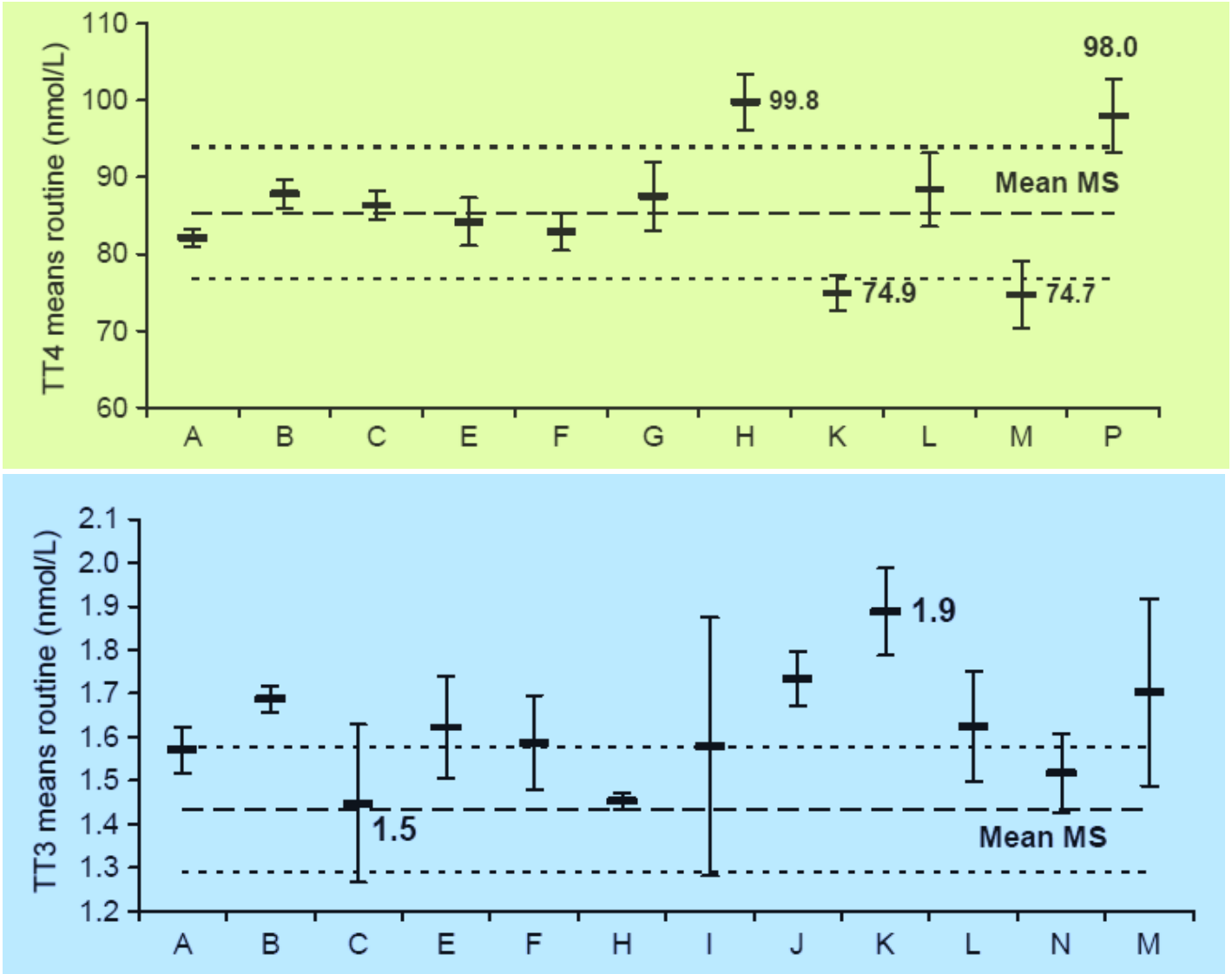
Data from: CAP Proficiency Testing Program, 2017 C-survey

Method Comparison Study of 11 TT4 and 12 TT3 Immunoassays vs. Tandem Mass Spectrometry (MS) using 40 serum samples



Assay means vs MS means. Dotted lines represent mean MS ± 10%.
4 of 11 TT4 assays >10% of MS mean; most TT3 assays have a positive bias

Free Thyroid Hormones: FT4 and FT3

- Free fraction of thyroid hormones exert biologic activity
- Free T4 measurement is preferred to total T4
- Total T3 usually suffices in preference to free T3

Direct Methods

- **Equilibrium dialysis (ED) or ultra filtration (UF) separate free from protein-bound hormone; prone to inaccuracies (under/over estimation)**
- **Reference method procedure for FT4 has been established by IFCC C-STFT (ED-ID-LC-MS/MS); technically demanding, inconvenient, expensive**

Indirect (estimate) methods

- **Total hormone measurements corrected for binding proteins with TBG or binding protein estimates**
- **Automated immunoassays employ antibody to sequester small amount of total that is proportional to concentration of free**
- **One-step; labeled antibody; two-step, back-titration**

Between method variability
(to be discussed by Dr. Uytfanghe)

Major between-method variability and significant biases relative to RMP far in excess of biological variation

Recalibrating method against RMP significantly reduces biases

What % of all health care decisions affecting diagnosis or treatment involve lab testing?

A 50%

B 60%

C 70%

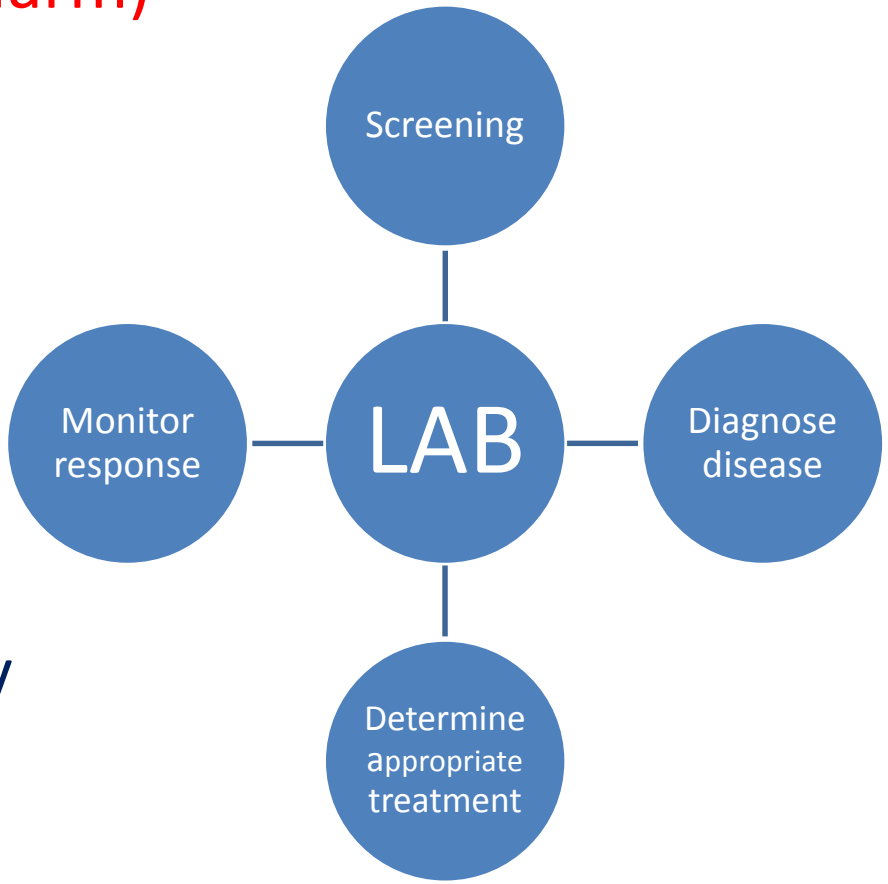
D 80%

E 90%

Primum non nocere

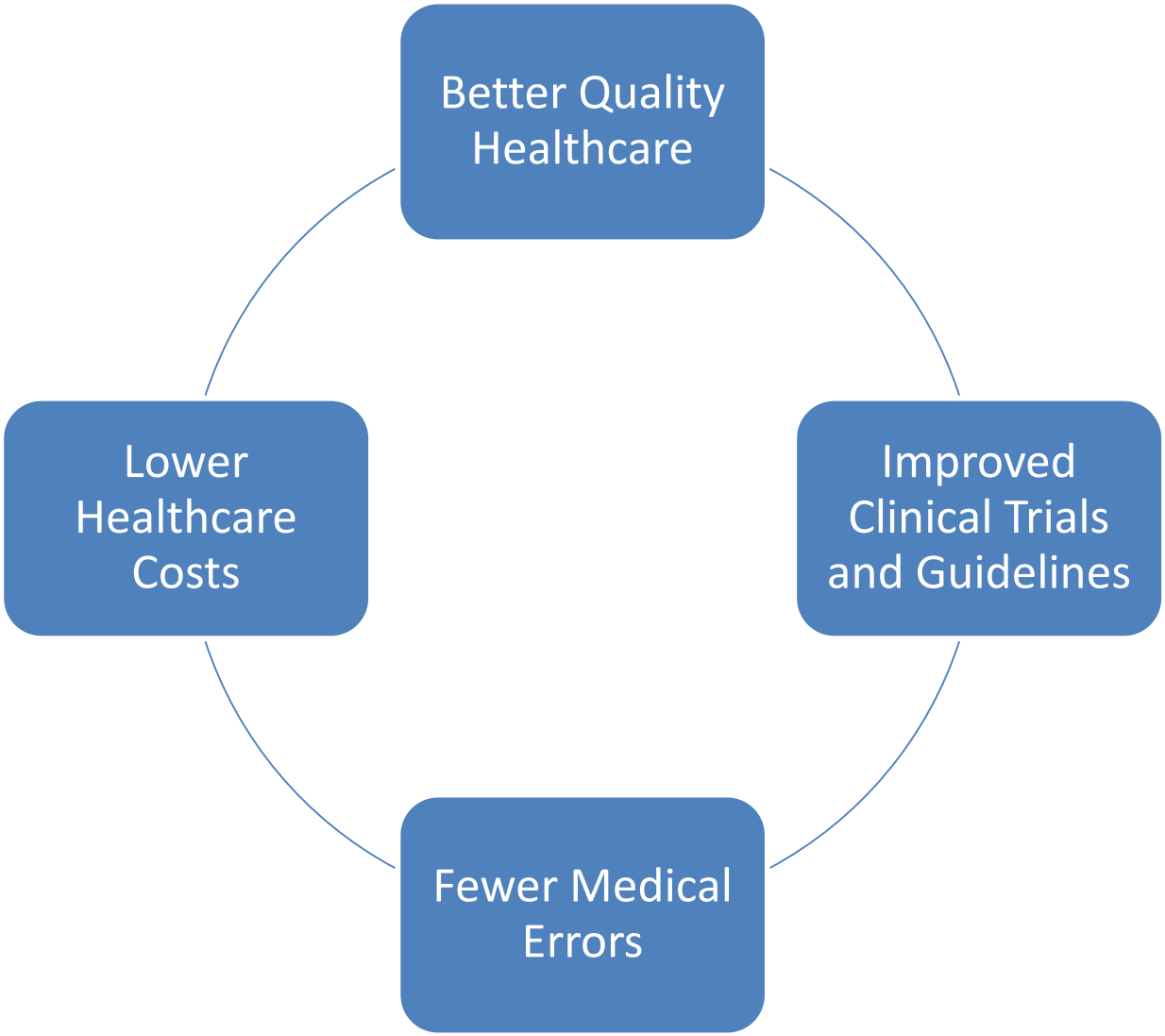
(Do No Harm)

- Clinical decisions often based on guidelines that use a fixed lab test result.
- Different assay methods that intend to measure the same hormone can give different results for the same patient specimen.
- International efforts underway to ensure test results are accurate and comparable across different measurement procedures, different locations and different times.



Right patient, right test, right result, right interpretation, right treatment

Important to Reduce Test Inaccuracy and Variability



Why do thyroid hormone assays give different results on a patient sample?

- IVD manufacturers
  - Different specimen requirements, methods, signal detection systems
- Reagent components
  - Different calibrators, enzymes, substrates, antigens, antibodies
- Assay conditions
  - Different reaction times, pH, temperature, software, curve fits, pre-analytical factors
- Targets
  - Different quotes for precision and accuracy

Improving Accuracy and Reliability

- Limitations of immunoassays for thyroid function tests are well known.
- Thyroid hormones (e.g., TT4 and FT4) are more accurately measured by mass spectrometry.
  - Technique for FT4 is demanding and equipment is expensive
  - Not practical for every hospital to perform free T4 measurements by mass spectrometry
- Thyroid hormone immunoassays can be improved by tracing calibration to a common reference system consisting of reference methods and materials.

Establishing Metrological Traceability

"The property of the result of a measurement, or the value of a standard, whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties."

EU Directive (IVDD; 2003)

- Represented a major step forward in promoting/driving international assay standardization/harmonization
- Applied to all manufacturers in the EU who provide calibrators for IVD devices
- New 2017 EU regulation (IVDR) requires traceability and appropriate accuracy with increased requirements for clinical evidence

Traceability

* Traceability requires both reference method procedures and certified reference materials
* For simple analytes (e.g., thyroxine) it is possible to get pure substance primary materials
* For complex analytes (e.g., TSH) pure substance materials may not be available
* Primary reference measurement procedures are often based on mass spectrometry

Reference measurement procedures

* **Primary reference measurement procedure**
* **Secondary reference measurement procedure**
* **Manufacturer measurement procedure**
* **Routine laboratory procedure**

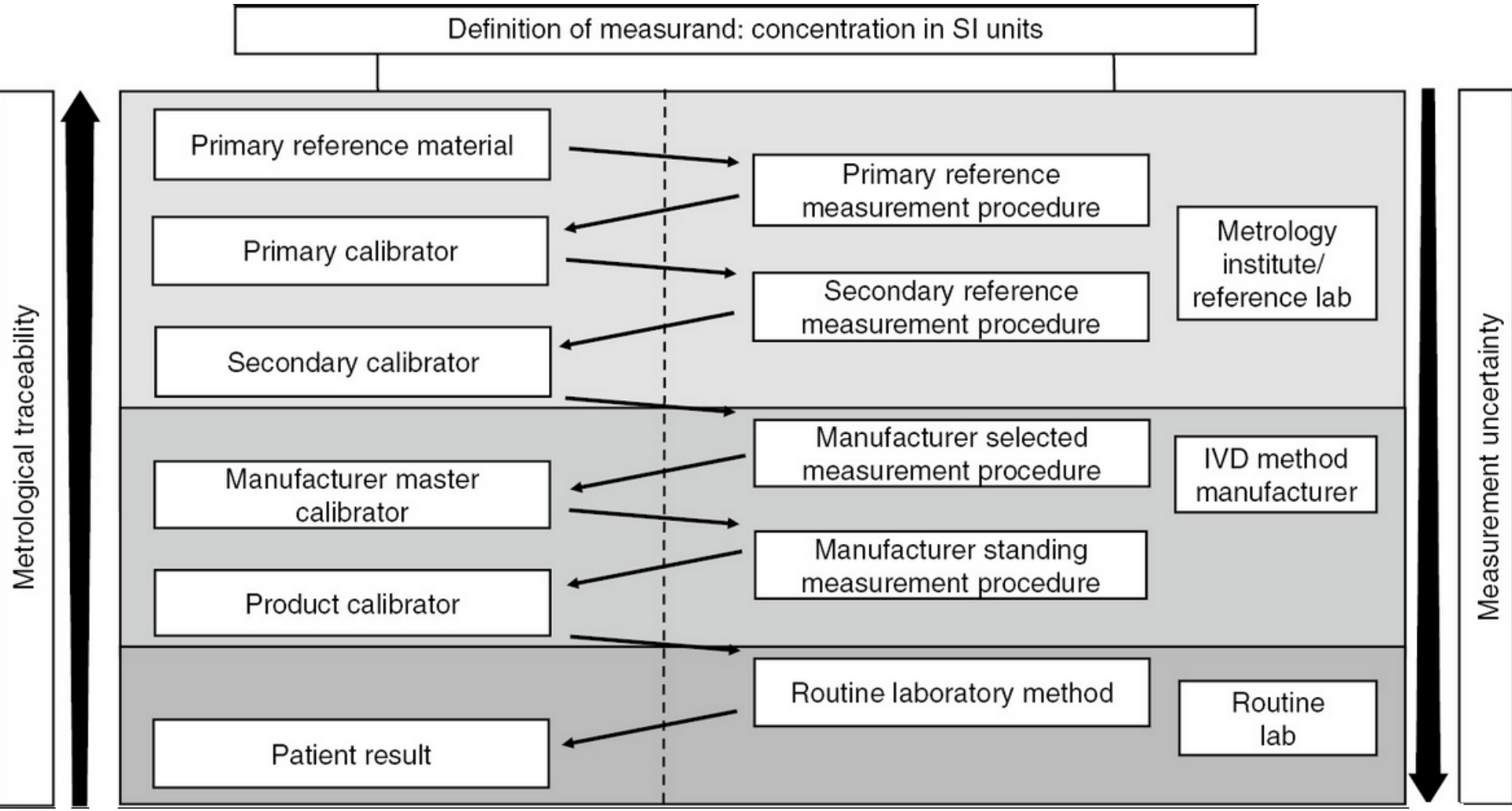
Reference materials (calibrators)

* Primary reference material
* Primary calibrator
* Secondary matrix-matched calibrator (serum)
* International conventional calibrator
* Manufacturer's calibrator (master or product)

Reference measurement laboratories

* Use primary reference methods & materials
* Are certified/accredited as calibration labs
* Participate in inter-laboratory comparisons

Metrological Traceability Chain



Adapted from: EN ISO 17511:2003

Traceability Categories:
Standardization vs. Harmonization

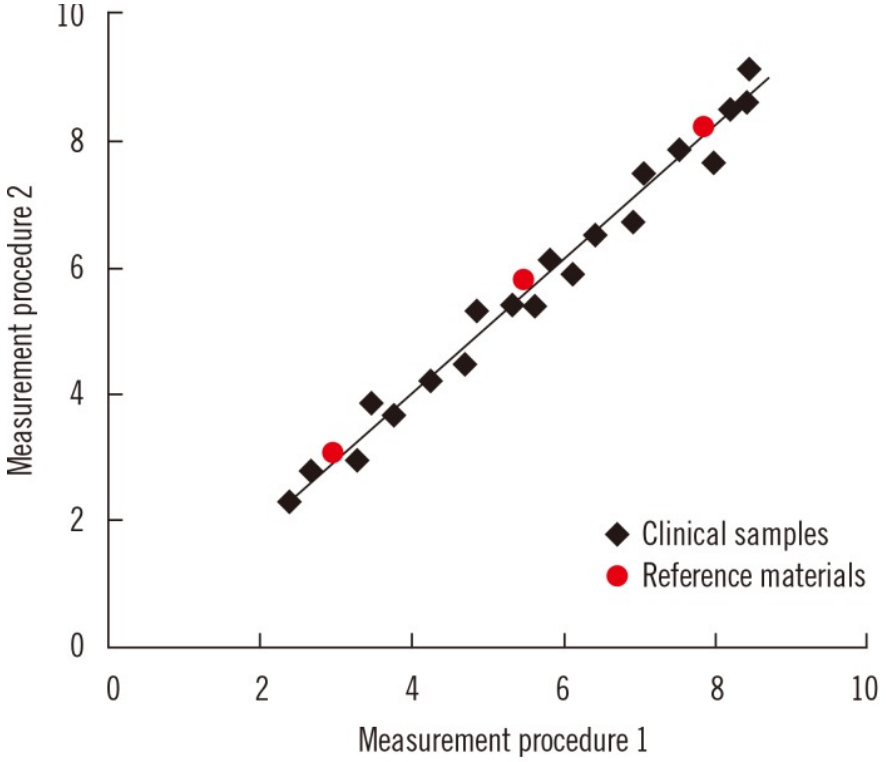
Category	Reference measurement procedure	Primary (pure substance) reference material	Secondary (value assigned) reference material	Examples
1	Yes	Yes	Possible	Electrolytes, glucose, cortisol
2	Yes	No	Possible	Enzymes
3	Yes	No	No	Hemostatic factors
4	No	No	Yes	Proteins, tumor markers, HIV
5	No	No	No	Proteins, EBV, VZV

Standardization

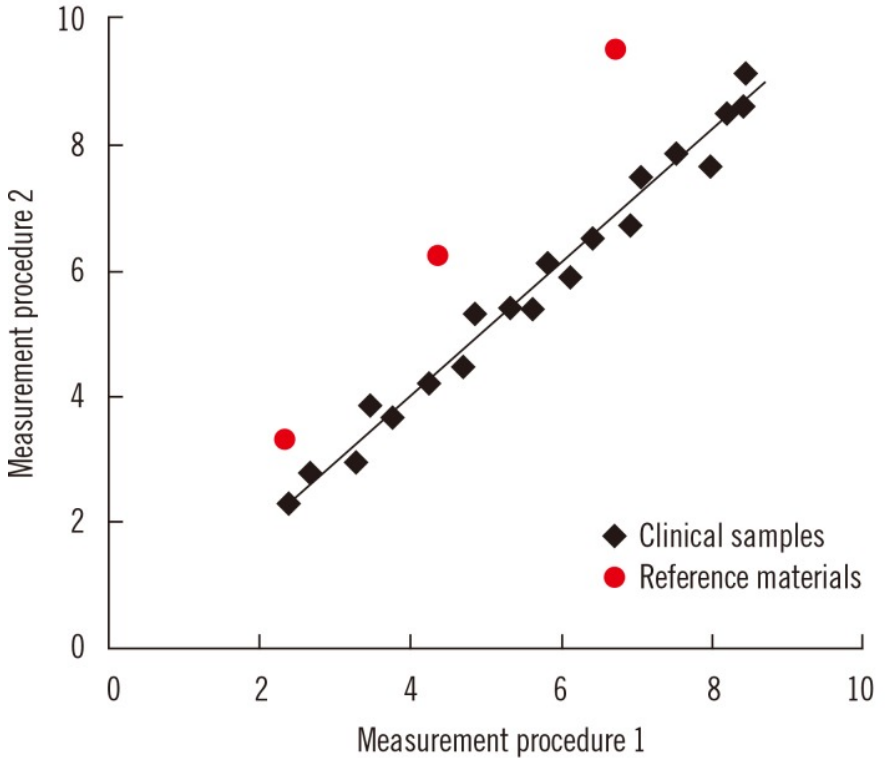
Harmonization

Adapted from: Miller Clin Chem 2011; 57:1108-17

Commutability of Reference Materials



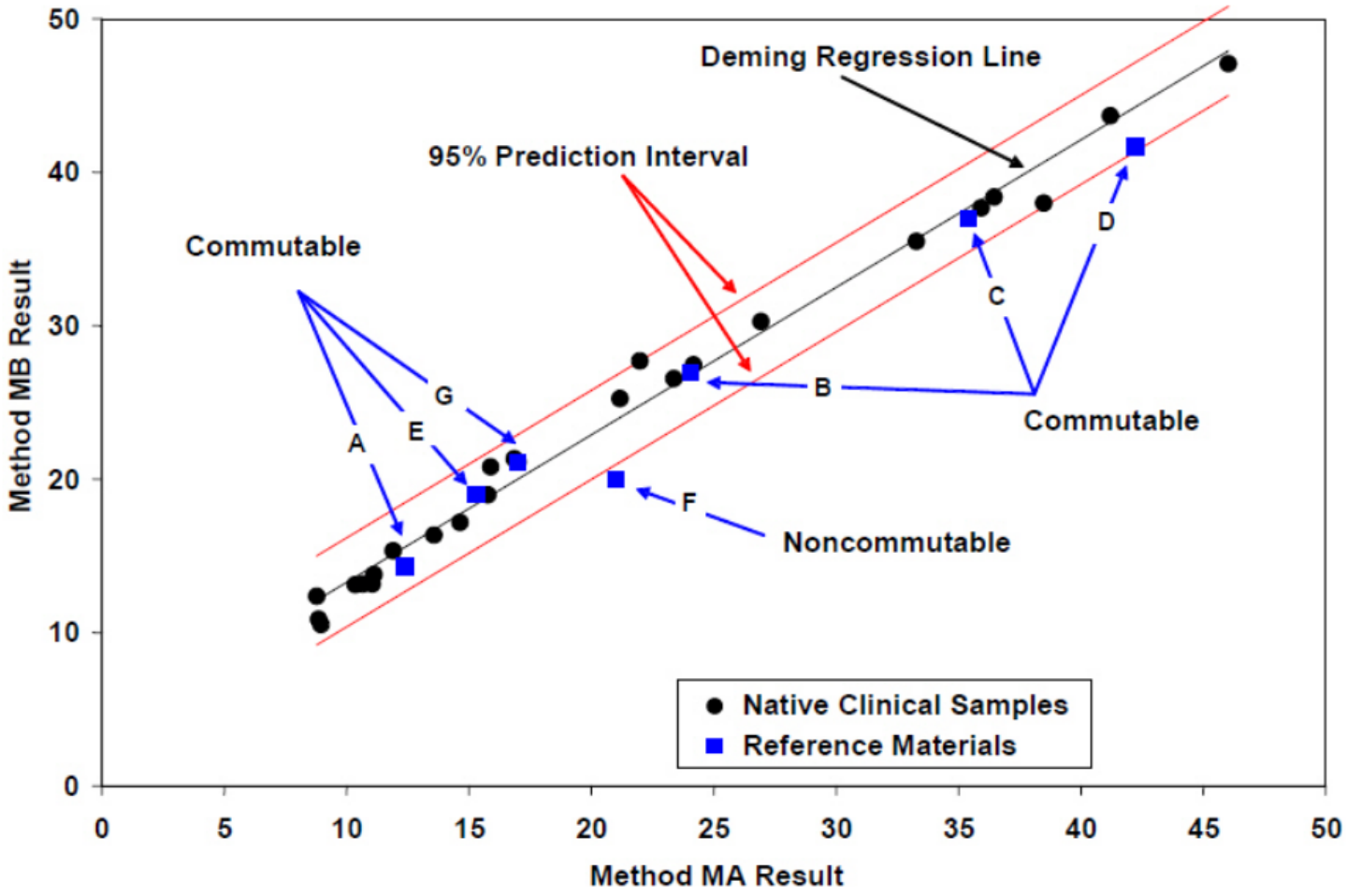
The three reference materials are commutable and yield results that are consistent with clinical samples.



The three reference materials are non-commutable and yield results that are inconsistent with clinical samples.

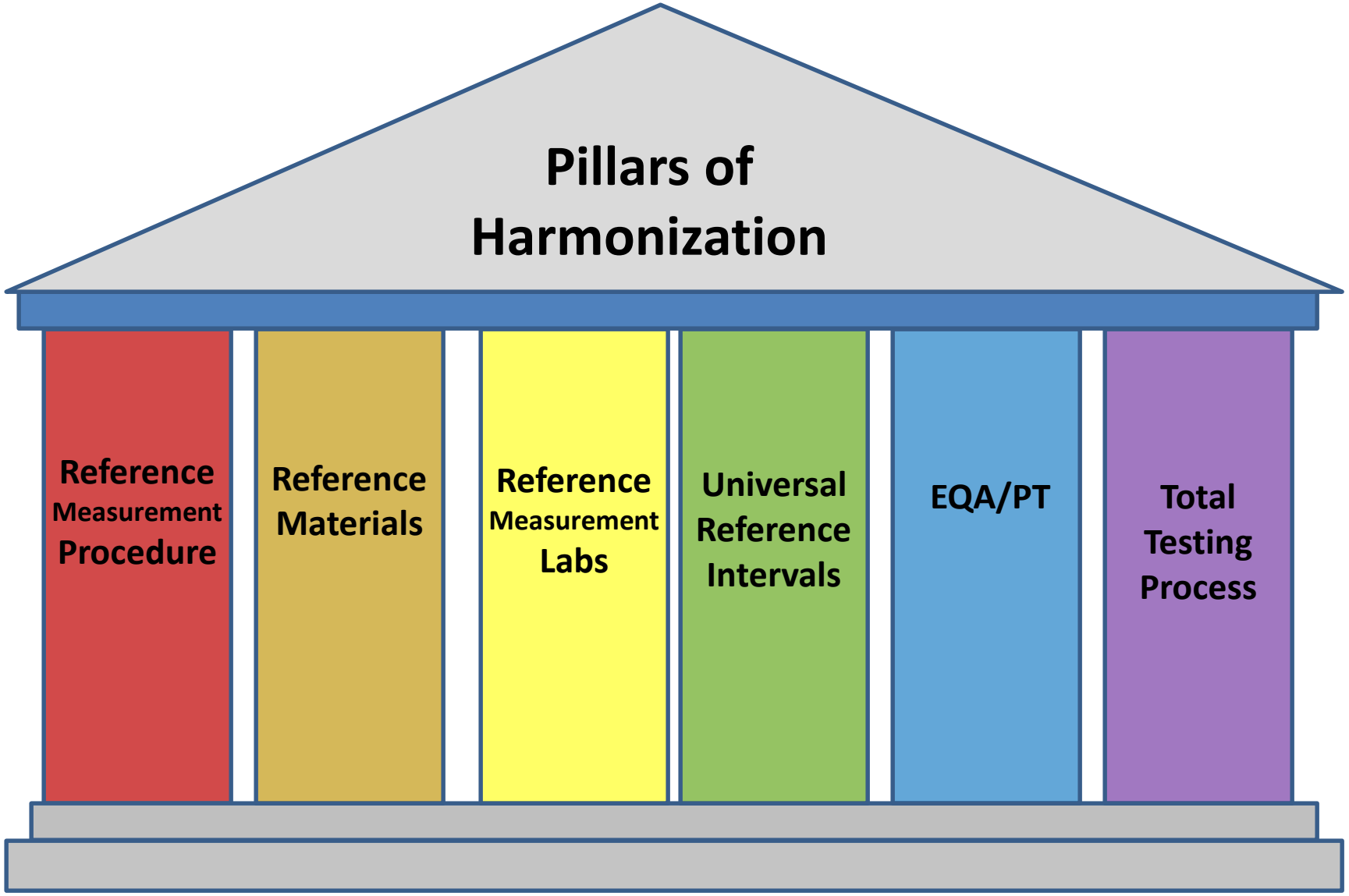
Source: Vesper Am J Clin Nutr 2016; 104: 907-12

Use of Regression Analysis to Evaluate Commutability of Reference Materials



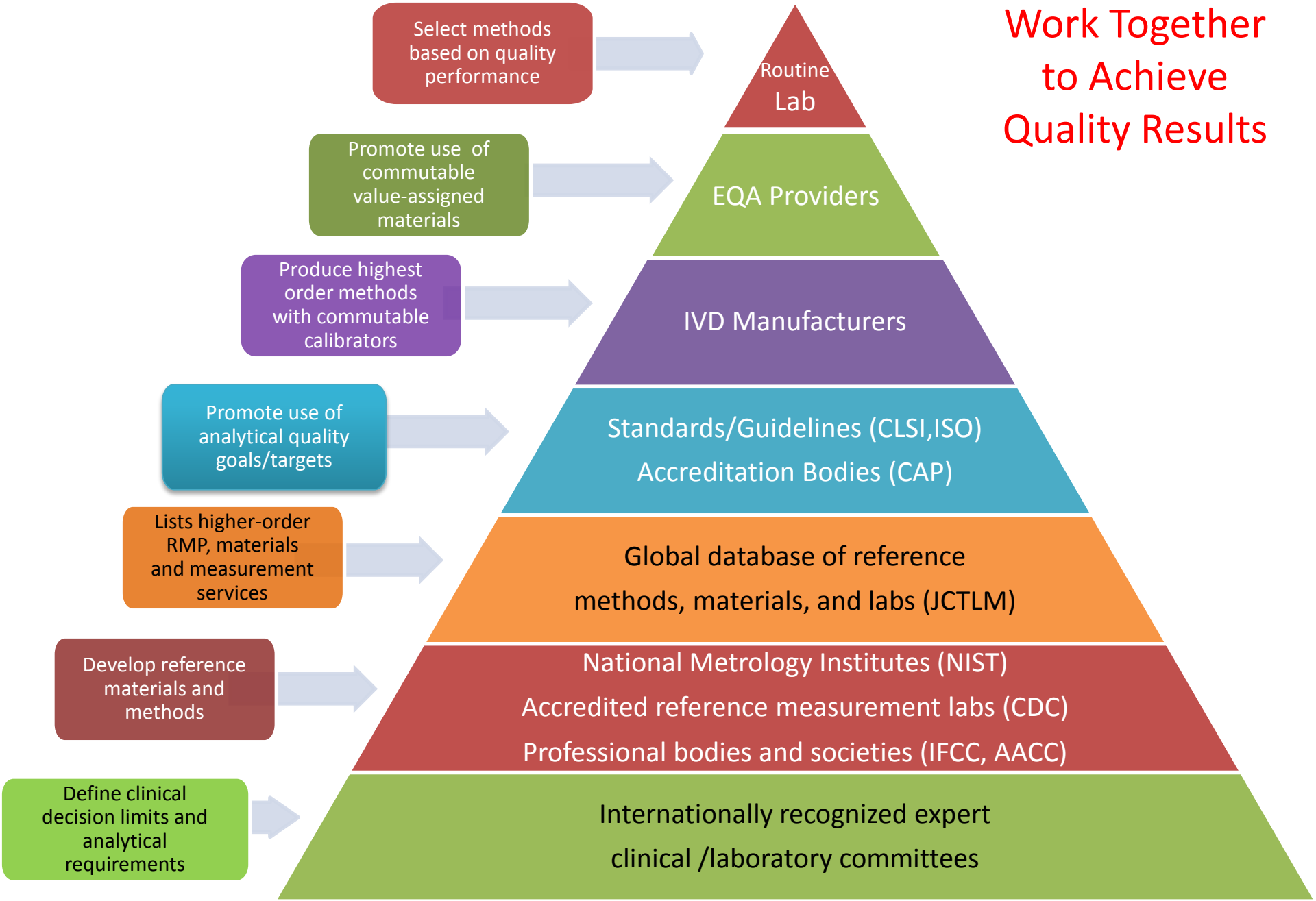
Reference materials with results that fall within the 95% prediction interval are considered commutable

Source: CLSI EP-30A, 2010


Pillars of Harmonization
Reference Measurement Procedure
Reference Materials
Reference Measurement Labs
Universal Reference Intervals
EQA/PT
Total Testing Process

International & National Initiatives for Improving Accuracy of Thyroid Hormone Measurement Results

Work Together to Achieve Quality Results



Adapted from: White Ann Clin Biochem 2011; 48:393-409

IFCC
International Federation of Clinical Chemistry and Laboratory Medicine

Sponsors committees and working groups to develop reference measurement procedures and materials

The Committee for Standardization of Thyroid Function Tests (C-STFT) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

C - STFT

- ✓**Standardization of TT4 and TT3 measurements**
- ✓**Standardization of FT4 measurements**
- ✓**Harmonization of TSH measurements**

Sources of Certified Reference Materials and Methods

The Joint Committee for Traceability in Laboratory Medicine

JCTLM

International Consortium for Harmonization of Clinical Laboratory Results

NIST National Institute of Standards and Technology U.S. Department of Commerce

European Commission Joint Research Centre

Australian Government National Measurement Institute

World Health Organization

National Institute of Advanced Industrial Science and Technology National Metrology Institute of Japan

NIBSC Confidence in Biological Medicines

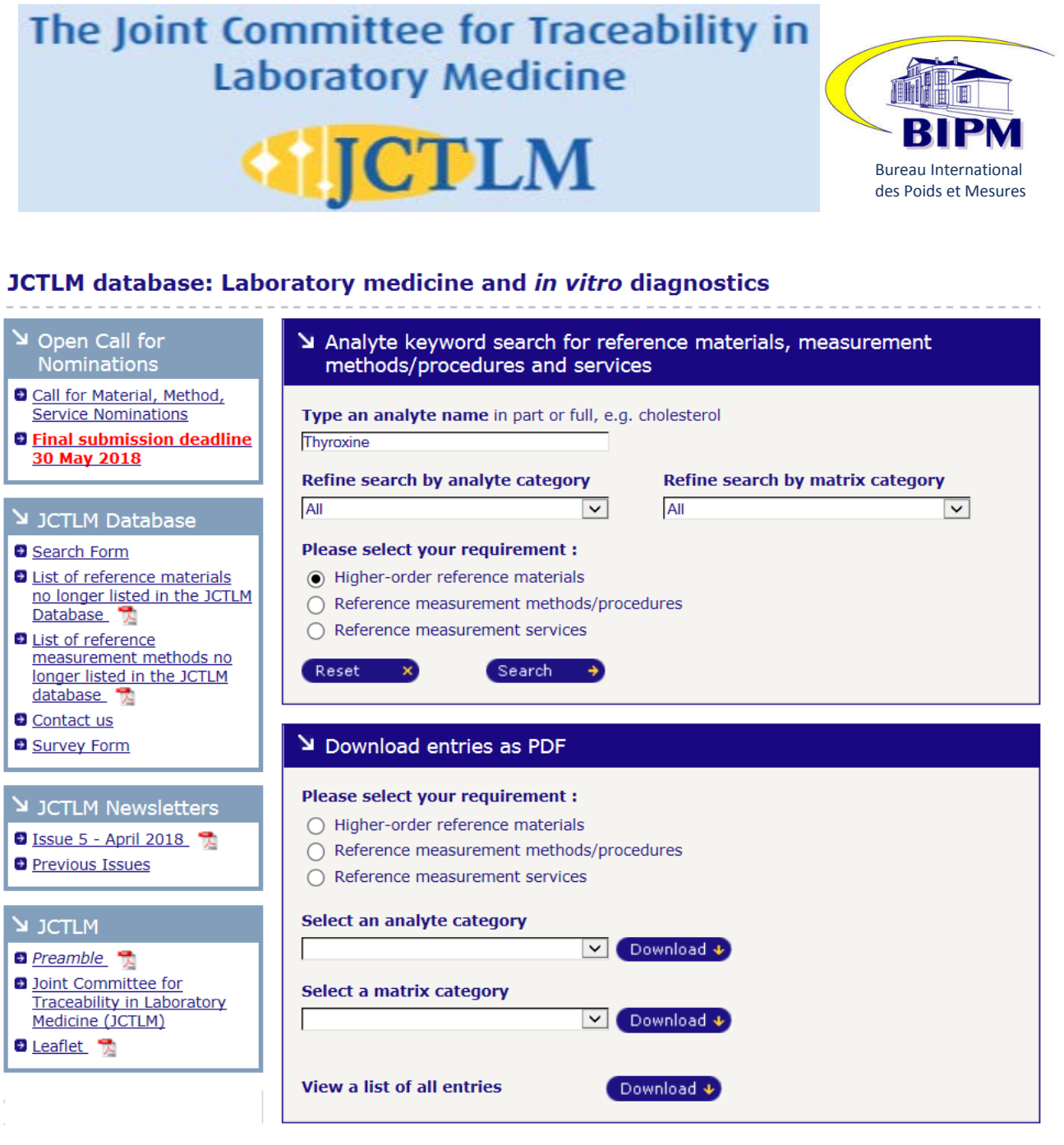
The Joint Committee for Traceability in Laboratory Medicine

JCTLM

BIPM
Bureau International des Poids et Mesures

ifcc
International Federation of Clinical Chemistry and Laboratory Medicine

ilac



Searchable Database

- 293 certified reference materials
- 180 reference measurement methods covering 80 analytes
- 146 reference measurement services covering 39 analytes

www.bipm.org/jctlm

Results of the search

Isotope dilution mass spectrometry method for free thyroxine in blood serum

- University of Ghent reference measurement procedure for free thyroxine in serum

Applicable matrice(s)	lyophilized, fresh, or frozen serum
Full description of technique(s)	equilibrium diaysis ID/LC/MS
Quantity	Amount-of-substance concentration
Applicable range	1.8 pmol/L to 80 pmol/L
Expected uncertainty (level of confidence 95%)	6.9 %
Reference(s)	*Clin. Chem. Lab. Med.*, 2011, **49**(8), 1275 - 1281
JCTLM DB identification number	C8RMP1

Results of the search

thyroxine (T4) in thyroxine crystalline material

European Commission, Joint Research Centre (JRC), European Union

Phone: +32 (0) 14 571 705
Fax: +32 (0) 14 590 406
Email: jrc-rm-distribution@ec.europa.eu
Web: https://crm.jrc.ec.europa.eu/

Name of the reference material	IRMM-468, Thyroxine (T4)
Quantity	Mass fraction
Analyte certified/assigned value	98.6 %
Expanded uncertainty (level of confidence 95 %)	0.7 %
Traceability	SI
CRM listing	List I

This (Certified) Reference Material has been reviewed for compliance with ISO 15194:2003 but not been reviewed against ISO 15194:2009

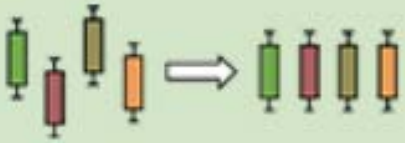
Sort by : (•) Analyte ( ) Matrix or Material ( ) Service provider

Select	Analyte	Matrix or Material	Country	Service provider
☐	free thyroxine	blood serum	Belgium	UGent - Ref4U
☐	thyroxine	blood serum	Germany	Instand e.V.
☐	total thyroxine (TT4)	blood plasma	China	Maccura
☐	total thyroxine (TT4)	blood plasma	Germany	RfB-DGKL
☐	total thyroxine (TT4)	blood plasma	Belgium	UGent - Ref4U
☐	total thyroxine (TT4)	blood serum	China	Maccura
☐	total thyroxine (TT4)	blood serum	Germany	RfB-DGKL
☐	total thyroxine (TT4)	blood serum	Belgium	UGent - Ref4U
☐	total thyroxine (TT4)	calibration solution	China	Maccura
☐	total thyroxine (TT4)	calibration solution	Germany	RfB-DGKL
☐	total thyroxine (TT4)	high purity material	China	Maccura

UGent - Ref4U, Belgium

Phone: +32 (0)9 264 81 21
Fax: +32 (0)9 264 81 98
Contact person: Dr. K. Van Uytfanghe
Email: Katleen.vanuytfanghe@ugent.be

Analyte	free thyroxine
Material or matrix	blood serum
Applicable material or matrix	lyophilized, fresh, or frozen serum
Quantity	Amount-of-substance concentration
Service measurement range	1.8 pmol/L to 80 pmol/L
Expanded uncertainty (level of confidence 95%)	7.2 % The expanded uncertainty is calculated for measurement protocol n = 6
Measurement principle	equilibrium dialysis - ID/LC/MS
JCTLM reference measurement method/procedure	University of Ghent reference measurement procedure for free thyroxine in serum



International Consortium
for Harmonization of Clinical Laboratory Results

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

AACC Better health through laboratory medicine

College of American Pathologists

JCCLS 特定非営利活動法人 日本臨床検査標準協議会
Japanese Committee For Clinical Laboratory Standards

대한진단검사의학회
Korean Society for Laboratory Medicine

The Consortium maintains

- a list of measurands that provides a priority for harmonization
- information on active harmonization projects being conducted by organizations worldwide to promote coordination and avoid duplication of effort
- information on resources available for harmonization and standardization of test results

www.harmonization.net

Toolbox of technical procedures to achieve harmonization

Information Required

To assess if harmonization
Is technically achieveable

1. Reproducibility
2. Linearity
3. Heterogeneity
4. Calibration
5. Overview

6. Commutability
7. Stability
8. Sustainability
9. Value Assignment
10. Availability
11. Costs
12. Will to Harmonize

13. Harmonization Achieveable

Experimental Design IHP

Samples included to get all information

32 Samples
of individual persons
healthy and diseased
assayed in triplicate

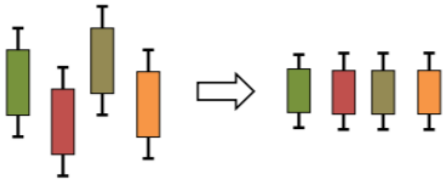
5 Mixtures
of the samples of the
individual persons

Linearity Panel
of 5 samples made from
the individual persons

3 Candidate Calibrators

2 Additional Batches
of one Candidate Calibrator

2 Stored Vials
of one Candidate Calibrator



Measurand Table

Summary of Harmonization/Standardization Activities

Measurand	Matrix	Medical Impact of Harmonization	Harmonization Status	Resources	Organization
Thyroid stimulating hormone (TSH)	Serum		Active		IFCC
Thyroxine (T4)	Serum		Active	JCTLM	IFCC
Thyroxine, free (FT4)	Serum		Active		IFCC
Triiodothyronine, free (FT3)	Serum	High	Needed		

Thyroid stimulating hormone (TSH)

The literature reports moderate biases among different measurement procedures for TSH. An IFCC committee is working to harmonize results from measurement procedures for TSH.

Thyroxine, free (FT4)

The literature reports substantial biases among different measurement procedures for FT4. An IFCC committee is working to harmonize results from measurement procedures for FT4.

Thyroxine (T4)

The literature reports substantial biases among different measurement procedures for T4. An IFCC committee is working to harmonize results from measurement procedures for T4.

Triiodothyronine, free (FT3)

A report that used a fresh-frozen presumably commutable sample in a College of American Pathologists Survey in 2003 found an approximate 2-fold difference in free-T3 results among 11 clinical laboratory immunoassay measurement procedures (1). A recent review reported that free-T3 results continue to have discrepancies among clinical laboratory immunoassay measurement procedures (2). To some extent, method specific reference intervals compensate for biases among different measurement procedures. The review reported that multiple studies have shown that T3, free-T3 and free-T4 for approximately 20% of patients on therapy for hypothyroidism are within the reference intervals by immunoassays when the values are below the reference interval by an ultrafiltration LC-MS/MS measurement procedure. Free thyroid hormone measurement by immunoassay is a challenging technology because of the influence of various binding protein abnormalities that alter the recovery and thus compromise the suitability of the reference interval determined for these methods.

Laboratory Standardization and Quality Assurance Programs

CDC's programs perform each step in the standardization process to improve laboratory measurements

Develop and Maintain Reference System

Establish Metrological Traceability

Verify "end-User" Test Performance

REFERENCE LABORATORY SERVICES

Develop reference methods and materials with target values assigned by reference methods. Provide reference value assignments to materials used in clinical and research labs.

STANDARDIZATION SERVICES

Reference materials created in the previous step are used by participants to calibrate their tests or to verify analytical accuracy and precision of their testing systems. Certification of performance is provided via HoSt & used by manufacturers to demonstrate they met new EU IVD regulations.

PERFORMANCE MONITORING SERVICES

Analytical accuracy/precision of tests calibrated in the previous step are assessed under routine testing conditions. Performance is monitored through accuracy-based EQA/PT programs, or accuracy-based blind QC.

Hormone Standardization Programs

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

Reference Methods

- Testosterone: LC-MS/MS
- Estradiol: LC-MS/MS
- 25-Hydroxyvitamin D: LC-MS/MS
- Free Thyroxine : ED MS/MS (in development)

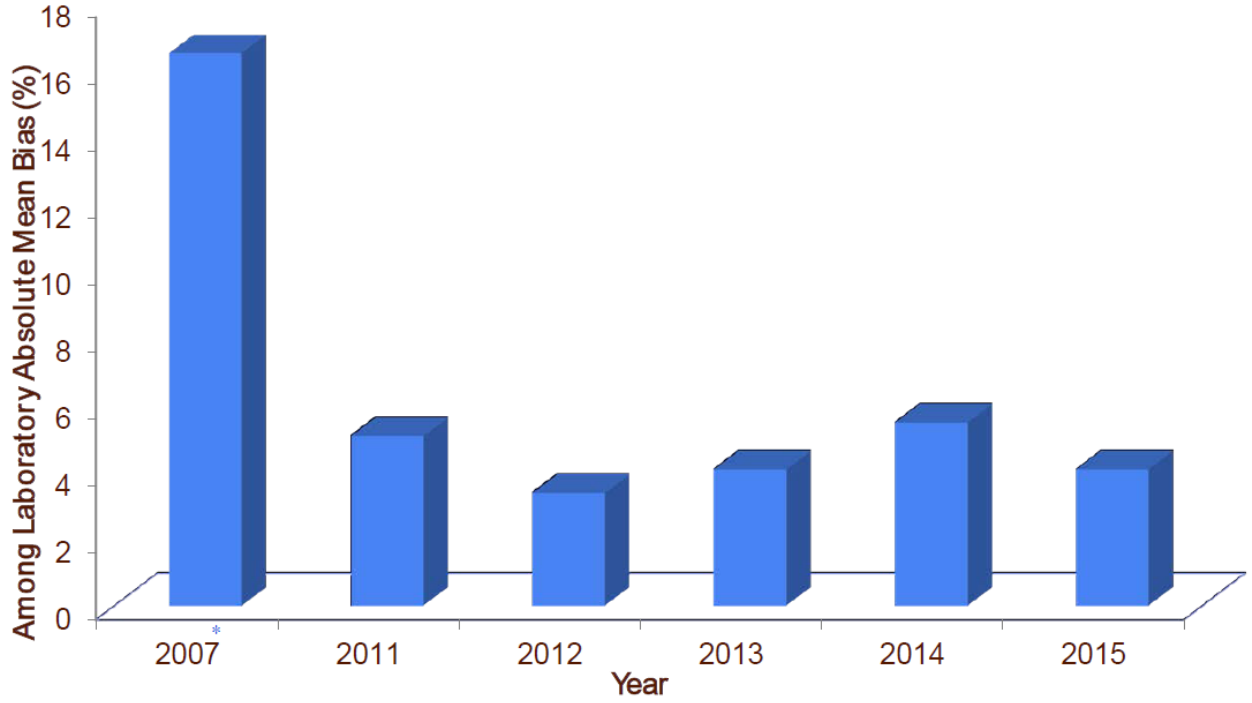
Certification Programs (HoST/VDSCP)

- 40 single-donor serum specimens
- Replicate measurements to assess imprecision
- Quarterly and yearly assessments
- Analyte concentrations customized to cover AMR

Performance Monitoring

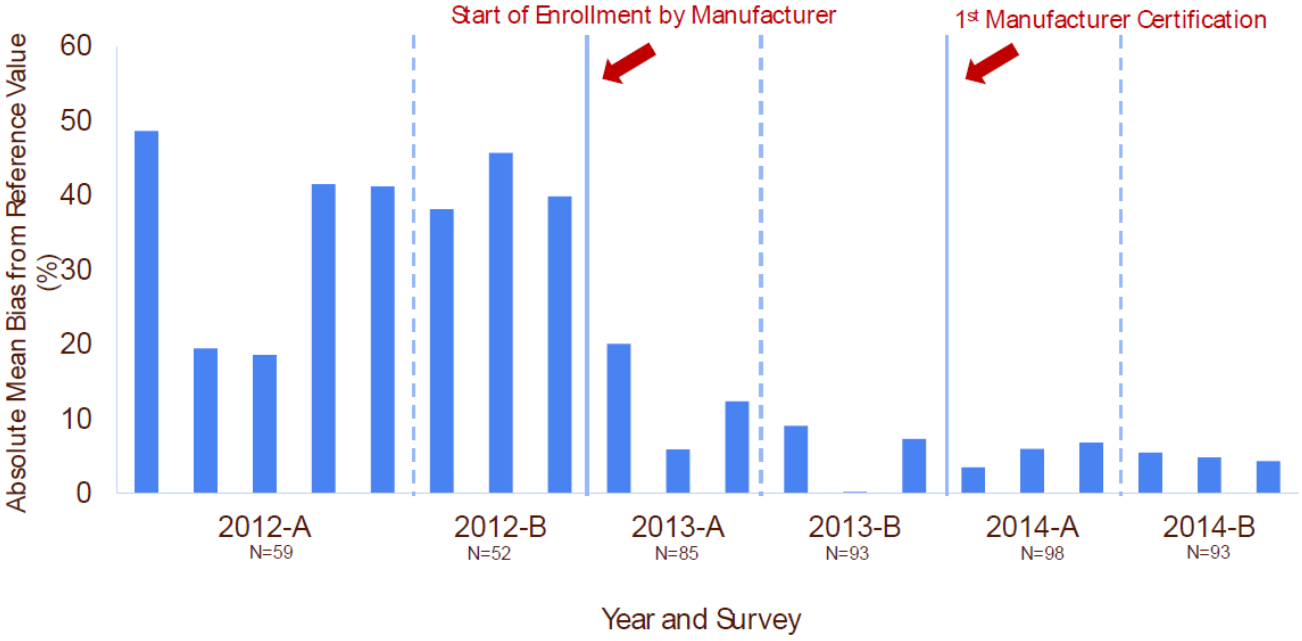
- Assigns target values for accuracy-based PT/EQA surveys conducted by CAP, RCPA, NY Dept Health
- Provides sets of 120 individual single-donor samples
- Conducts ad-hoc interlaboratory/commutability studies

www.cdc.gov/labstandards



CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

Calibration bias of testosterone measurements has improved since start of CDC HoST program



Certification of manufacturers has led to improved vitamin D measurement accuracy in patient care.

Source: Vesper JCTLM Meeting, 2015

PARTNERSHIP for the ACCURATE TESTING of HORMONES
Better Tests, Better Health

Supports and Promotes Standardized Hormone Tests for Better Healthcare and Research

PATH is a stakeholder organization

- Public and private organizations from the clinical, medical and public health fields
- Main areas of work
  - Education
  - Advocacy
  - Technical support

www. Hormoneassays.org

PATH Members

American Association for Clinical Chemistry
American Society for Bone and Mineral Research
American Thyroid Association
American Urological Association
Androgen Excess/PCOS Society
Association of Public Health Laboratories
Centers for Disease Control and Prevention
College of American Pathologists
Endocrine Society
International Society of Andrology
LabCorp
LA Biomed
NIH
North American Menopause Society
Pediatric Endocrine Society
Quest Diagnostics
National Assoc of Chronic Disease Directors
U.S. Food and Drug Administration

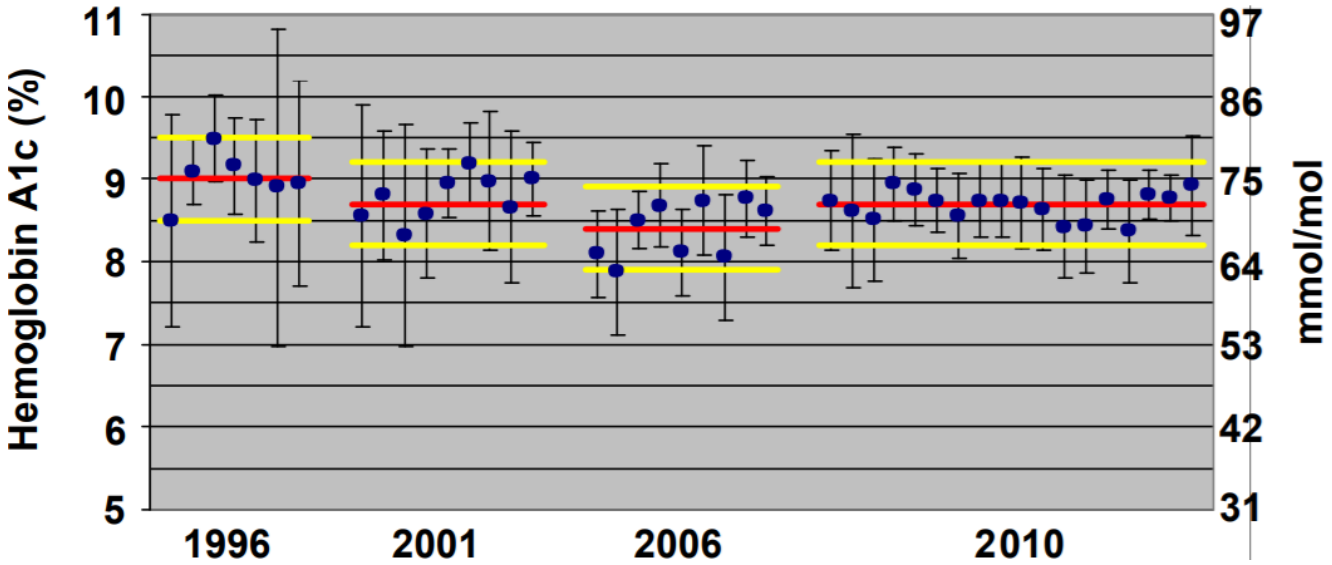
PARTNERSHIP for the ACCURATE TESTING of HORMONES
Better Tests, Better Health

Objectives/Strategies

Goal 1. Standardization	• Increase the number of standardized hormone assays • Increase the number of assays that demonstrate a rigorous assessment for meeting performance requirements
Goal 2. Education	• Deepen the knowledge of stakeholders …importance of accurate and reliable hormone assays …assay quality in patient care, research and public health
Goal 3. Implementation	• Increase the use of standardized assays in patient care, public health and research
Goal 4. Sustainability	• Establish a sustainable system to standardize hormone assays and to keep hormone assays accurate and reliable

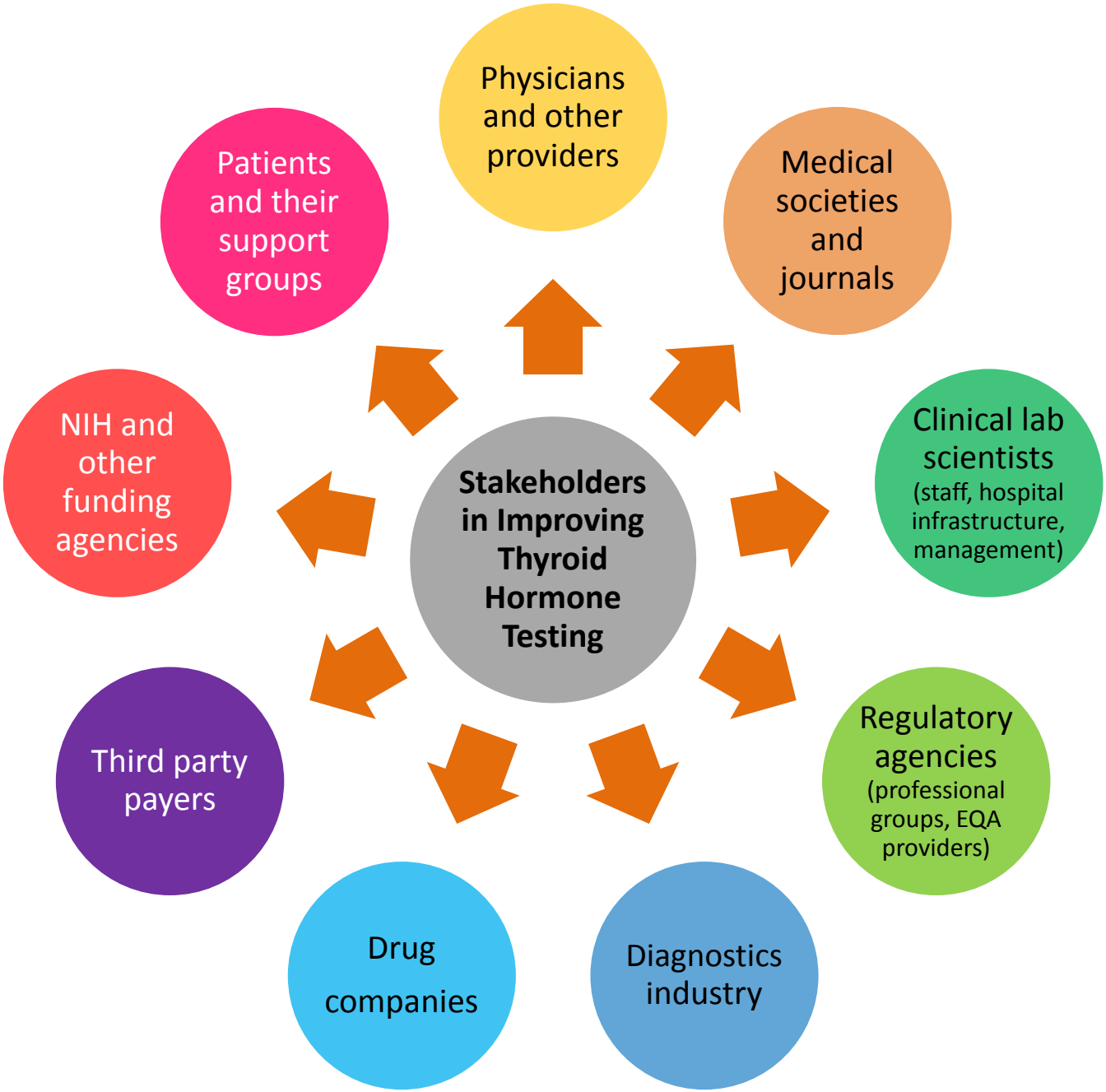
Sustaining Improvements

- Accuracy-based EQA/PT
  - Clinical labs and manufacturers should be encouraged to participate
  - Commutable materials should cover the AMR with data made public
- Certification process
  - Manufacturers can document harmonization/traceability (required by EU)



Example of role of EQA/PT to improve performance (HbA1c)

Source: Miller Clin Chem 2011; 57:1670-80


Physicians and other providers
Medical societies and journals
Clinical lab scientists (staff, hospital infrastructure, management)
Regulatory agencies (professional groups, EQA providers)
Diagnostics industry
Drug companies
Third party payers
NIH and other funding agencies
Patients and their support groups
Stakeholders in Improving Thyroid Hormone Testing

Manufacturers

1. Comply w/ traceability directives & professional society guidelines.
2. Select suitable reference materials/methods, and transfer values to calibrators accurately.
3. Subscribe to CDC's HoST

Researchers

1. Use standardized tests in clinical studies.
2. Understand how reliable tests strengthen data from different studies.
3. Be aware that accurate testing allow quicker use of research studies to improve patient care.

Health Insurers, Regulators, Legislators

1. Aware accuracy lowers costs & improves pt care.
2. Note some tests more reliable than others; support coverage for standardized tests
3. Require clinical trials to use standardized tests.

Clinicians

1. Look into the quality of ordered tests.
2. Obtain details about how test accuracy & precision are gauged and achieved.
3. Educate colleagues about how standardized tests lead to better patient care, less retesting, more consistent diagnoses.

Lab Directors

1. Check traceability status of methods in use.
2. Select methods based on quality performance.
3. Subscribe to EQA/PT program that include accuracy-based commutable materials.

What Can I Do to Assure Accuracy of Thyroid Hormones?

THANK YOU!

Kentucky Derby

http://www.clockworkhare.com/tag/horse-racing

Questions or Comments

Tap the question mark icon to submit a question or comment

Accurate Measurement of Thyroid Hormones in Disease and Pregnancy

|

70th AACC Annual Scientific Meeting & Clinical Lab Expo - Chicago, IL

Clinical Challenges of Thyroid Hormone Testing

Gregory A. Brent, MD

Departments of Medicine and Physiology

Endocrinology and Diabetes Division

David Geffen School of Medicine at UCLA

VA Greater Los Angeles Healthcare System

Los Angeles, CA, USA

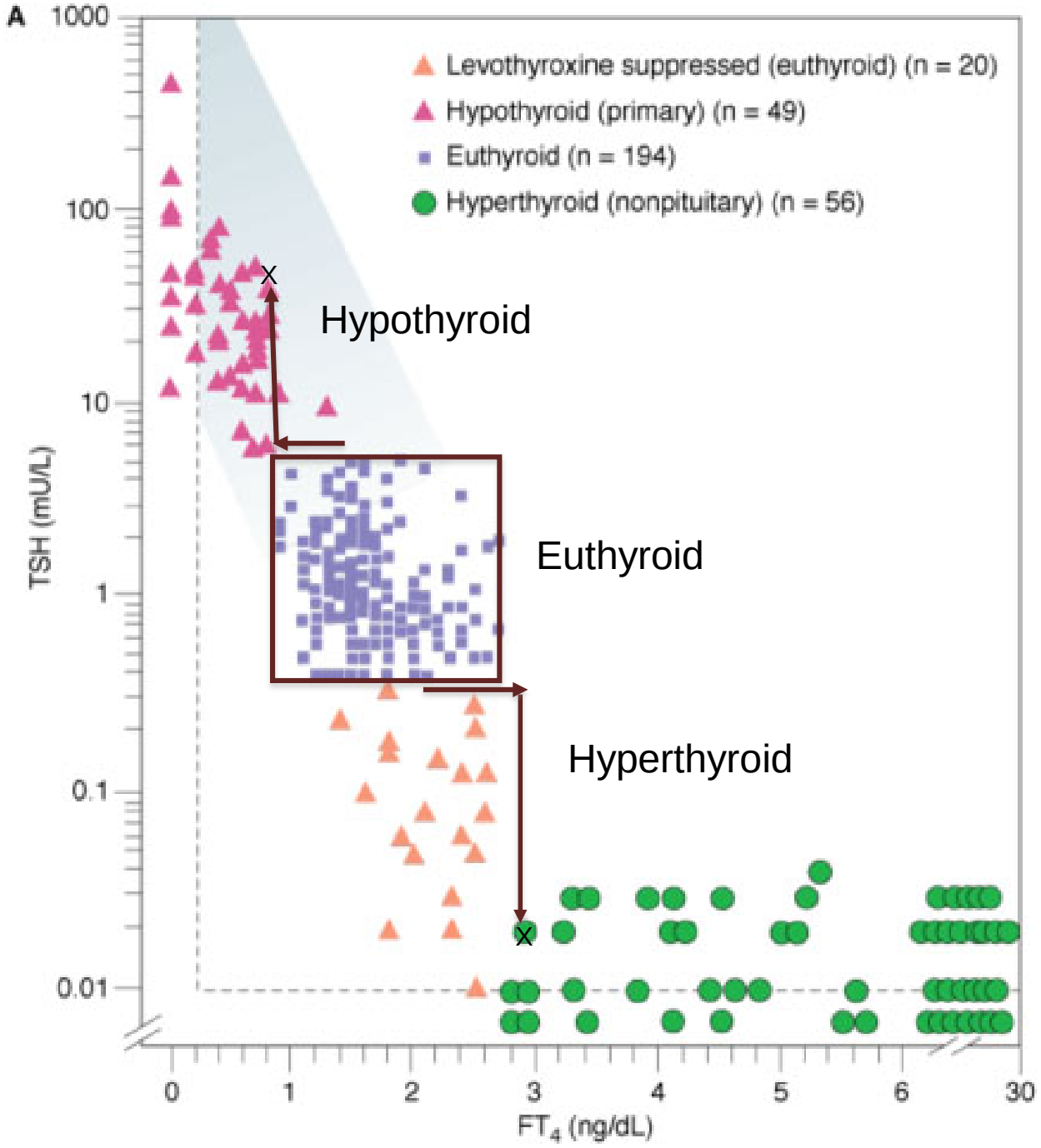
DISCLOSURE

Nothing to Disclose

Clinical Challenges of Thyroid Hormone Testing

- Laboratory Testing in the Diagnosis and Treatment of Hypothyroidism
- Laboratory Testing in the Diagnosis and Treatment of Hyperthyroidism
- Evaluating Thyroid Function in Pregnancy
- Factors that Interfere With Thyroid Function Testing

Log-Linear Relationship of TSH and Free T4 (FT4)



Thyroid Antibodies

- **Anti-Thyroid Peroxidase Antibody (TPO) –** mediates thyroid destruction in Hashimoto's disease.
- **Anti-thyroglobulin antibodies (Tg) –** only obtain in conjunction with thyroglobulin measurement for thyroid cancer.
- **Thyroid Stimulating Immunoglobulin (TSI) –** bioassay of Graves' TSH receptor stimulating activity. Helpful in euthyroid Graves' ophthalmopathy, Graves' in pregnancy, assess remission (?).
- **TSH Receptor Ab (TRAb)** – Graves' immunoglobulin that binds the TSH receptor.

Recent Clinical Practice Guidelines for the Management of Thyroid Diseases

- Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer-ATA-2016
- Hyperthyroidism and other Causes of Thyrotoxicosis-ATA/AACE-2011, ATA-2016
- Clinical Practice Guidelines for Hypothyroidism in Adults-ATA/AACE-2012
- Guidelines for the Treatment of Hypothyroidism-ATA-2014
- The Use of L-T4 +L-T3 in the Treatment of Hypothyroidism-ETA-2012
- Guidelines for the Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum-ATA-2011, Endo Soc 2012, ETA 2014, ATA-2017

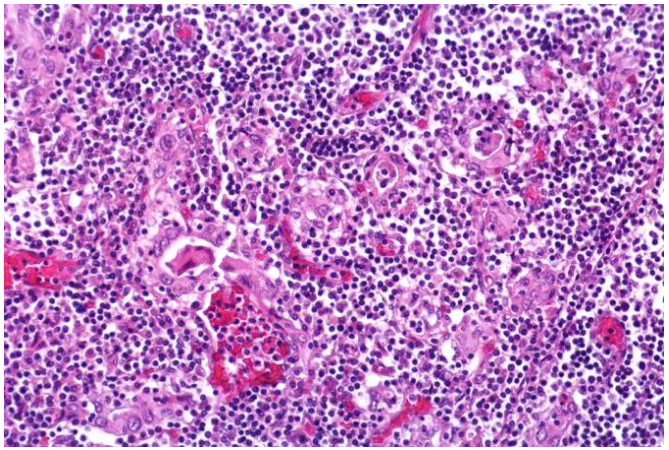
ATA = American Thyroid Association; Endo Soc =The Endocrine Society; AACE = American Association of Clinical Endocrinologists; ETA = European Thyroid Association.

HYPOTHYROIDISM

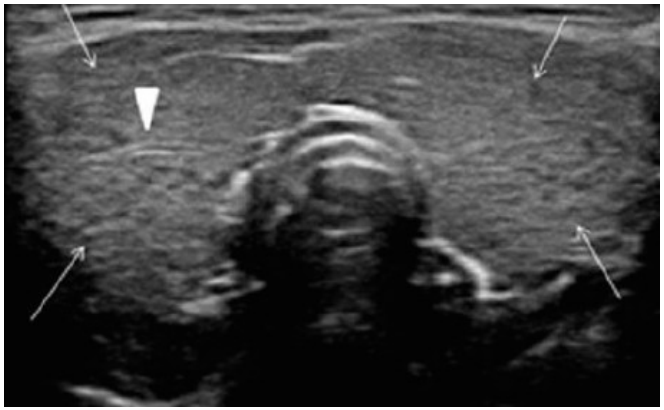
Hypothyroidism

- Evaluation of patient with suspected hypothyroidism
- Decision to treat subclinical hypothyroidism
- TSH targets

Hashimoto's Thyroiditis

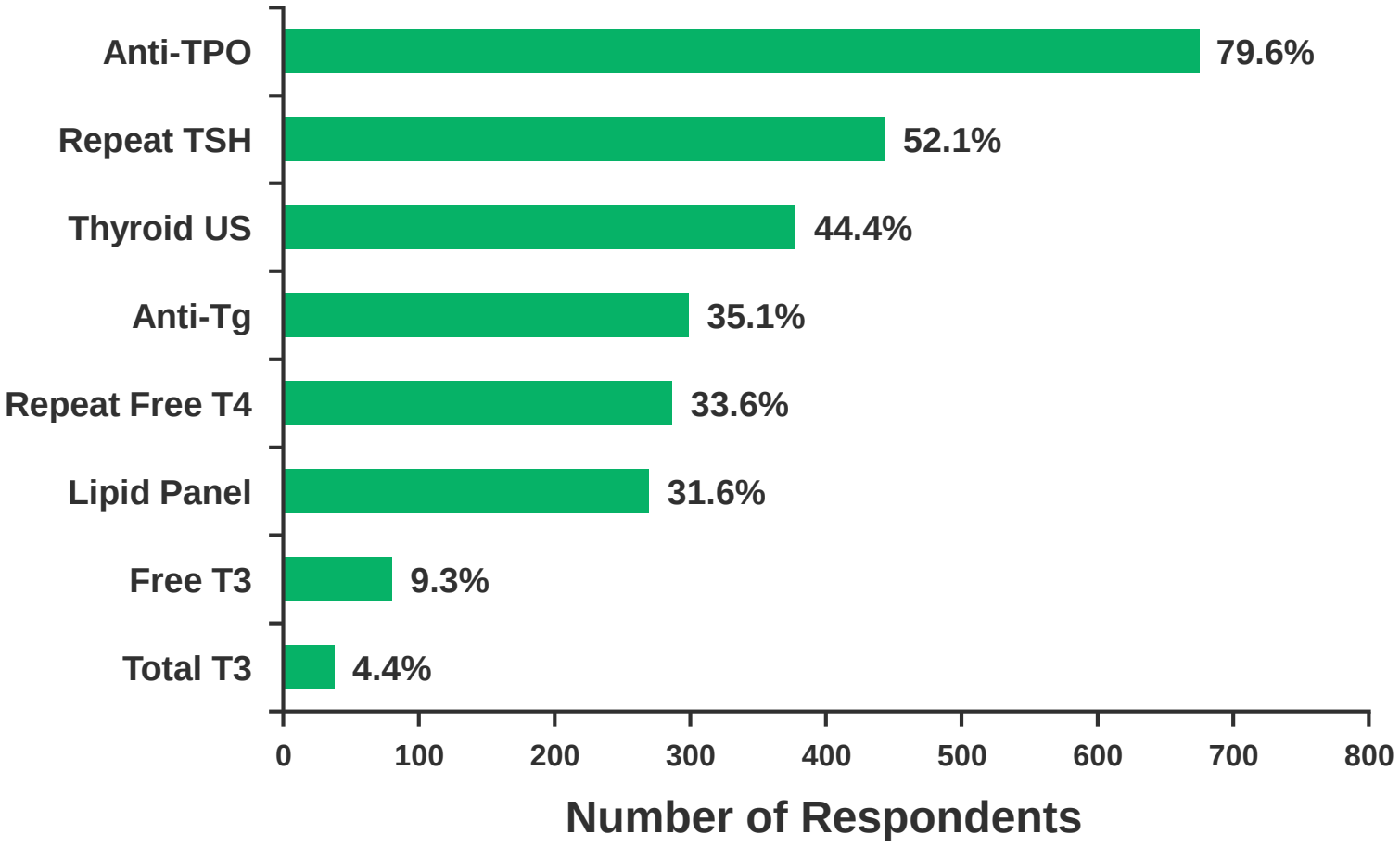


Histology
Lymphocytic Infiltration



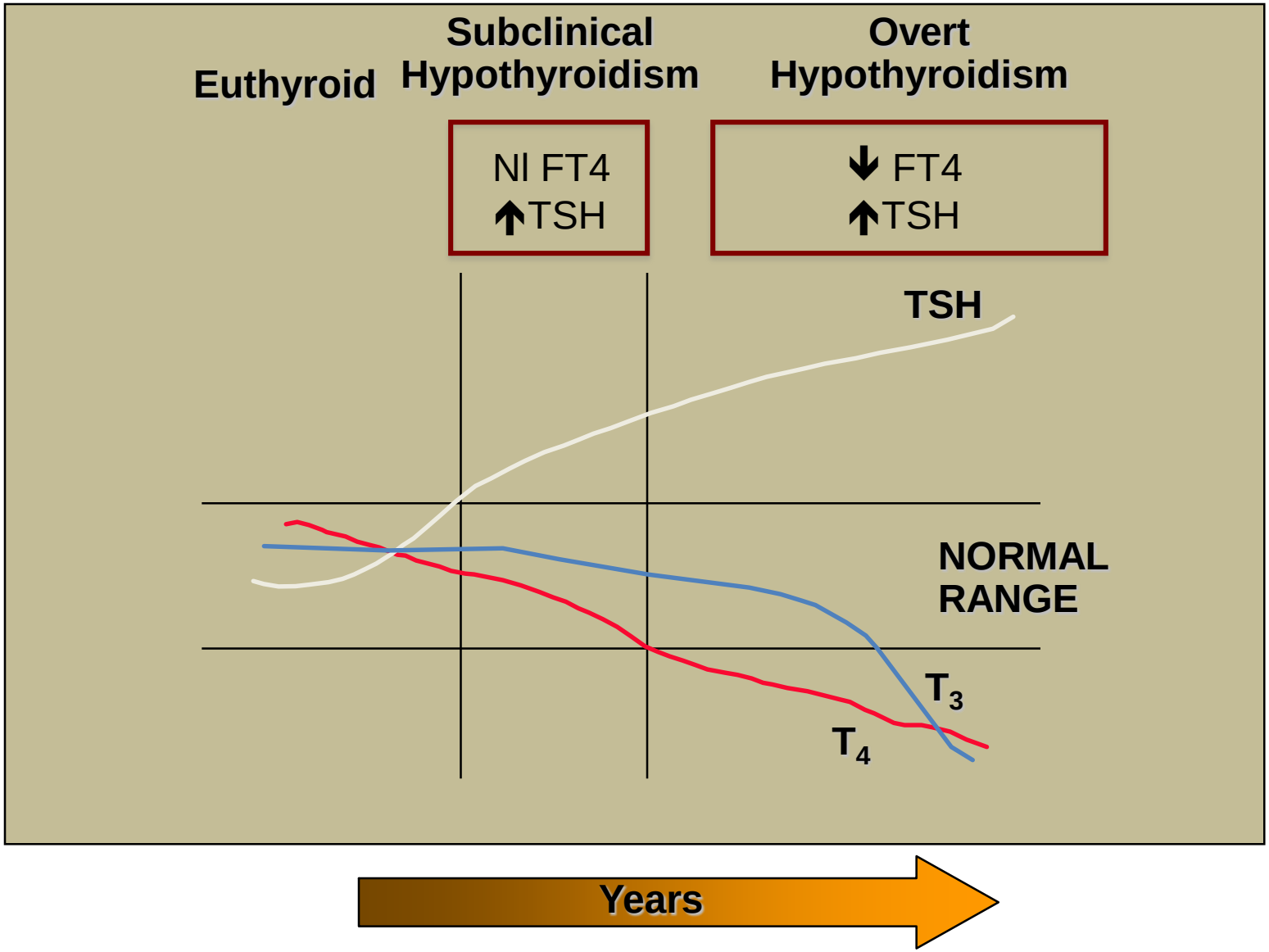
Ultrasound
Enlarged lobe, heterogeneity

Initial Testing in Overt Hypothyroid Patient Based on Expert Survey



Burch, et al. *J Clin Endocrinol Metab*. 2014;99:2077

Progression of Thyroid Failure



Indications for Thyroxine Treatment of Subclinical (mild) Hypothyroidism

Recommend Thyroxine Treatment	Consider Thyroxine Treatment
TSH>10 mU/L	TSH 5-10 mU/L
Anti-TPO+ and goiter	Anti-TPO+ no goiter
Undergoing infertility treatment	Symptoms (eg. Persistent Fatigue)
Recurrent miscarriage	Hyperlipidemia
Pregnant	Depression

Anti-TPO-thyroid peroxidase antibody

Garber et al Clinical Practice Guidelines for Hypothyroidism in Adults-ATA/AACE-Thyroid 22:1200, 2012

What is the best approach to initiating and adjusting levothyroxine therapy?

- Thyroid hormone therapy should be initiated as an initial full replacement (1.6-1.8 ug/kg), or as partial replacement with gradual increments in the dose titrated upward using serum TSH as the goal.
- Dose adjustments should be made when there are large changes in body weight, with aging, and with pregnancy.
- TSH assessment 4-6 weeks after any dosage change.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Jonklaas J, et al. *Guidelines for the Treatment of Hypothyroidism Thyroid.* 2014; 24:1670.

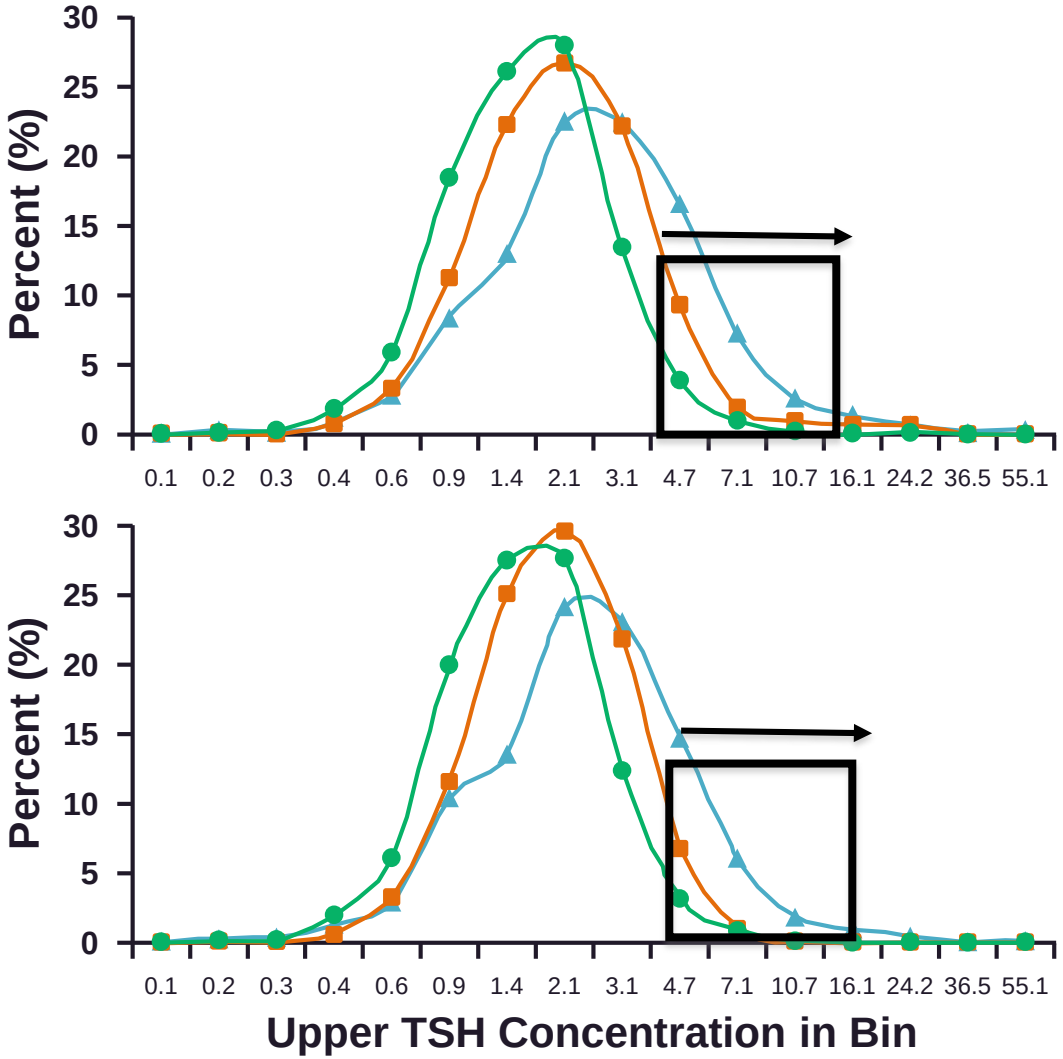
What are the potential deleterious effects of excessive levothyroxine?

- The deleterious health effects of iatrogenic thyrotoxicosis include atrial fibrillation and osteoporosis.
- Avoid subnormal serum TSH values, particularly TSH values below 0.1 mIU/L, especially in older persons and postmenopausal women.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Jonklaas J, et al. *Guidelines for the Treatment of Hypothyroidism Thyroid.* 2014; 24:1670.

Age Distribution of TSH from NHANES III Survey Data



Disease Free
No report of thyroid disease or meds

Age 20-29
Age 50-59
Age 80+

Reference Population
No pregnancy, thyroid antibodies, related med

Surks and Hallowell. *J Clin Endocrinol Metab*. 2007;92:4575.

How should levothyroxine therapy be managed in the elderly with hypothyroidism?

- Levothyroxine should be initiated with low doses, and the dose titrated slowly based on serum TSH measurements.
- Normal serum TSH ranges are higher in older populations (such as those over 65 years), and higher serum TSH targets may be appropriate for those on thyroxine replacement.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Jonklaas J, et al. *Guidelines for the Treatment of Hypothyroidism Thyroid.* 2014; 24:1670.

What is the appropriate degree of TSH suppression in patients with Differentiated Thyroid Cancer?

- For high-risk thyroid cancer patients, initial TSH suppression to below 0.1 mU/L is recommended.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

- For intermediate-risk thyroid cancer patients, initial TSH suppression to 0.1– 0.5 mU/L is recommended.

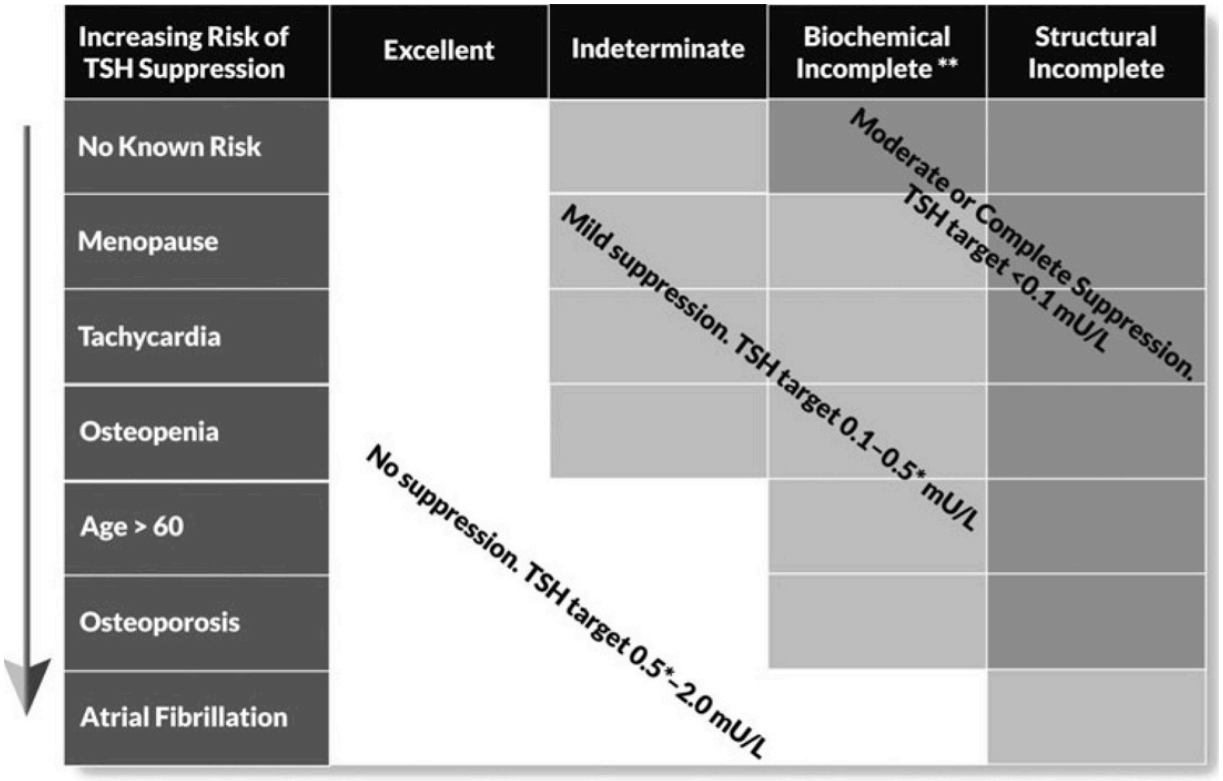
WEAK RECOMMENDATION **LOW-QUALITY EVIDENCE**

- For low-risk patients who have undergone remnant ablation and have undetectable serum Tg levels, TSH may be maintained at the lower end of the reference range (0.5–2mU/L) while continuing surveillance for recurrence. Similar recommendations hold for low-risk patients who have not undergone remnant ablation and have undetectable serum Tg levels.

WEAK RECOMMENDATION **LOW-QUALITY EVIDENCE**

Haugen et al Thyroid 26:1, 2016

Initial TSH Targets in Differentiated Thyroid Cancer Patients According to Risk Assessment



* 0.5 mU/L represents the lower limit of the reference range for the TSH assay which can be 0.3–0.5 mU/L depending on the specific assay

** TSH target for patients with a biochemical incomplete response can be quite different based on original ATA risk, Tg level, Tg trend over time and risk of TSH suppression

No suppression. TSH target 0.5*–2.0 mU/L

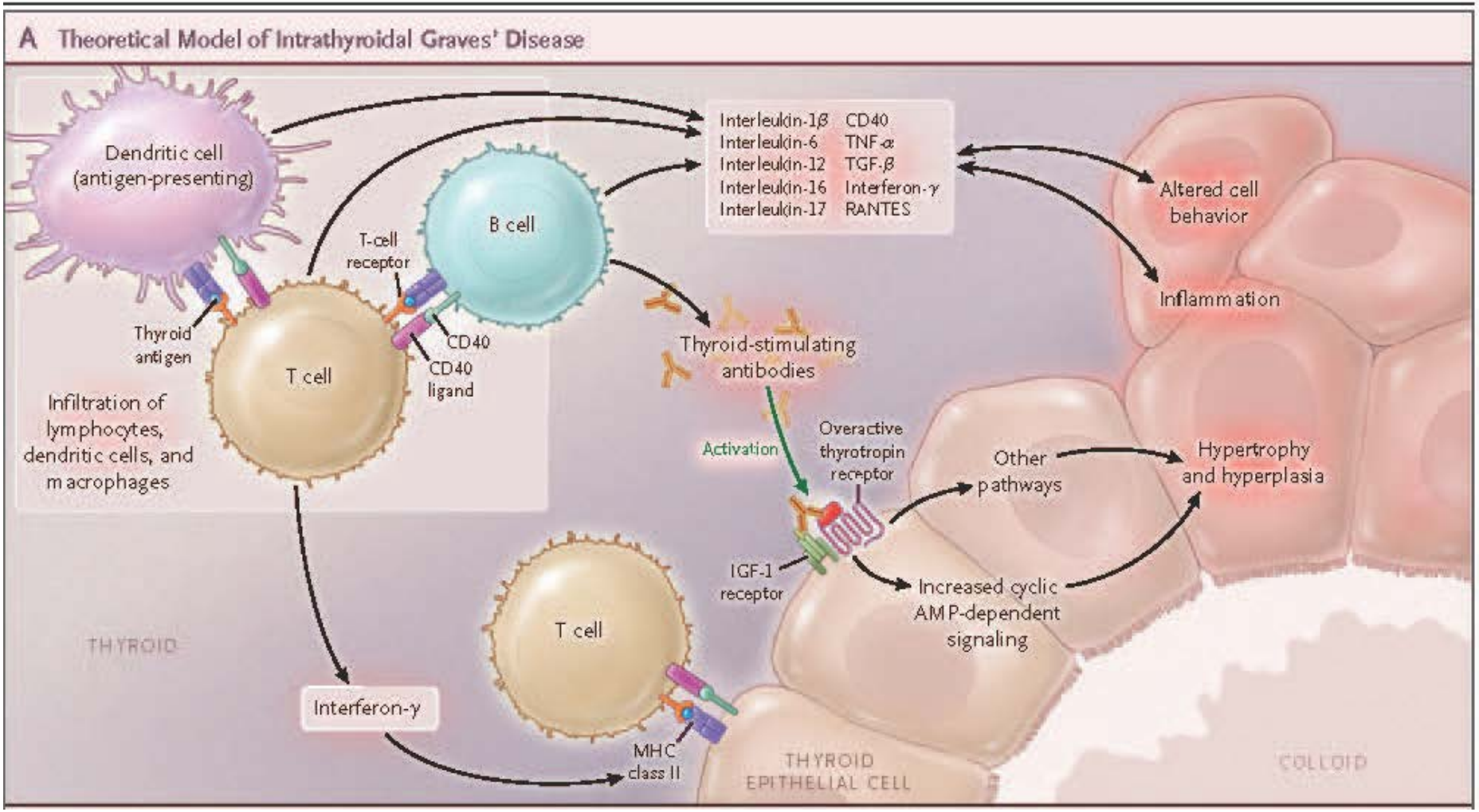
Mild suppression. TSH target 0.1–0.5* mU/L

Moderate or Complete suppression. TSH target <0.1 mU/L

Haugen et al Thyroid 26:1, 2016

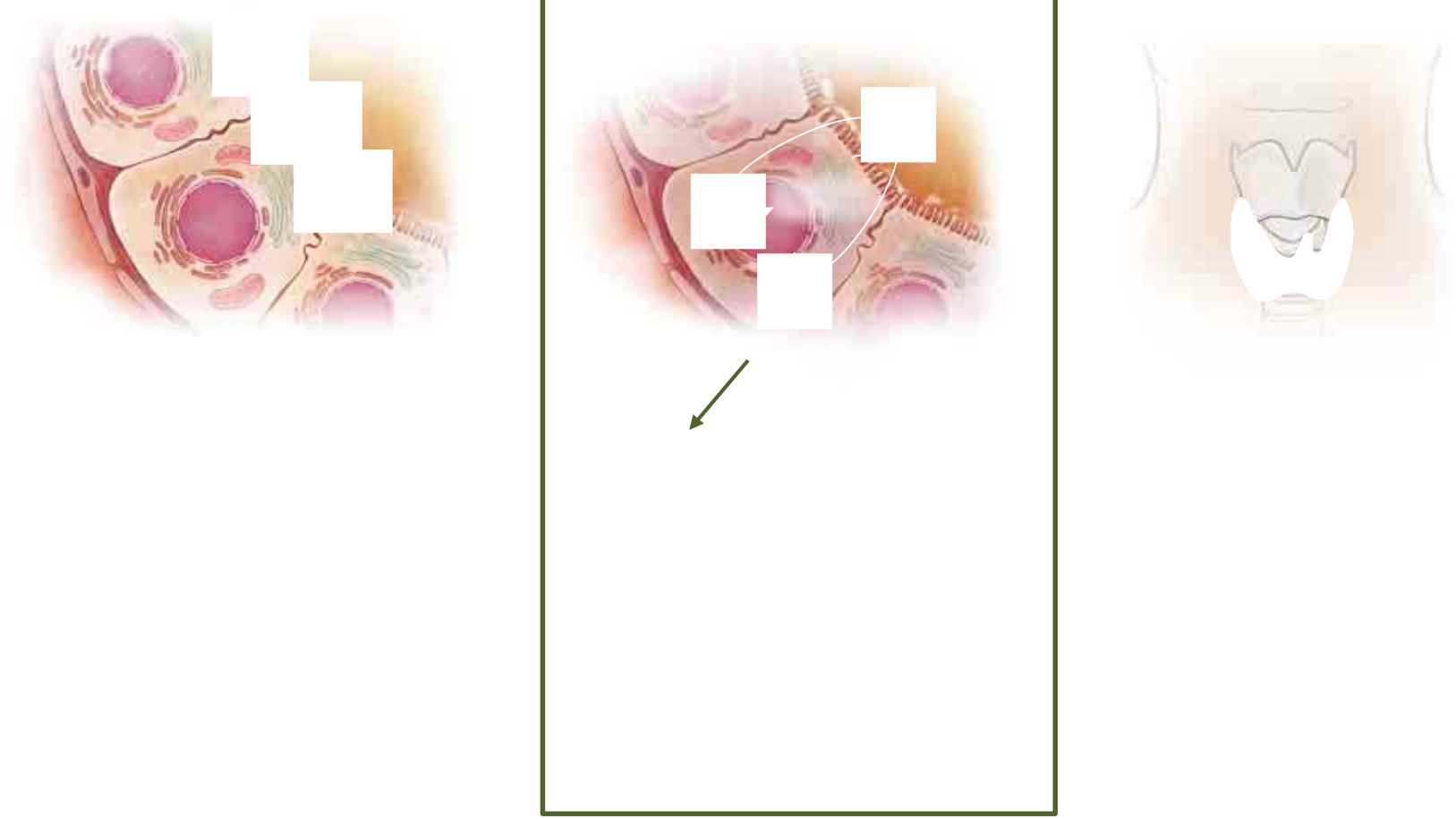
HYPERTHYROIDISM

Pathogenesis of Graves' Disease



Smith TJ N Engl J Med 375:1552, 2016

Primary Treatments for Graves' Disease



Burch and Cooper JAMA. 314:2544, 2015

Changes in Guidelines for Management of Hyperthyroidism 2017

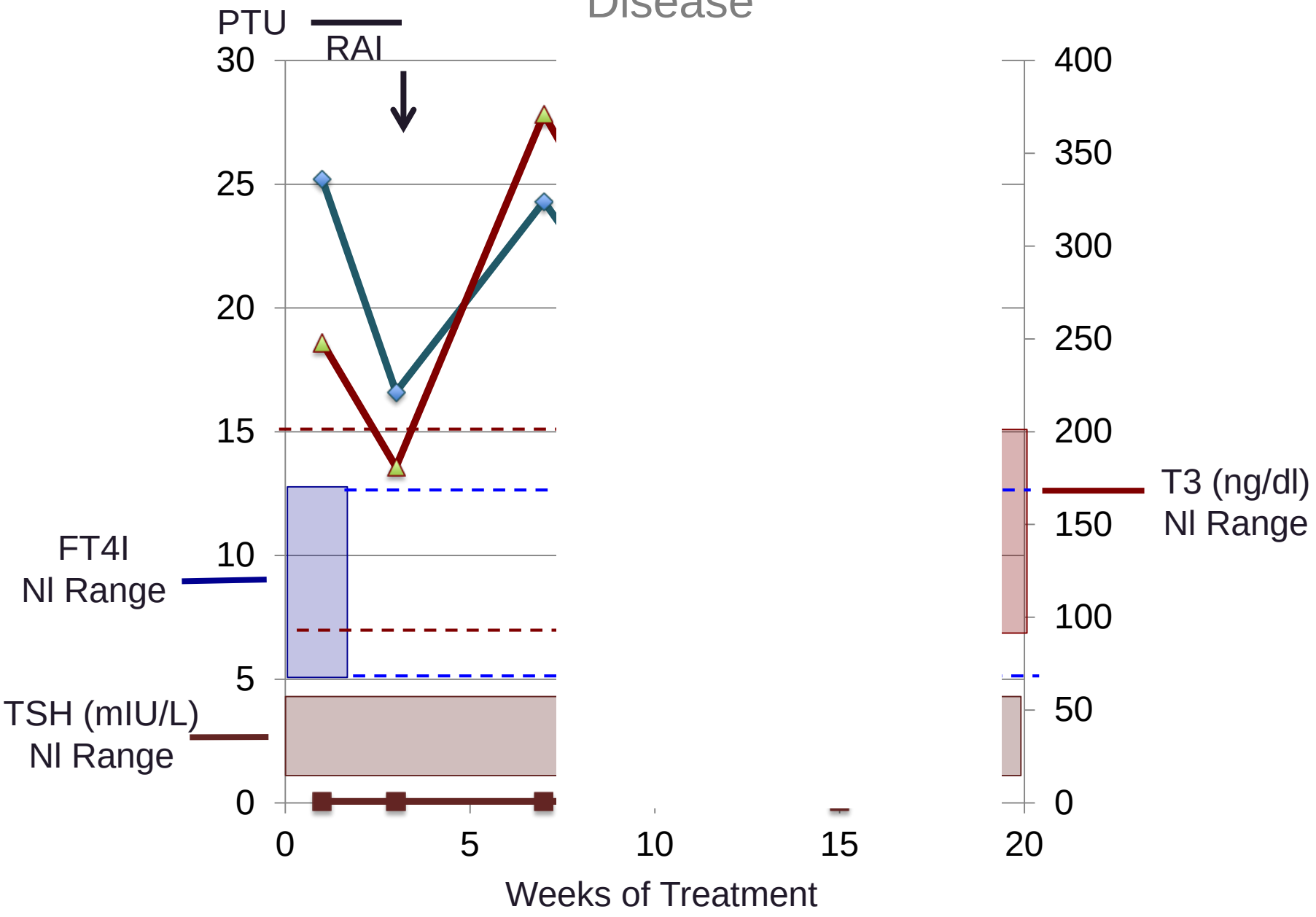
- Reduced need for RAI Uptake and Scan for diagnosis, emphasis on ultrasound for blood flow.
- Emphasis on TR-Ab for diagnosis, monitoring, and predicting remission.
- Patient education in reducing impact of ATD side-effects
- Support of chronic therapy with ATD's
- Recognition that both MMI and PTU are associated with embryopathy in pregnancy.
- Avoid ATD in first trimester, PTU preferable if ATD is needed
- Assess calcium and vitamin D preoperatively for surgery in Graves' disease, to prevent postoperative hypocalcemia, consider treating prophylactically.

Antithyroid Drug Therapy Dosing and Monitoring Response to Treatment

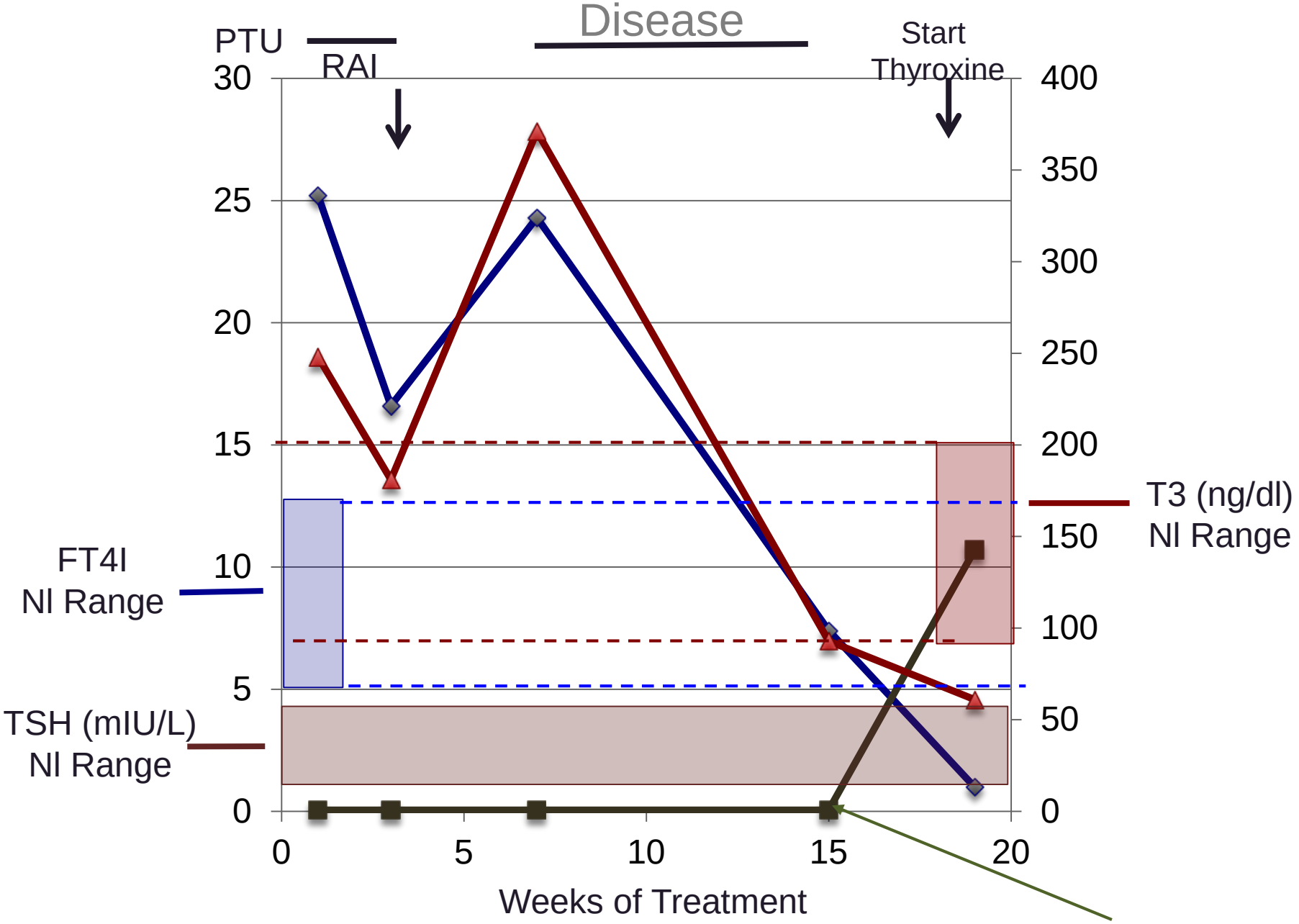
- MMI dose based on fold-FT4 above Upper Limit Normal; 5-10 mg 1-1.5 X, 10-20 mg 2X, 30-40 mg 2-3X.
- T4 and T3 levels begin to reduce after 2-6 weeks of therapy
- Reduction in heart rate and increase in body weight are typically seen in the 4-8 week range.
- TSH often remains suppressed even when T4/T3 are normal or even reduced.
- When T4/T3 are normalized, only **20%** of patients have a normal range TSH.
- After 3 months of a normal T4/T3, 80% of patients have a normal range TSH.

Ross et al Thyroid 26:1343, 2016

Changes In Thyroid Function With Treatment of Graves' Disease



Changes In Thyroid Function With Treatment of Graves' Disease



How can TSH Receptor Antibodies (TRAb) be used in managing Graves' disease?

- Measurement of TRAb levels prior to stopping ATD therapy is suggested because it aids in predicting which patients can be weaned from the medication, with normal levels indicating greater chance for remission.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

- If MMI is chosen as the primary therapy for GD, the medication should be continued for approximately 12–18 months, then discontinued if the TSH and TRAb levels are normal at that time.

STRONG RECOMMENDATION **HIGH-QUALITY EVIDENCE**

Ross et al Thyroid 26:1343, 2016

Guidelines for Treatment of Subclinical Hyperthyroidism (suppressed TSH, normal T4/T3)

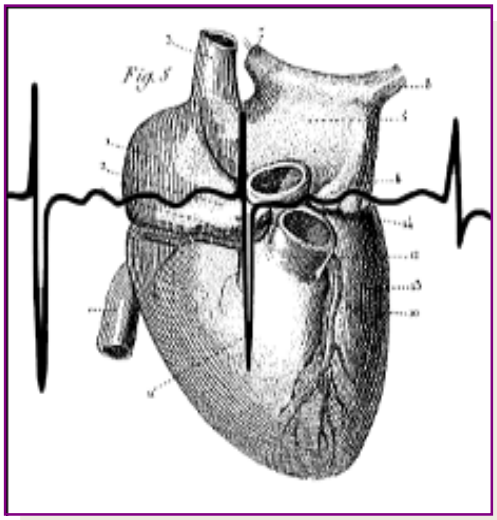
Factor	TSH (<0.1 mU/L)	TSH (0.1-0.4 mU/L*)
Age >65	Yes	Consider treating
Age <65 with Comorbidities		
Heart Disease	Yes	Consider treating
Osteoporosis	Yes	Consider treating
Menopausal	Consider treating	Consider treating
Hyperthyroid Symptoms	Yes	Consider treating
Age <65, Asymptomatic	Consider treating	Observe

*Where 0.4 is lower limit of normal range

Ross et al Thyroid 26:1343, 2016

Physiological Effects of Subclinical Hyperthyroidism

↓ bone density
↑ serum osteocalcin
↑ urinary hydroxyproline and pyrrolidine links



↑ heart rate
↑ risk of atrial fibrillation
↑ cardiac contractility[2]
↑ LV mass index
↑ intraventricular septal and posterior wall thickness

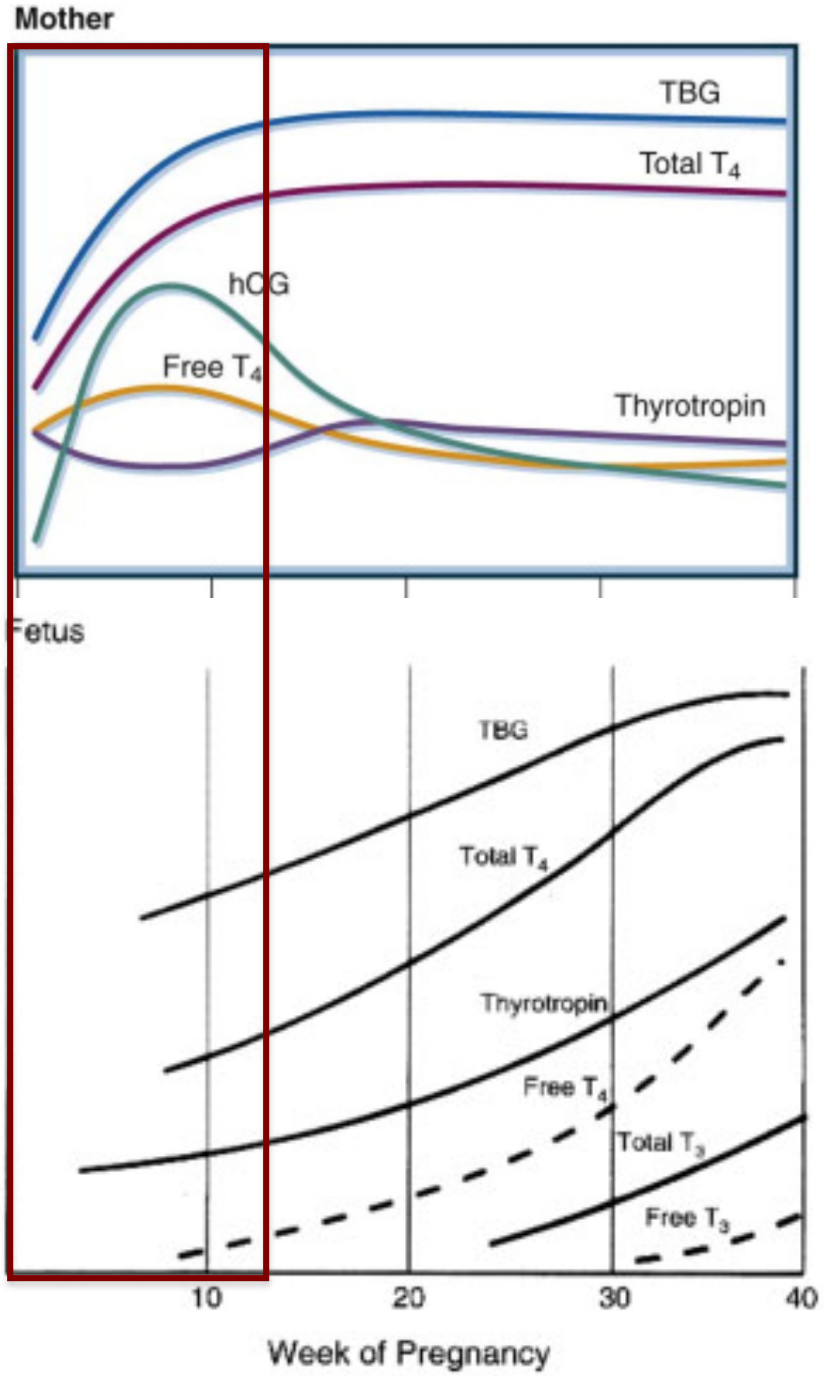
1. Ross DS. In: *Werner and Ingbar's The Thyroid,* 7th ed. 1996:1016.
2. Biondi B et al. *J Clin Endocrinol.* 1993;77:334.

THYROID FUNCTION TESTING IN PREGNANCY

Changes in Thyroid Function in Pregnancy in Mother and Fetus

Suppressed TSH, <0.01
Seen in normal pregnant Women:
1st Trimester-15%
2nd Trimester-10%
3rd Trimester-5%

hCG-human chorionic gonadotropin
TBG-thyroxine binding globulin



Burrow et al N Engl J Med 331:1072, 1994

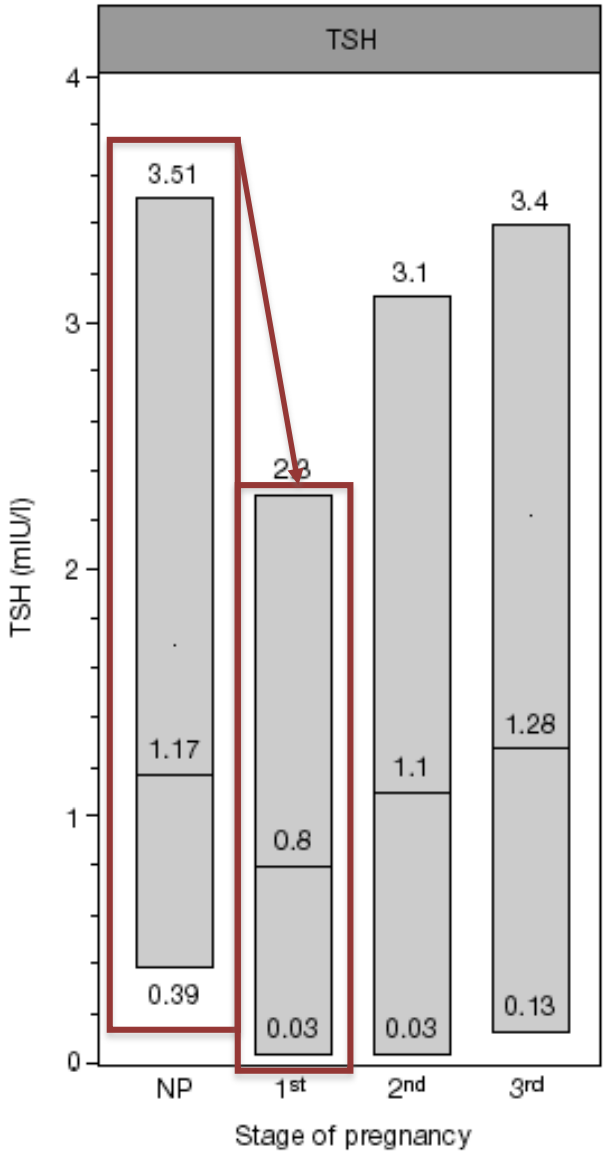
RECOMMENDATION 37

When a suppressed serum TSH is detected in the first trimester (TSH less than the reference range), a medical history, physical examination, and measurement of maternal serum Free T4 or total T4 concentrations should be obtained. Measurement of TSH receptor antibodies (TRAb), and in some cases maternal total T3 may prove helpful in diagnosing and clarifying the etiology of thyrotoxicosis.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Alexander et al Thyroid 27:315, 2017

Median TSH (95% CI) By Trimester in Normal Pregnancy



Chan GW, Mandel SJ. Nature Clin Prac Endocrinol Metab 3:470, 2007

Trimester-Specific TSH Ranges

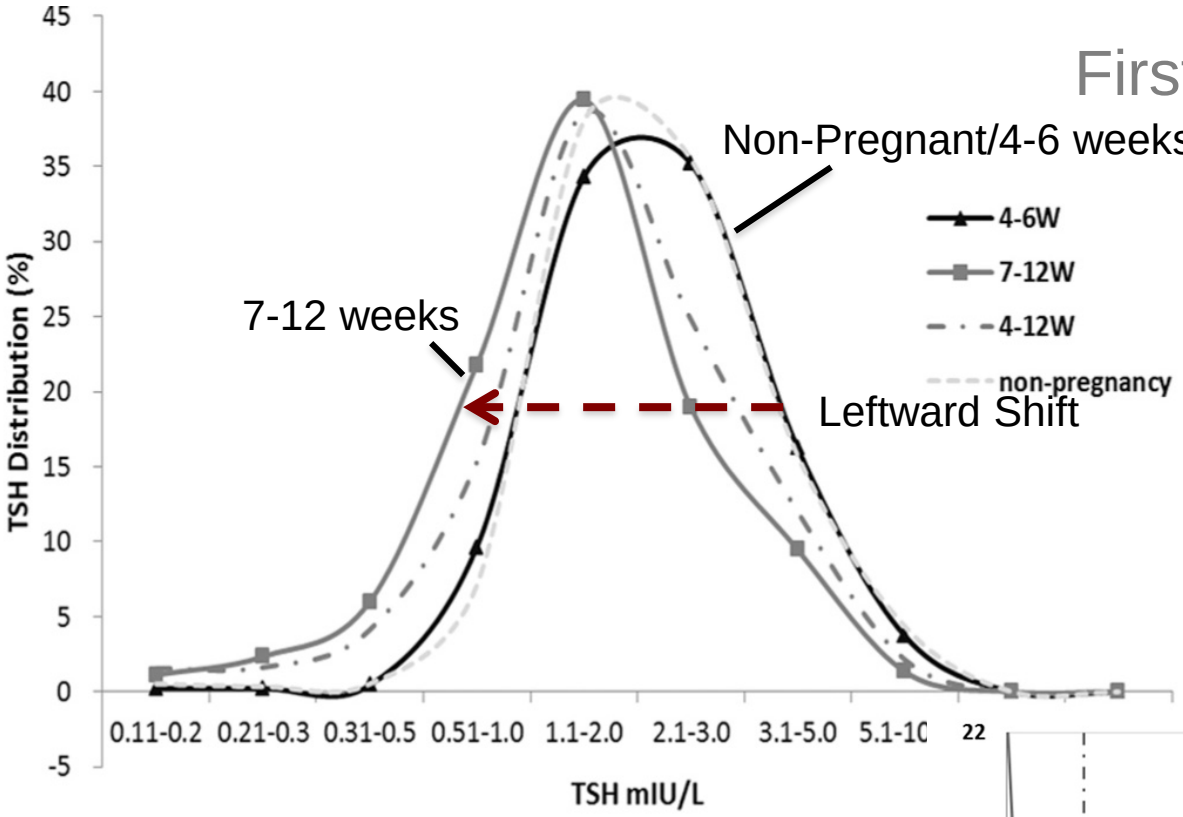
Thyrotropin reference ranges in different populations

Reference	Population	Thyrotropin reference range (mIU/L)		
		1st trimester	2nd trimester	3rd trimester
Stagnaro-Green[8]	US*	0.1-2.5	0.2-3.0	0.3-3.0
De Groot[9]	US†	0.1-2.5	0.2-3.0	0.3-3.5
Yan[19]	Chinese	0.03-4.51	0.05-4.50	0.47-4.54
Li[20]	Chinese	0.14-4.87		
Marwaha[21]	Indian	0.6-5.0	0.44-5.78	0.74-5.7
Korevaar[22]	Mixed (Dutch, Moroccan, Turkish, Surinamese)	0.06-4.51		

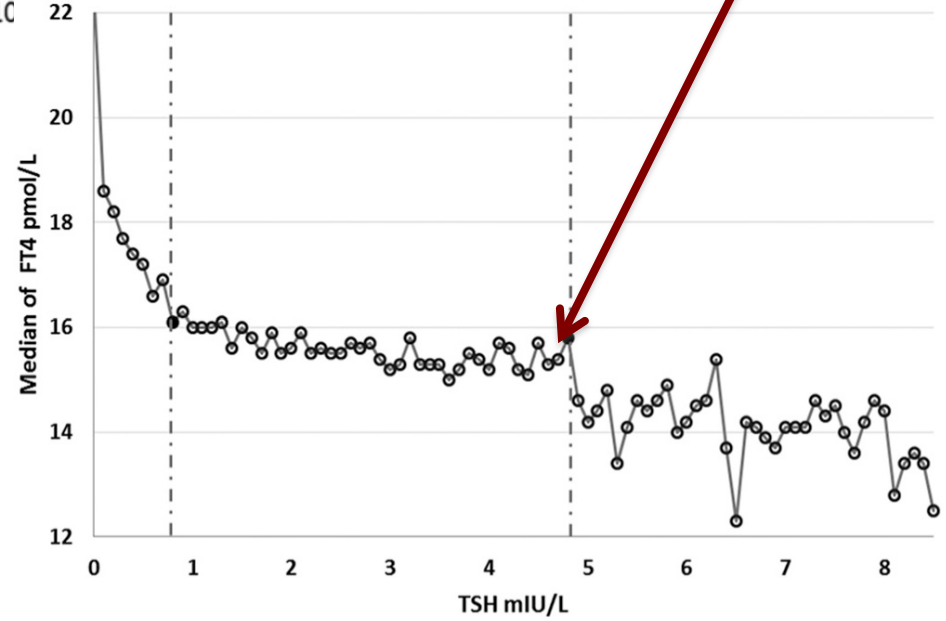
*American Thyroid Association guideline recommendations.
†Endocrine Society guideline recommendations.

Negro and Stagnaro-Green BMJ 349:g4929, 2014

First Trimester TSH Range



"flexion" point for fall in T4 TSH 4.8



First trimester TSH in 4,800 pregnant women

Li et al J Clin Endocrinol Metab 99:73, 2014

Recommendation 1

When possible, trimester-specific reference ranges for serum TSH should be defined through assessment of local population data representative of a healthcare provider's practice. Reference range determinations should only include pregnant women with no known thyroid disease, optimal iodine intake, and negative TPO Ab status.

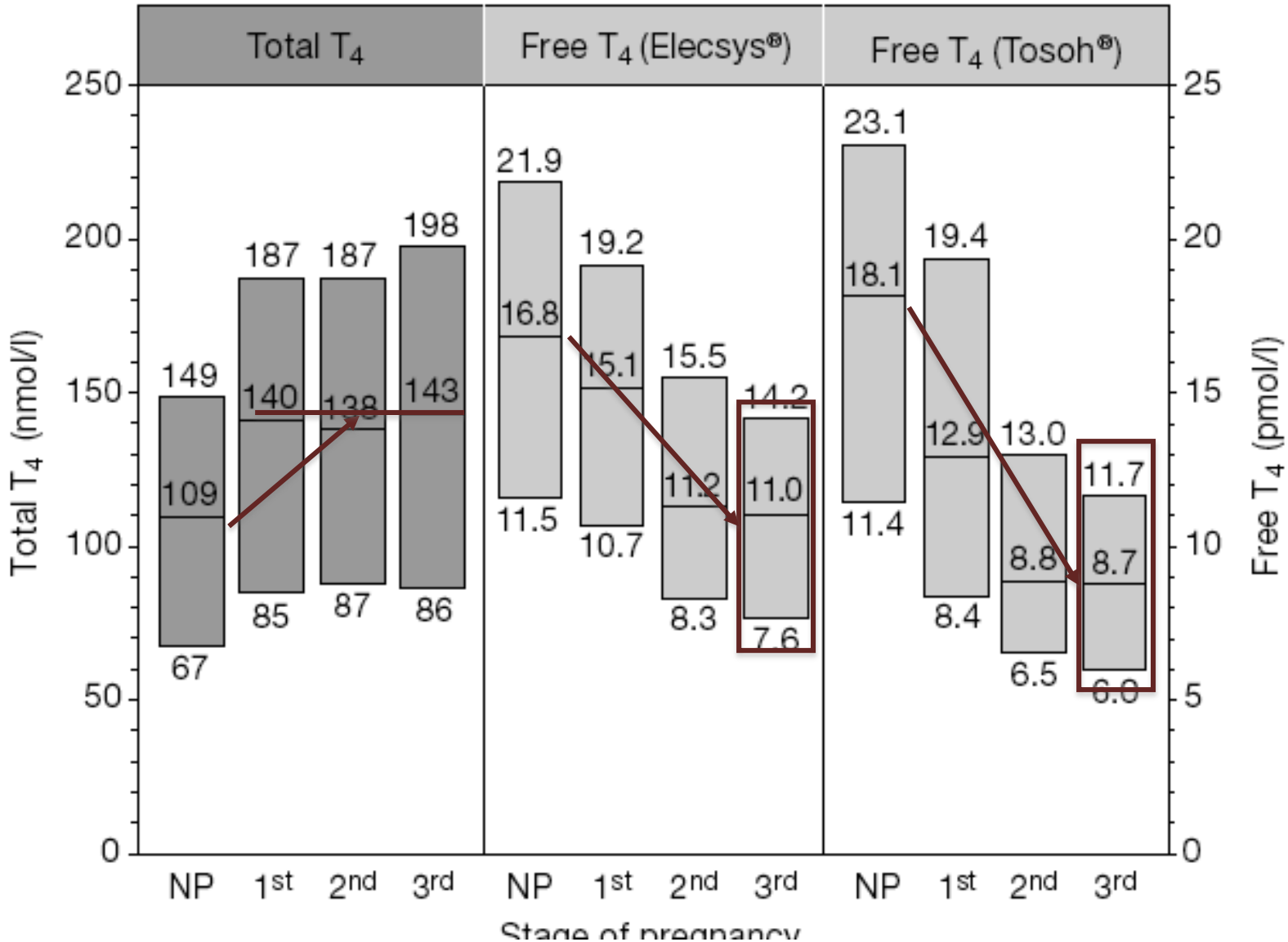
STRONG RECOMMENDATION | MODERATE-QUALITY EVIDENCE

If internal or transferable reference ranges are not available, the upper limit of the TSH reference range as applied to non-pregnant patients (usually ~ 4.0mU/l) should be used.

STRONG RECOMMENDATION | MODERATE-QUALITY EVIDENCE

Alexander et al Thyroid 27:315, 2017

Thyroxine Measurements in Pregnancy



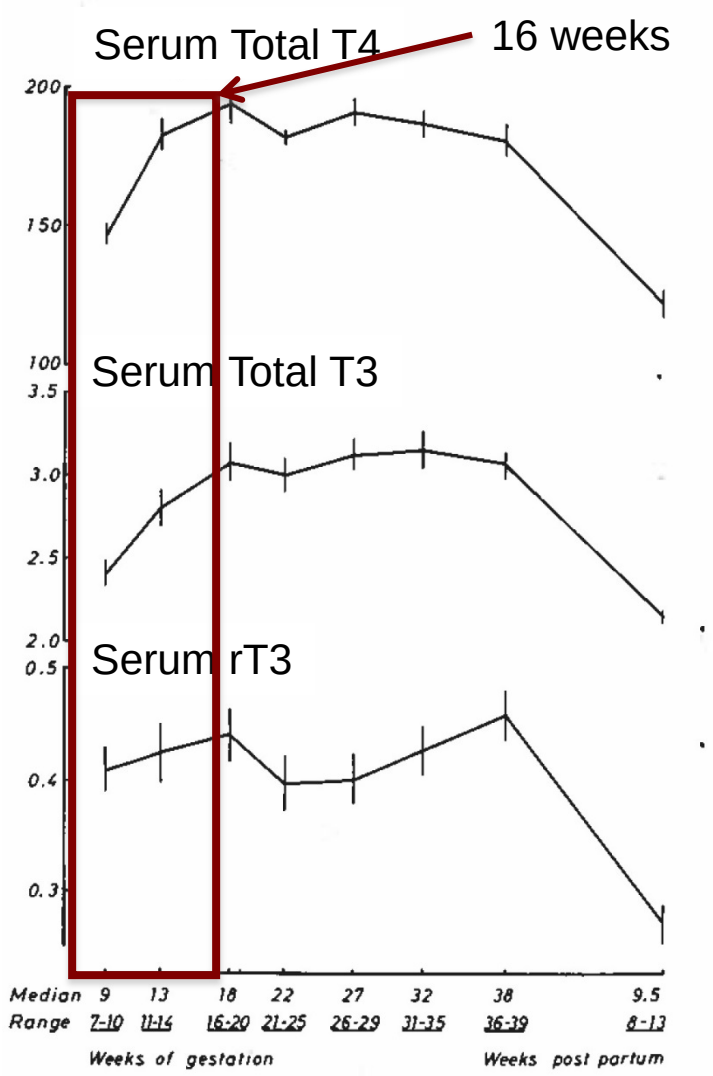
Chan GW, Mandel SJ. Nature Clin Prac Endocrinol Metab 3:470, 2007

Recommendation 2

The accuracy of serum Free T4 measurement by the indirect analog immunoassays is influenced by pregnancy and also varies significantly by manufacturer. If measured in pregnant women, assay method-specific and trimester-specific pregnancy reference ranges should be applied.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Alexander et al Thyroid 27:315, 2017



Guidelines to Use Total Thyroxine (T4) Measurement During Pregnancy

- Total T4 upper reference range increases to 50% above the pre-pregnancy level (eg. upper level 12.0 ug/dl is then 18).
- The increase in Total T4 occurs weeks 7-16 and is then sustained throughout pregnancy
- Gestational-specific week values can be calculated as 5% above normal per week in weeks 7-16, and weeks 17-40 50%

Weeke et al Acta Endocrinologica 101:531, 1982

Recommendation 3

In lieu of measuring freeT4, total T4 measurement (with a pregnancy-adjusted reference range), is a highly reliable means of estimating hormone concentration during pregnancy. Accurate estimation of the freeT4 concentrations can be done by calculating a free thyroxine index.

STRONG RECOMMENDATION **MODERATE-QUALITY EVIDENCE**

Alexander et al Thyroid 27:315, 2017

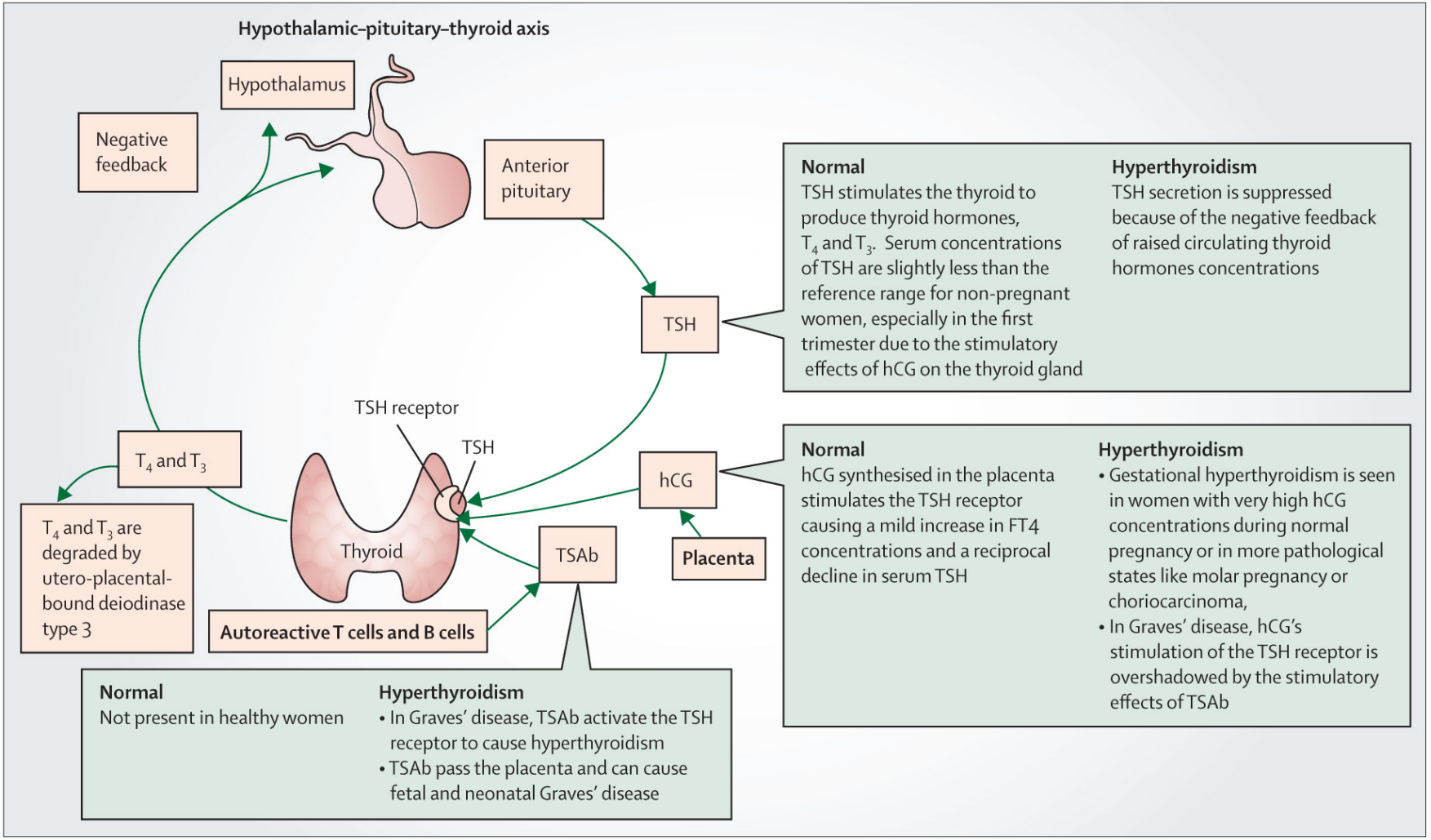
Table 1. American Thyroid Association Recommendations for the Management of Subclinical Hypothyroidism and Hypothyroxinemia in Pregnancy.*

Laboratory Data	Levothyroxine Therapy	Recommendation Strength	Evidence Quality
Anti-TPO–positive and thyrotropin level > pregnancy-specific reference range	Yes	Strong	Moderate
Anti-TPO–negative and thyrotropin level >10 mU/liter	Yes	Strong	Low
Anti-TPO–positive and thyrotropin level >2.5 mU/liter and < upper limit of the reference range	Consider	Weak	Moderate
Anti-TPO–negative and thyrotropin level > upper limit of the reference range and <10 mU/liter	Consider	Weak	Low
Isolated maternal hypothyroxinemia†	No	Weak	Low

* Adapted from Alexander et al.[3] Anti-TPO denotes antithyroperoxidase antibody.
† Isolated maternal hypothyroxinemia is defined as a free thyroxine level that is less than the 2.5th or 5th percentile and a normal thyrotropin level.

Cooper and Pearce N Engl J Med 376;9, 2017
Alexander et al Thyroid 27:315, 2017

Hyperthyroidism in Pregnancy



Cooper and Laurberg Lancet Diabetes Endocrinol 1:238, 2013

RECOMMENDATION 45

a. In women being treated with antithyroid drugs in pregnancy, FT4/TT4 and TSH should be monitored approximately every 4 weeks.

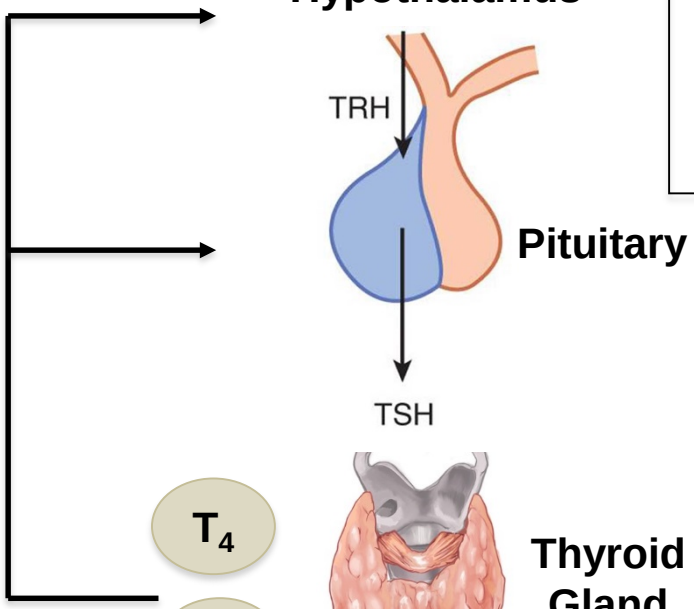
STRONG RECOMMENDATION | MODERATE-QUALITY EVIDENCE

b. Antithyroid medication during pregnancy should be administered as the lowest effective dose of methimazole or PTU, targeting serum FT4/TT4 at or moderately above the reference range.

STRONG RECOMMENDATION | HIGH-QUALITY EVIDENCE

FACTORS THAT INTERFERE WITH THYROID FUNCTION TESTING

Drugs and Site of Action on Thyroid Axis



Central Suppression
Glucocorticoids
Dopamine
Octreotide
Bexarotene

Thyroxine Binding Globulin

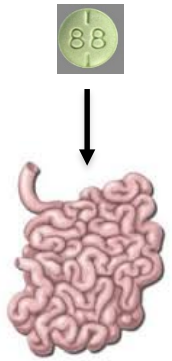
T_4 T_3

Free T_4 Free T_3

Alter TBG
↑ Estrogen
↓ Androgen

Displace Binding
Furosemide
Slicylates

Thyroidal Secretion
↓ ↑ Iodine
↓ Lithium
↓ ↑ Amiodarone



Impaired Absorption
Calcium
Iron
Omeprazole
Aluminum Hydroxide

Altered Metabolism
Phenobarbital
Rifampin
Phenytoin

Agents and Conditions Having an Impact on L-thyroxine Therapy and Interpretation of Thyroid Tests

Interference with Absorption:

- **Bile acid sequestrants** (cholestyramine, colestipol, colesevelam)
- **Sucralfate**
- **Cation exchange resins** (Kayexelate)
- **Oral biophosphonates**
- **Proton pump inhibitors**
- **Raloxifene**
- **Multivitamins** (containing ferrous sulfate or calcium carbonate)
- **Ferrous sulfate**
- **Phosphate binders** (sevelamer, aluminum hydroxide
- **Calcium salts** (carbonate, citrate, acetate)
- **Chromium picolinate**
- **Charcoal**
- **Orlistat**
- **Ciprofloxacin**
- **H2 receptor antagonists**[a]
- **Malabsorbtion syndromes**
  - Celiac disease
  - Jejunoileal bypass surgery
  - Cirrhosis (biliary)
  - Achlorhydria
- **Diet**
  - Ingestion with a meal
  - Grapefruit juice[a]
  - Espresso coffee
  - High fiber diet
  - Soybean formula (infants)
  - Soy

ATA/AACE Guidelines for Hypothyroidism. Garber JR, et al. *Thyroid*. 2012;22:1200-1235.

Case

A 48 year old woman is referred for suspected thyrotoxicosis. She initially had thyroid tests performed for complaints of fatigue, but rather than an elevated serum TSH, she was found to have a suppressed TSH of 0.01 mIU/L (nl 0.5-4.3). She has struggled with weight gain as well as complaints of low energy and dry hair. She has Type 2 diabetes and is on metformin 1000 mg BID. She is on no other medication, but takes a number of vitamins and nutritional supplements. On physical exam she has no thyroid eye signs, thyroid enlargement, or tremor.

Case (continued)

- Additional thyroid studies include:
- Free T4 4.5 ng/dl (nl 0.9-1.8)
- Free T3 720 pg/dl (nl 230-420)
- TRAb level was >40 IU/liter (nl <1.7)

Case Question

What is the next step to confirm the likely etiology of the abnormal thyroid function tests?

A. Perform a 24 hour radioiodine uptake measurement and scan.

B. Discontinue nutritional supplements for 2-3 days and repeat thyroid function tests.

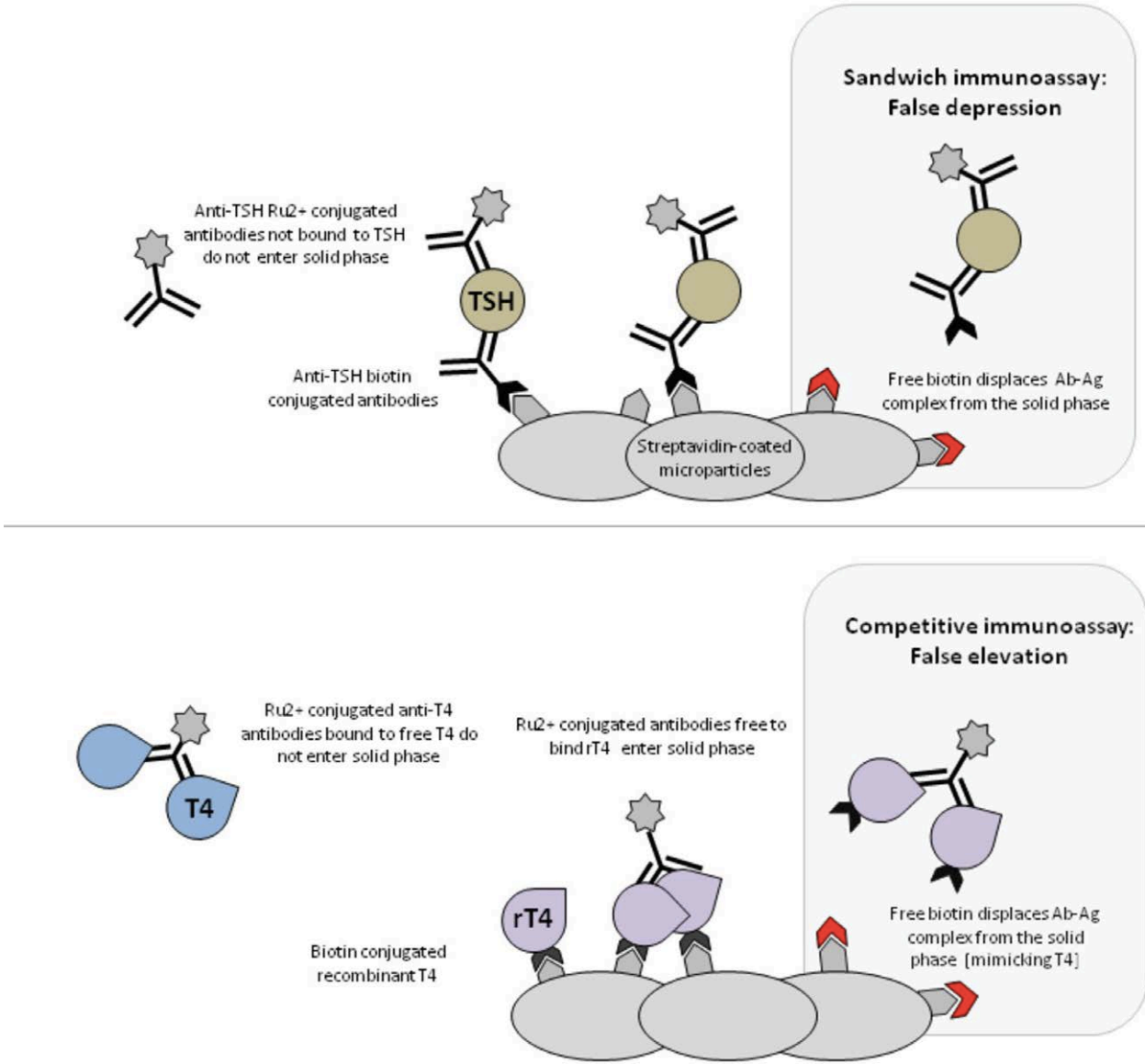
C. Measure human anti-mouse antibodies.

D. Dilute serum and repeat thyroid function measurements.

Answer B Discontinue nutritional supplements for 2-3 days and repeat thyroid function tests.

- Performing a radioiodine uptake (Answer A), human anti-mouse antibodies (Answer C) or diluting the serum (Answer D) are unlikely to lead to a specific diagnosis.
- The absence of any clinical findings suggestive of thyrotoxicosis and the abnormality of measurements of TSH, free T4, free T3, and thyroid receptor antibodies, strongly suggests the impact of ingested biotin on thyroid testing
  - Kummer et al N Engl J Med 375:704, 2016
  - Elston et al J Clin Endocrinol Metab 101:3251, 2016

Mechanism of Biotin Interference With Hormone Assays



TSH

Free T4
Free T3
TRAb

Minkovsky et al AACE Clin Case Reports 2(4):e370 2016

Answer B Discontinue nutritional supplements for 2-3 days and repeat thyroid function tests.

- Biotin is a water-soluble vitamin that is widely used in over-the-counter dietary supplements as well as treating patients with disorders of mitochondrial energy metabolism and multiple sclerosis. Moderate to high doses are associated with interference with a range of thyroid assays that rely on the interaction of biotin and strepavidin
- Cessation of biotin for 1-3 days, rapidly reverses the interference and thyroid function tests should return to normal. In addition to thyroid function testing, a large number of immunoassays, such as for folate and vitamin B12 and many hormone measurements, can also be altered by biotin

Standardization of thyroid function tests

Dr. Katleen Van Uytfanghe
Ref4*U*

On behalf of the IFCC C-STFT
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Clin Chem 2010, 56-6

Speakers financial disclosure information

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  Abbott Diagnostics, Beckman Coulter, Inc., bioMérieux s.a., DiaSorin S.p.A., Fujirebio Inc., LSI Medience Corporation, Mindray Medical International Limited, Ortho-Clinical Diagnostics, Roche Diagnostics GmbH, Sanyo Chemical Industries, LTD, Snibe Co.,Ltd, Sichuan Maccura Biotechnology Co., Ltd, Siemens Healthcare Diagnostics Inc., Sysmex Corporation, Tosoh Corporation, Wako Pure Chemical Industries, LTD
- **Salary/Consultant Fees: None**
- **Board/Committee/Advisory Board Membership: None**
- **Stocks/Bonds: None**
- **Honorarium/Expenses: None**
- **Intellectual Property/Royalty Income: None**

Thyroid Diseases are a global major public health burden

Estimated prevalence among selected countries:

Hyperthyroidism: 0.1% - 2.9%,

Hypothyroidism: 0.3% - 10.95%

(Taylor Nature Reviews Endocrinology, 2018;14:301)

Tests reimbursed by U.S. Medicare Plan B in 2014:

TSH: 21.3 million - $477 million

T4: 6.6 million - $79 million

(HHS OIG Data Brief OEI-09-15-00210)

The problem

Suppose a patient is used to have his tests done in lab A, now receives, per exception, results from lab B. Knowing his "numbers" – he thinks his thyroid function is out of control. Do you agree?

Lab A – Assay X

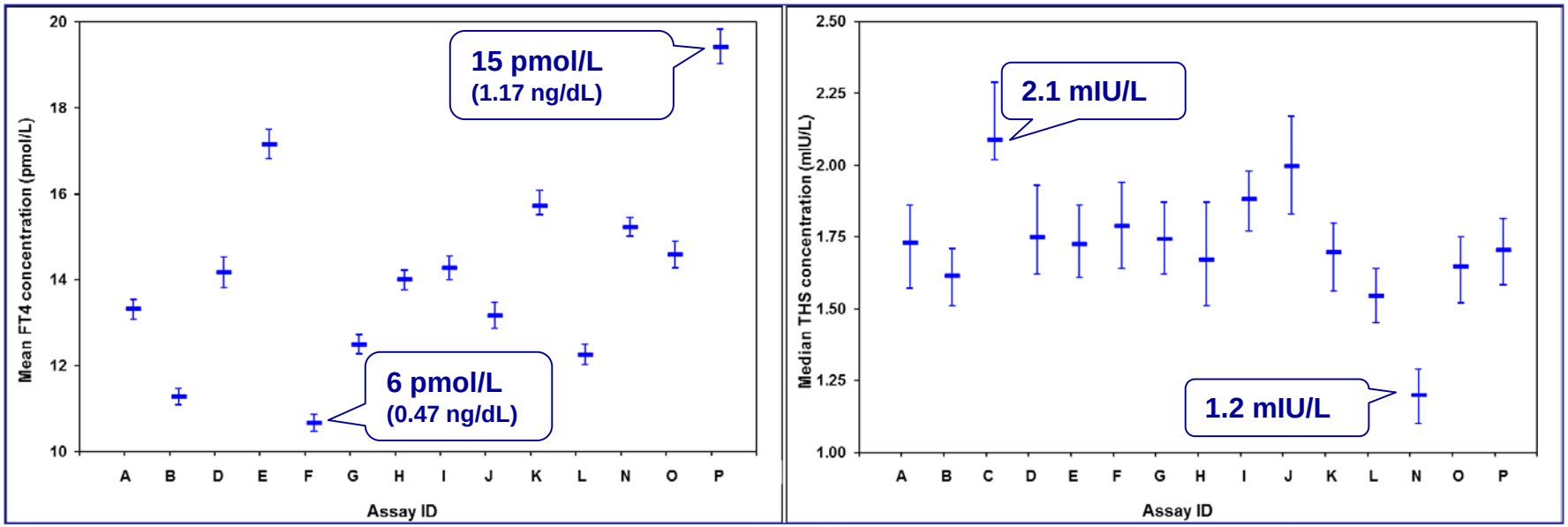
Analyte	Result	Reference Interval
TSH (mIU/L)	2.02	0.55 - 4.78
FT4 (pmol/L) FT4 (ng/dL)	13.4 1.04	12.0 - 22.7 0.93 – 1.76

Lab B – Assay Y

Analyte	Result	Reference Interval
TSH (mIU/L)	1.90	0.50 – 4.30
FT4 (pmol/L) FT4 (ng/dL)	11.0 0.85	10.3 - 21.9 0.80 – 1.70

Concerning high varability among TSH and fT4 assays has been reported

Mean/median concentrations for a panel of 120 healthy volunteers.



(Thienpont LM et.al. Clin Chem 2017;63:1248 – Thienpont LM et.al. Clin Chem 2017;63;1642)

Accurate and reliable TSH and fT4 tests are critical for ensuring correct diagnosis and treatment of patients

Patient diagnosis relies on TSH and fT4 tests

Accurate and reliable TSH and fT4 tests

- **Minimize misdiagnosis**
- **Allow for effective treatment and monitoring, even when the testing is performed in different labs**
- **Enable establishment of normal ranges that can be used all labs**
- **Facilitates the formulation and implementation of patient treatment guidelines**

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

Established in light of the prevalence of thyroid disease, the frequency of laboratory testing, and multiple reports on discrepant measurement results

www.ifcc-cstft.org

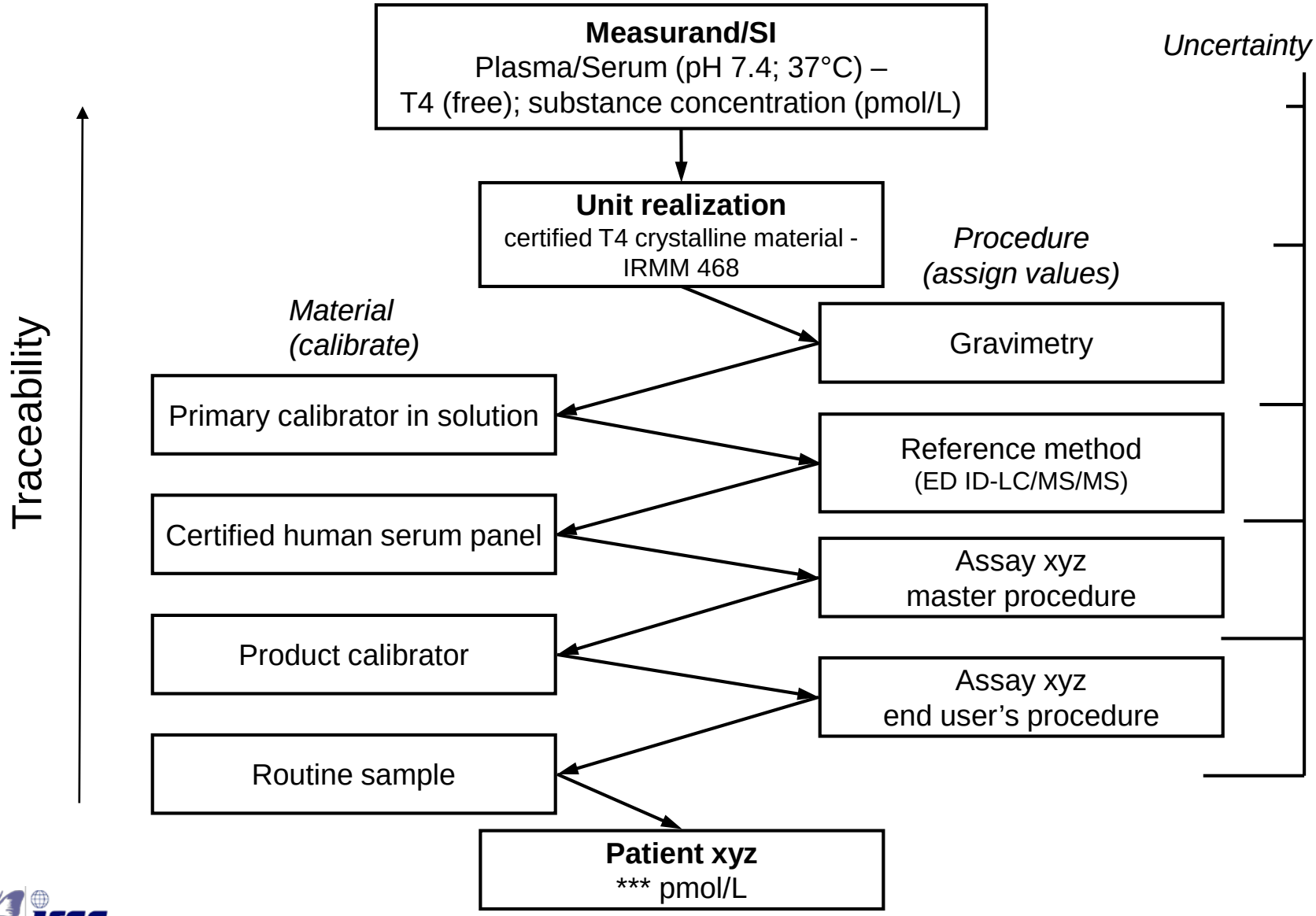
http://www.ifcc.org/ifcc-scientific-division/sd-committees/c-stft

Development of reference measurement systems for FT4 and TSH

Aim

Have a reference measurement system (RMS) that is conform with global standards and in line with local regulations

FT4 reference measurement system



Reference measurement procedure (RMP)

Equilibrium dialysis (ED) with ID-LC/MS/MS calibrated with certified reference material IRMM 468

(Van Houcke SK et al. Clin Chem Lab Med 2011;49:1275)

RMP is considered a “conventional RMP”

For which the convention applies for …

A. The conditions for equilibrium dialysis

B. The ID-LC/tandem MS method

C. Both equilibrium dialysis and ID-LC/tandem MS

Reference measurement procedure (RMP)

Equilibrium dialysis (ED) with ID-LC/MS/MS calibrated with certified reference material IRMM 468

(Van Houcke SK et al. Clin Chem Lab Med 2011;49:1275)

Dialysis conditions defined by convention to ensure that the equilibrium between bound and free T4 is minimally disturbed. → International conventional RMP

(CLSI guideline C45)

Measurand is operationally defined as

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

(Thienpont LM. Clin Chem Lab Med 2007;45:934)

Key parameters of the fT4 RMP

JCTLM-listed RMP C8RMP1

- Calibration: IRMM-468, [$^{13}C_9$]T4 as internal standard
- Dialysis: 1 mL serum vs 1 mL HEPES-buffer for 4 hours, at pH 7.4 and 37°C using a membrane of regenerated cellulose
- Sample clean-up: solid phase extraction on Sep Pak C18 columns
- Chromatographic separation: 2D-column switching on a Acquity UPLC C4 and BEH C18 column (2.1 x 50 mm, 1.7 µm)
- Tandem mass spectrometry: m/z 778 → 732 & 784 → 738

Quality specifications

- Imprecision (max): 3.7% (5%)
- Bias (max): -0.2% (2.5%)
- Expanded measurement uncertainty: 7.6%
- LoD/LoQ: 0.5/1.3 pmol/L (0.04/0.10 ng/dL)

(Van Houcke SK et al. Clin Chem Lab Med 2011;49:1275)

Serum-based reference materials

Panel of unmodified, individual donor serum

- **clinically relevant concentrations (euthyroid, hypo- and hyperthyroid)**
- **Commutable**
- **Target values assigned with RMP**

Intended use:

- **Calibrator for routine assays**
- **Trueness control**

Note on commutability

A commutable material is a material that has inter-assay properties comparable to those of patient samples, both measured with two or more analytical methods.

A range of procedures often applied to sera was found to lead to non-commutability (i.e., filtration, spiking).

➔ Use of unmodified, individual donor samples were found to be the most suitable reference materials

(Van Houcke SK et al. Clin Chem Lab Med 2013;51:967)

TSH reference measurement system

Definition of the measurand:

- **TSH not yet unequivocally defined**
- **TSH is heterogeneous:**
  - **Two noncovalently linked subunits**
  - **Disease-specific glycosilation**

  ➔ Heterogeneity in both

(Thienpont et al. Clin Chim Acta 2010;411:2058)

➔ TSH analysis is “mixture” analysis

Heterogeneity reflected in the definition of the measurand:
arbitrary amount-of-substance concentration (mIU/L)
(defined by the WHO IRP 80/558 & 81/565)

Challenges for TSH reference measurement system

- No RMP exist to quantitate all relevant variations of TSH
- Primary reference materials are non-commutable
- A new approach is needed to improve the variability among TSH assays

➔ harmonization

New approach to establish a reference system for TSH

Surrogate RMP:

Statistical "all-procedure trimmed mean" (APTM) from a method comparison with a clinically relevant panel and participation by (as many as possible) assays

(Van Houcke et al. Clin Chem Lab Med 2013;51:e103)

➔ **Statistical basis: robust factor analysis model**

(Stöckl et al. Clin Chem Lab Med 2014;52:965)

➔ **Working calibrator: panel of serum samples**

.

Milestones achieved with the developed FT4 and TSH reference measurement systems

Method comparison studies

Four studies performed (2008 - 2016): Phase I – IV

Objectives:

- **Determine calibration status of current FT4 & TSH assays (RMPs: fT4 – ED-LC/MS/MS, TSH: APTM)**
- **Assess analytical performance of the assays**
- **Assess feasibility of standardization (use of patient samples to recalibrate assay master calibrators)**
- **Estimate impact of recalibration**

Phase IV study for FT4 and TSH

Aim

- **Perform method comparison studies after recalibration**
- **Provide a proof-of-concept for reference interval (RI) studies**

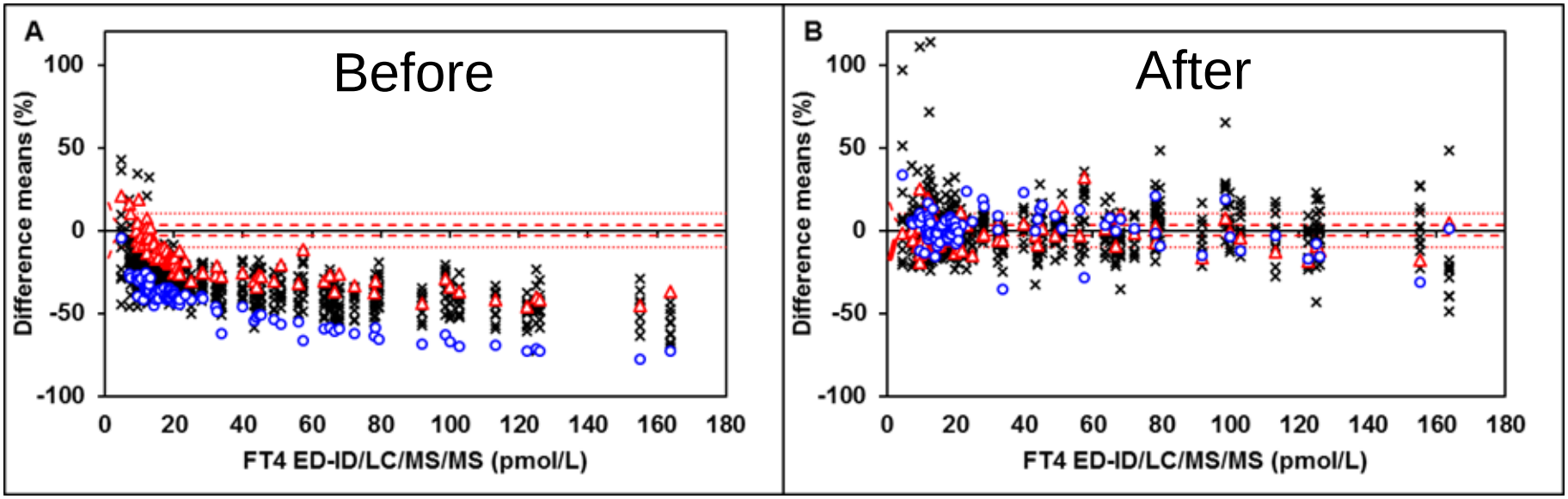
Phase IV study serum samples

Analyte	Panel Name	Concentration Range	Intended Use
FT4	Standardization Panel (n=91)	4.5 – 164 pmol/L (0.35 – 12.7 ng/dL)	Recalibration
	Reference Interval Panel (n=120)	12.7 – 25.8 pmol/L (0.99 – 2.00 ng/dL)	Proof-of-concept Preliminary reference interval
	Follow-up Panel (n=95)	4 – 202 pmol/L (0.31 – 15.7 ng/dL)	Sustainability
TSH	Harmonization Panel (n = 101)	~0.002 – 193 mIU/L	Recalibration
	Reference Interval Panel (n=120)	0.3 – 7.7 mIU/L	Proof-of-concept Preliminary reference interval
	Follow-up Panel (n=95)	~0.002 – 169 mIU/L	Sustainability

Outcome of Phase IV study

Phase IV – FT4 standardization

Measurement bias of individual samples before and after recalibration



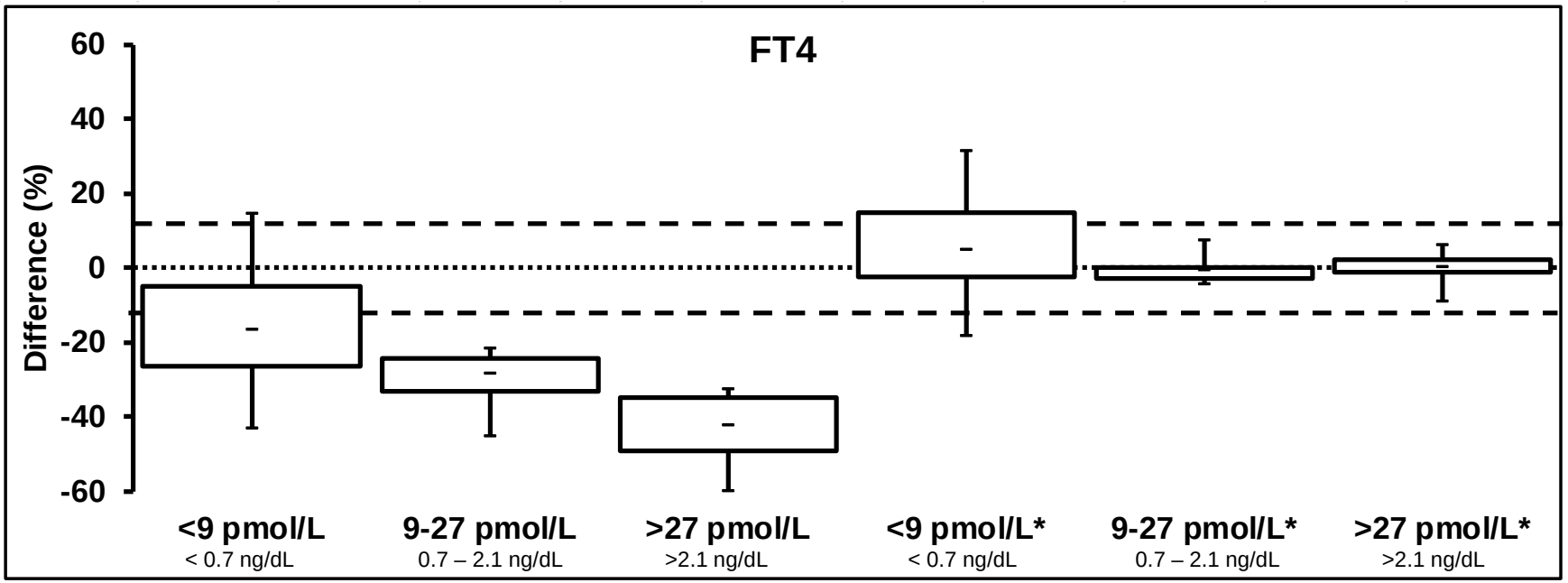
(Thienpont LM et al. Clin Chem 2017;63;1642)

➔ **On a scale of 1 – 10:**
how would you consider the impact of standardization?

1-10

Recalibration has highest impact in the hyperthyroid range

Bias distribution by hypo-, eu-, and hyperthyroid concentration before and after recalibration



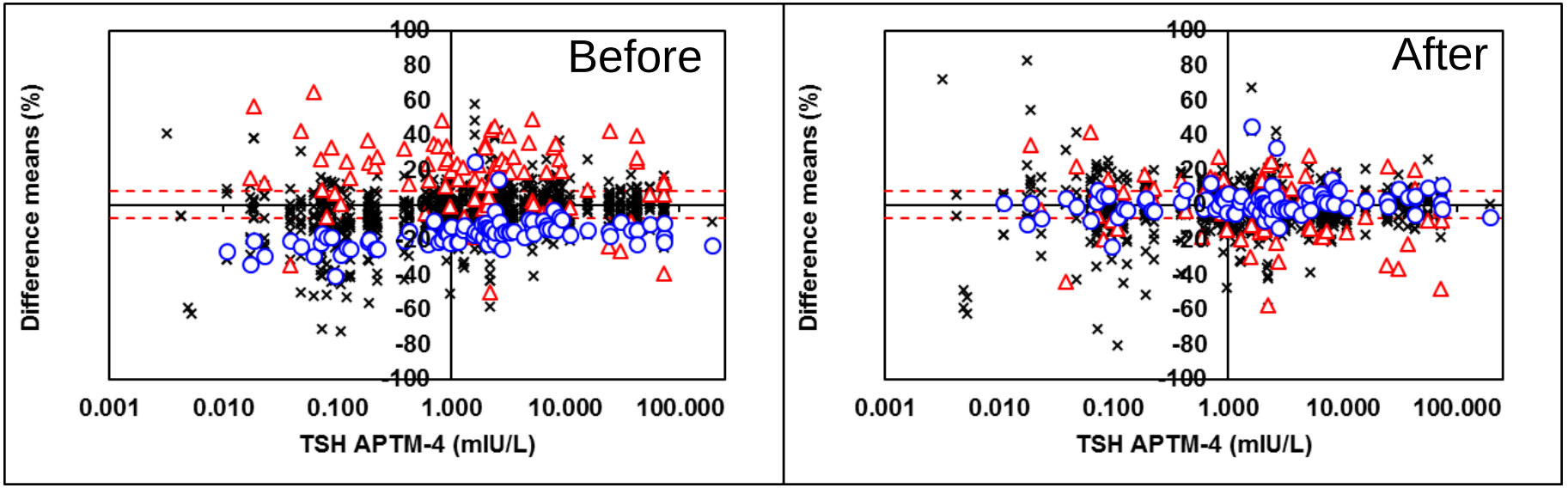
(Thienpont et al. Clin Chem 2017;63;1642)

*** After recalibration**

➔ **Recalibration will result in new *reference intervals***

Phase IV – TSH harmonization

Measurement bias of individual samples before and after recalibration



(Thienpont LM et al. Clin Chem 2017;63:1248)

➔ **Impact of harmonization is moderate**

Reference interval proof-of-concept study

Objective

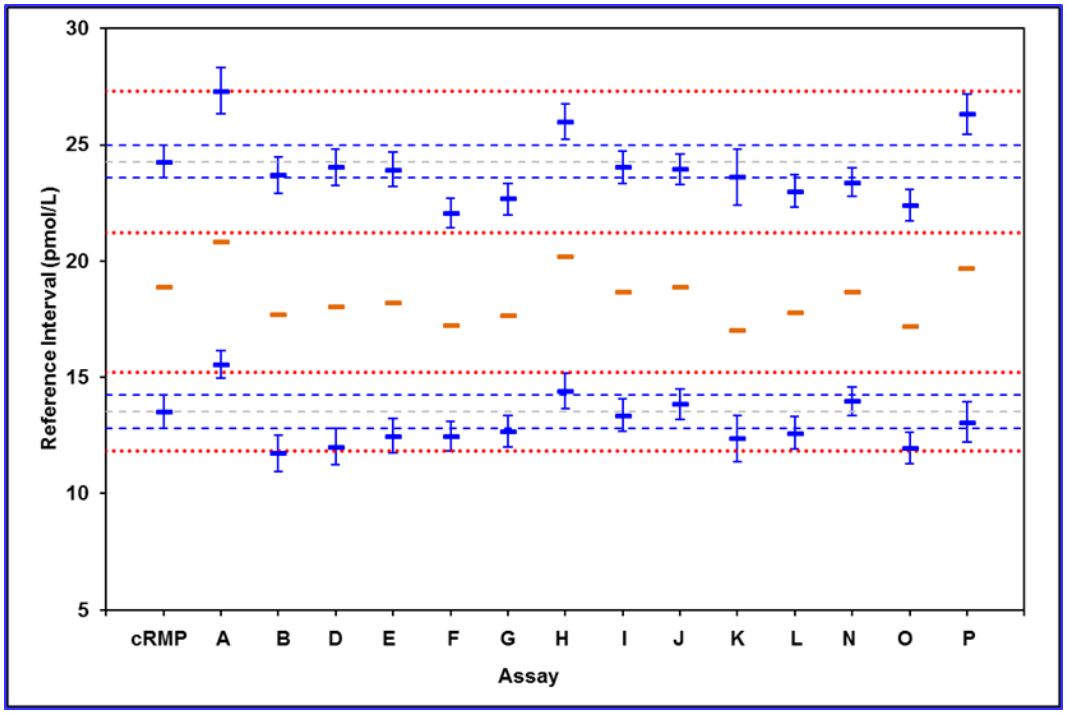
Assess whether common reference interval can be used after recalibration

Approach

- **Two panels of 120 apparently healthy volunteers**
- **Samples measured by recalibrated immunoassays**
- **Assess variability at 2.5th and 97.5th percentile**

Reference interval study

FT4 – RI by direct parametric procedure (apart from 1 IA)



RI by the RMP

Mean: 18.9 pmol/L (1.47 ng/dL)
Width: 10.7 pmol/L (0.83 ng/dL)

2.5 Percentile (90% CI):
13.5 pmol/L (1.05 ng/dL)
(12.8 – 14.2 pmol/L) (0.99 – 1.10 ng/dL)

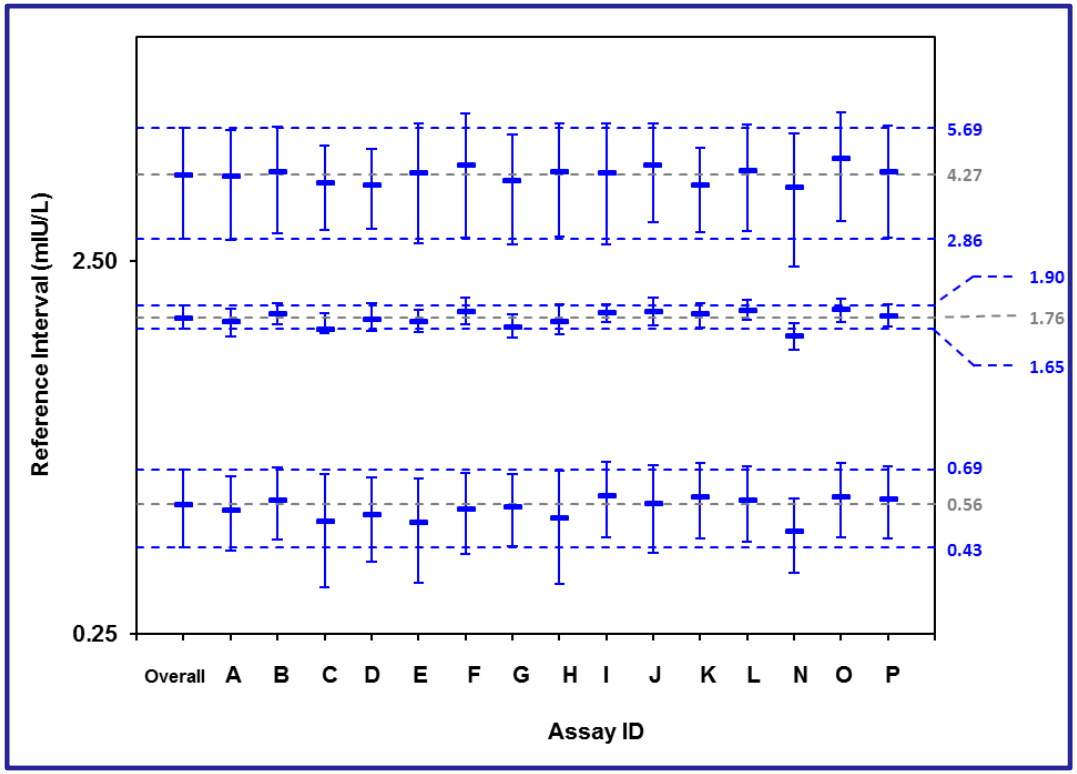
97.5 Percentile (90% CI):
24.3 pmol/L (1.89 ng/dL)
(23.6 – 25.8 pmol/L) (1.83 – 2.00 ng/dL)

➔ **Variability at 2.5th and 97.5th percentiles appears sufficiently small for use of common limits**

(Thienpont LM et al. Clin Chem 2017;63;1642)

Reference interval study

TSH – RI by non-parametric procedure



RI based on all method mean

Median: 1.76 mIU/L
Width: 3.72 mIU/L

2.5 Percentile (90% CI): 0.56 mIU/L (0.43 – 0.69 mIU/L)

97.5 Percentile (90% CI): 4.27 mIU/L (2.86 – 5.69 mIU/L)

➔ **Variability at 2.5th and 97.5th percentiles appears sufficiently small for use of common limits**

(Thienpont LM et.al. Clin Chem 2017;63:1248)

Way forward

IFCC C-STFT terms of reference

- **Establish a system to maintain traceability of free thyroid hormone and TSH measurements.**
- **Coordinate programs to evaluate free thyroid and TSH assays with regards to their analytical performance.**
- **Develop reference intervals for free thyroid hormones and TSH.**
- **Liaise with key stakeholders to promote the use of the standardized assays.**

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

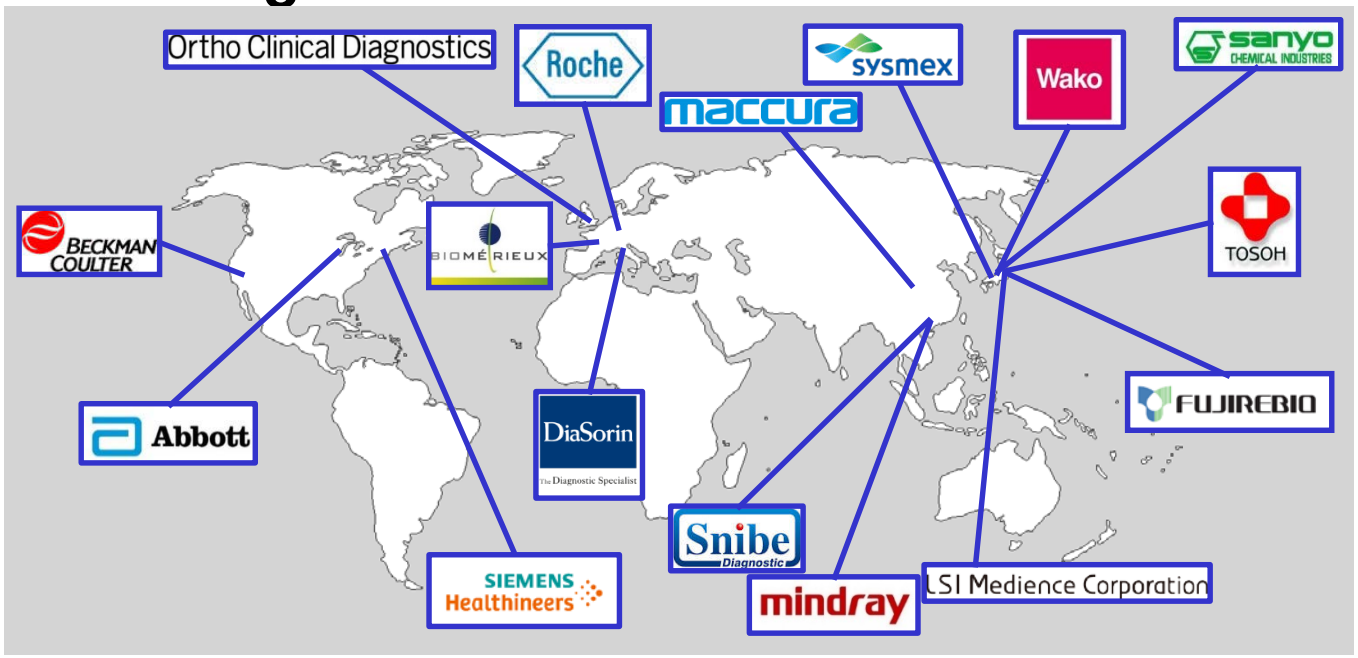
Current projects

Network of reference laboratories

- **4 candidate members**
  - Ref4U (Belgium)
  - CDC (USA)
  - Radboud University Medical Center of Nijmegen (The Netherlands)
  - Reference Material Institute for Clinical Chemistry Standards (ReCCS, Japan)
- **Implemented the IFCC endorsed FT4 RMP**
  i.e. convention conditions for ED combined with an SI traceable ID-LC/MS method quantification of T4 in the dialysate
- **Performance criteria under development**
- **First method comparison on-going**

Acknowledgements

- **Past and current Chair of the C-STFT**
  Prof. Em. Linda Thienpont and Dr. Hubert Vesper
- **Past and current members, consultants and stakeholders of the C-STFT**
- **PhD students and staff of Ref4*U*, Ghent University**
- **Collaborating IVD-manufacturers**



Want to find out more?

http://ifcc-cstft.org/