Published in http://www.thyroidmanager.org/

THYROID FUNCTION TESTS: ASSAY OF THYROID HORMONES AND RELATED SUBSTANCES

Carole Spencer,PhD Professor of Medicine University of Southern California, Director USC Endocrine Laboratories, USC Endocrine Laboratory 126 W. Del Mar Blvd., Pasadena, CA 91105-2508, Department of Medicine Univ of Southern Calif, Medical School 2025 Zonal Ave. Los Angeles, CA 90033-4526

Revised February 2017

ABSTRACT

This chapter reviews how improvements in sensitivity and specificity of thyroid function tests [total and free thyroid hormones, TSH, thyroid autoantibodies (TRAb, TPOAb and TgAb) and thyroglobulin (Tg)] have dramatically improved clinical strategies for detecting and treating thyroid disorders. The review discusses the strengths and limitations of the different methodologies currently used (RIA, IMA and LC-MS/MS) and their propensity for analyte-specific interferences caused by heterogeneity (TSH, TgAb and Tg) or analyte-specific autoantibodies (T4Ab, T3Ab, TSHAb and TgAb). In addition, non-analyte related interferences from heterophile antibodies, including human anti-mouse antibodies (HAMA) and Rheumatoid Factor (RF), and interferences related to the use of Biotin and Streptavidin reagents, are discussed. The review provides an update on collaborations between the International Federation of Clinical Chemistry (IFCC) committee for the standardization of thyroid function tests (C-STFT) and the in-vitro diagnostic (IVD) industry- the goal being to eliminate between-method biases. Although re-standardization of thyroid hormone tests against established reference measurement procedures, and harmonization of TSH tests to the all-method mean has proved effective, recalibration has yet to be implemented by the IVD. Until between-method biases are eliminated, it is not feasible to propose universal reference ranges that would apply across methods. The review contains a comprehensive discussion of the clinical utility of Tg methodology (RIA, IMA or LC-MS/MS), used to monitor patients with differentiated thyroid cancer (DTC). Mechanisms for in-vitro and possible in-vivo TgAb interference with Tg testing are proposed. The methodologic and clinical strengths and weakness of each test are discussed relative to current guidelines.For complete coverage of this and related areas in Endocrinolofy, visit our free web-books, www.endotext.org and www.thyroidmanager.org.

INTRODUCTION

Over the past forty years, improvements in the sensitivity and specificity of thyroid testing methodologies have dramatically impacted clinical strategies for detecting and treating thyroid disorders. In the 1950s, only one thyroid test was available - an indirect estimate of the serum total (free + protein-bound) thyroxine (T4) concentration, using the protein bound iodine (PBI) technique (1). Since 1970, technological advances in radioimmunoassay (RIA) (2-6), immunometric assay (IMA) (7-11) and most recently liquid chromatography-tandem mass spectrometry (LC-MS/MS) (12-23) have progressively improved the specificity, reproducibility and sensitivity of thyroid tests (24,25). Currently, serum-based immunoassays and LC-MS/MS techniques are available for measuring total and free thyroid hormones, [Thyroxine (T4) and Triiodothyronine (T3)] (23,26-28), as well as the pituitary thyroid stimulator, Thyrotropin (Thyroid Stimulating Hormone, TSH) (8,29) and the thyroid hormone precursor protein, Thyroglobulin (Tg) (9,16,21,30-33). In addition, measurements can be made of the thyroid hormone binding proteins, Thyroxine Binding Globulin (TBG), Transthyretin (TTR)/Prealbumin (TBPA) and Albumin (34-36). Methods to detect the thyroid autoantibodies (24,37): TSH receptor antibodies (TRAb) (38-43), thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) (31,44,45) have been developed in response to the recognition that autoimmunity is a major cause of thyroid dysfunction (46-48). Currently, most thyroid testing is performed on serum specimens using manual or automated immunoassays employing specific antibody reagents targeting these ligands (22,24).

Over the last ten years the International Federation of Clinical Chemistry (IFCC) committee for the standardization of thyroid function tests (C-STFT)* has been working with manufacturers to identify and reduce between-method variability for total and free thyroid hormones as well as TSH (49). Reference measurement procedures (RMP) for TT4 and TT3 using primary calibrators have been developed (12,13,26) and used to establish isotope-dilution liquid chromatography/tandem mass spectrometry (ID-LC-MS/MS) as the RMP for FT4 and FT3 after isolating free hormone by equilibrium dialysis (26,27,50-53) or ultrafiltration (14,23,27,28,54,55). Thyroglobulin has also been detected by LC-MS/MS after trypsinization (16,19-21). Despite technical improvements, sensitivity, specificity and standardization issues still result in substantial between-method variability for many thyroid analytes (8,9,16,32,44,49,53,56-58). The C-STFT studies have shown that recalibrating thyroid hormone methods to their RMPs (50-53) and harmonizing TSH methods to the all-method mean, derived by a robust factor analysis model, significantly reduces between-method biases (29,52,53,59,60). It is hoped that the industry will shortly recalibrate their thyroid tests to remove current biases thereby allowing establishment of universal reference ranges that could apply to all methods and improve the clinical utility of thyroid testing. This chapter is designed to give an overview of the current status and limitations of the thyroid testing methods most commonly used in clinical practice, as recommended by current guidelines (24,61-74). Table 1

Table 1-Reference ranges for Thyroid Function Tests Used in USC Clinical Laboratory

Test	Method	USC Reference Ranges *
Total Thyroxine (TT4)	Roche Cobas	57-159 nmol/L (4.5-12.5 ug/dL)
Total Triiodothyronine (T3)	Roche Cobas	1.2-2.8 nmol/L (80-180 ng/dL)
Thyroid Hormone Binding Ratio (THBR)	Roche Cobas	0.72 - 1.24 (unitless)
Thyrotropin (TSH)	Roche Cobas	0.3 - 4.0 mIU/L
Thyroxine Binding Globulin (TBG)	Siemens Immulite	14.0-31.0 mg/L (14.0-31.0 µg/mL)
Thyroid Peroxidase Antibody (TPOAb)	Kronus/RSR	<1.0 kIU/L
Thyroglobulin (2G-Tg-IMA)	Beckman Access	3-40 µg/L (3-40 ng/mL) #
Thyroglobulin RIA (Tg-RIA)	USC LDM [31]	3-40 µg/L (3-40 ng/mL) #
Tg Autoantibody (TgAb)	Kronus/RSR	<0.4 kIU/L

***These ranges are only applicable to the method listed. They were established for a non-pregnant <60 year-old euthyroid cohort recruited by USC.**

Tg range should be adjusted for thyroid mass and TSH status [see below].

* *My sincere thanks to the C-STFT committee chair Professor Linda Thienpont for informative discussions and for providing some of the data contained in this chapter.*

TOTAL THYROID HORMONE MEASUREMENTS (TT4 AND TT3)

Thyroxine (T4) circulates 99.97% bound to the plasma proteins, primarily TBG (60-75%) but also Transthyretin TTR/TBPA (15-30%) and Albumin (~10%)(Table 2) . In contrast, approximately 99.7% of Triiodothyronine (T3) is protein-bound, primarily to TBG [34,35,75]. Total (free + protein-bound) concentrations of thyroid hormones (TT4 and TT3) circulate at nanomolar concentrations and are considerably easier to measure than the free hormone moieties (FT4 and FT3) that circulate in the picomolar range. Serum TT4 measurement has evolved over the past four decades from the protein-bound iodine and competitive protein binding tests [1,76] to non-isotopic immunometric assays [77] and LC-MS/MS methods [13,78-80].

Table 2: Conditions that Influence Thyroid Hormone Binding Proteins

	Increased TBG	Decreased TBG	Albumin Transthyretin (TTR) Abnormalities
Drugs	Estrogens Tamoxifen 5-Fluorouracil Heroin/Methadone Clofibrate Nicotinic Acid Perphenazine	Thyroid hormones Androgens Anabolic steroids Glucocorticoids L-asparaginase Interleukin-6	
Pathophysiologic conditions	Pregnancy Hypothyroidism Acute/chronic hepatitis HCC/PBC Adrenal insufficiency AIDS Angioneurotic edema Acute intermittent porphyria Oat cell carcinoma	Hyperthyroidism Critical illness Sepsis Hepatic failure Nephrotic syndrome Diabetic ketoacidosis Chronic alcoholism Malnutrition Acromegaly Cushing's syndrome Extreme prematurity	Nonthyroidal illness Malnutrition Inflammation Pregnancy
Congenital conditions	TBG excess	TBG deficiency	Familial Dysalbuminemic Hyperthyroxinemia, FDH Transthyretin-Associated Hyperthyroxinemia, TTR-AH

From: references 35 and 36

Serum TT4 measurement has evolved over the past four decades from the protein-bound iodine and competitive protein binding tests [1,76] to non-isotopic immunometric assays [77] and LC-MS/MS methods [13,78-80]. Total hormone methods require the inclusion of inhibitors, such as 8-anilino-1-napthalene-sulphonic acid, to block hormone binding to serum proteins in order to facilitate binding to the antibody reagent [81]. Methodology for TT4 measurement has changed over the decades and been paralleled by changes in TT3 methodology. However TT3 measurement presents a greater sensitivity and precision challenge, because TT3 concentrations are ten-fold lower than TT4 [13,82-86]. Most laboratories currently measure TT4 and TT3 concentrations by non-competitive immunometric assays performed on automated platforms using enzymes, fluorescence or chemiluminescent molecules as signals [25,75,87]. A recent IFCC C-STFT study compared eleven TT4 and twelve TT3 immunoassays marketed by eight diagnostic companies [80]. TT4 and TT3 measurements were made in sera from healthy individuals using the various immunoassays and compared with values reported by isotope dilution tandem mass spectrometry (ID-LC-MS/MS) - the reference measurement procedure (RMP) based on using primary T4 and T3 standards for calibration [80,88]. Although most methods fell short of the optimal 5 percent goal established by the C-STFT, 4/11 TT4 assays agreed within 10 percent of the reference, whereas most TT3 assays exhibited a positive bias that would necessitate re-standardization [80, 88] (Figure 1). Thus, as would be expected, TT4 assays are more reliable than TT3 although assay variability persists, likely as a result of matrix differences between calibrators and patient sera, the efficiency of the blocking agent employed by different manufacturers and lot-to-lot variability [53,56,89,90].

Figure 1- Between-method TT4 and TT3 Variability

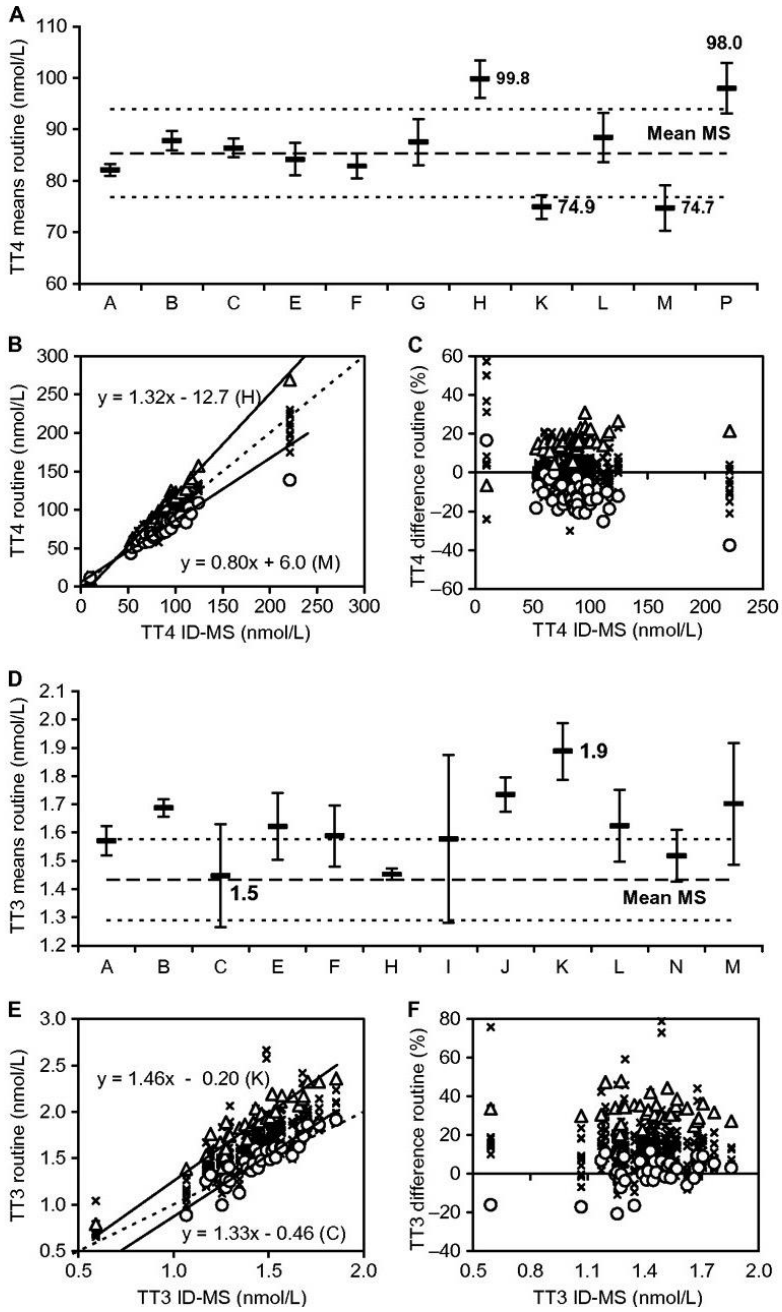


Figure 1. (A), (TT4); (D) (TT3): assay means (1-sided 95% CIs) vs the mean by the RMPs.The x axis gives the codes of the different assays, the dotted lines represent the mean of the RMP _10%. For the assays differing >10% from the mean of the RMP, the numerical value of the mean is listed. (B), (TT4); (E), (TT3): scatter plot (x = mean of the RMP, y = mean of singlicate results per assay) with indication of the line of equality (dotted) and the most extreme Deming regression lines/equations. The results for the most deviating assays are indicated by circles and triangles; all other assays are indicated with the same symbol, X. (C), (TT4); (F), (TT3): percent-difference plot with indication of the strongest negatively (circles) and positively (triangles) biased assays. Note that (B), (C), (E), and (F)

are extended to show the complete range (10–221 nmol/L for TT4, 0.6 –1.9 nmol/L for TT3) [80].

Clinical Utility of TT4 and TT3 Measurements

The diagnostic accuracy of total hormone measurements would be equivalent to that of free hormone tests if all patients had similar binding protein concentrations [35,75]. In fact, a recent study has reported that a screening cord blood TT4 < 7.6 µg/dL (< 98 nmol/L) can be used as a screening test for congenital hypothyroidism [91]. Unfortunately, many conditions are associated with TBG abnormalities that distort the relationship between total and free thyroid hormones (Table 1). Additionally, some patients have abnormal thyroid hormone binding albumins (dysalbuminemias) [92-94], thyroid hormone autoantibodies [95-98], or are taking drugs [25,99-101] that render total hormone measurements diagnostically unreliable [Table 1]. Consequently, TT4 and TT3 measurements are rarely used as stand-alone tests, but are typically employed in conjunction with a direct TBG measurement or an estimate of binding proteins [i.e. a thyroid hormone binding ratio test, THBR, that can be used to calculate a free hormone index (FT4I or FT3I). This index approach effectively corrects for the most common thyroid hormone binding protein abnormalities that distort total hormone measurements [[102-104]. Because free hormone immunoassays are more technically challenging than total hormone measurements [49,86] total hormone tests can useful confirmatory when a free hormone immunoassay result appears questionable, especially in pregnancy and critical illness where changes in binding protein concentrations and affinity for thyroid hormones can occur [22,104-106]. Suboptimal FT3 assay sensitivity limits reliable FT3 measurements to the high (hyperthyroid) range [86]. However, since T3 is typically only a 3rd-line test of thyroid status used for diagnosing unusual cases of hyperthyroidism, TT3 measurement can usually suffice in preference to FT3, especially when TT3 is used as a ratio with TT4 to eliminate binding protein effects [107]. In fact, in Graves' hyperthyroidism preferential thyroidal T3 secretion resulting from increased deiodinase activity secondary to thyroidal stimulation by TSH receptor antibodies (TRAb) [108] such that a high serum TT3/TT4 or FT3/FT4 ratio that can be used to differentiate Graves' from other causes of hyperthyroidism [107,109,110].

TT4 and TT3 Reference Ranges

Total T4 reference ranges have approximated 58 to 160 nmol/L (4.5-12.5 µg/dL) for more than four decades, although some between-method differences and sample-related variability remains [80, 104]. The IFCC C-STFT found that most TT4 methods report values within 10 percent of the ID-LC-MS/MS RMP (Figure 1) [80]. In euthyroid pregnant subjects the major influence on TT4 is the TBG concentration that rises approximately two-fold by mid-gestation. As a consequence, TT4 steadily increases from the first trimester to plateau at approximately 1.5-fold pre-pregnancy levels by mid-gestation [104,106,111-114]. Thus the non-pregnant TT4 reference range, adjusted by a factor of 1.5 can be used to assess thyroid status in the latter half of gestation [66,67,104,106,115,116].

TT3 reference ranges generally approximate 1.2 - 2.7 nmol/L (80 –180 ng/dL) [84]. However, TT3 methods display far more between-method variability than TT4, and most display more than a 10 percent bias relative to the reference method [79,80,86]. The IFCC C-STFT continues to work with manufacturers to the reduce variability and improve the calibration of TT3 methods against the RMP.

Free Thyroid Hormone Tests (FT4 and FT3)

In accord with the free hormone hypothesis, it is the free fraction of the thyroid hormones (0.02% of TT4 and 0.2% of TT3) that exerts biologic activity at the cellular level [117], whereas protein-bound hormone is considered as biologically inactive. Since binding-protein abnormalities are highly prevalent (Table 1) [35], free hormone measurement is considered preferable to total hormone testing [22,118]. However, free hormone measurement that is independent of thyroid hormone binding proteins remains challenging [22,118-120]. Free hormone methods fall into two categories – **direct** methods, that employ a physical separation of the free from protein-bound hormone, and **estimate** tests, that either calculate a free hormone "index" from a measurement of total hormone corrected for binding proteins with either a TBG measurement or a binding-protein estimate, or immunoassays that employing an antibody to sequester a small amount of the total hormone that is purportedly proportional to the free hormone concentration [22,75,118]. All free hormone tests are subject to limitations. Both index tests (FT4I and FT3I) and FT4 and FT3 immunoassays are typically protein-dependent to some extent, and may under- or overestimate free hormone, when binding proteins are abnormal [52,92,118-128]. Even direct methods that employ equilibrium dialysis or ultrafiltration to separate free from protein-bound hormone are not immune from technical problems relating to dilution, adsorption, membrane defects, temperature, the influence of endogenous binding protein inhibitors, fatty acid formation and sample-related effects [22,128-133]. The IFCC C-STFT has now established a reference measurement procedure (RMP) for free thyroid hormones that is based on equilibrium dialysis-dilution-mass spectrometry (ED-ID-MS) and primary calibrators [15,51,54,134]. An evaluation of current FT4 immunoassays has revealed major between-method variability and significant biases relative to the RMP that are far in excess of FT4 biological variation [50,53]. Recalibrating methods against the RMP was shown to significantly reduce biases that currently preclude implementing universal reference intervals that would apply across methods. The C-STFT is actively working with the in vitro diagnostic industry to re-standardize free hormone methods against the RMP to reduce current biases.

Direct FT4 and FT3 Methods

Direct free hormone methods have employed equilibrium dialysis [51,54,135-137], ultrafiltration [14,17,18,23,131,138-142] or gel filtration [143] to separate free hormone from the dominant protein-bound moiety. These separation techniques can be prone to inaccuracies causing under- or overestimate of free hormone due factors relating to dilution, adsorption, membrane defects, temperature, pH, the influence of endogenous binding protein inhibitors, fatty acid formation and sample-related effects [22,118,128,130-133,141,142,144-146]. The IFCC C-STFT has now established the RMP for FT4 as ED ID-LC-MS/MS. Specifically, equilibrium dialysis of serum is performed under defined conditions before FT4 is measured in the dialysate by isotope-dilution-liquid chromatography/tandem mass spectrometry [15,51,54]. Manufacturers are recommended to use this RMP to recalibrate their FT4 immunoassay tests [52-54,134]. Because direct free hormone methods are technically demanding, inconvenient and expensive, they are typically only readily available in reference laboratories. Most FT4 and FT3 testing is made using estimate tests - either the two-test "index" approach or an immunoassay "sequestration" method [118]. However, all current FT4 and FT3 estimate tests are binding-protein dependent to some extent [118,147-150], and a direct free hormone test can be especially useful for evaluating thyroid status when immunoassay values appear discordant with the clinical presentation and/or the TSH measurement [22].

Equilibrium Dialysis

Early equilibrium dialysis methods used I^{-131} and later I^{-125} labeled T4 tracers to measure the free T4 fraction, that when multiplied by a total hormone measurement gave an estimate of the free hormone concentration [135]. Subsequently, symmetric dialysis in which serum was dialyzed without dilution (or employing a near-physiologic medium) was used to overcome dilution effects [132]. By the early 1970s higher affinity T4 antibodies (>1x10^{11} L/mol) and high specific activity T4-I^{125} tracers were used to develop sensitive RIA methods that could to directly measure FT4 and FT3 in dialyzates and ultrafiltrates [82,136-138,142,151-154]. Subsequent improvements have involved employing more physiologic buffer diluents and improving the dialysis cell design [132,137]. More recently, isotope-dilution liquid chromatography/tandem mass spectrometry (ID-LC-MS/MS) [155] has been used to measure FT4 in ultrafiltrates [14,156,157] and dialyzates [27,50,51,134]. The FT4 RMP recently established by the IFCC C-STFT is based on ED followed by ID-LC-MS/MS [15,51].

Figure 2. FT4 and FT3 Immunoassay Method Comparison

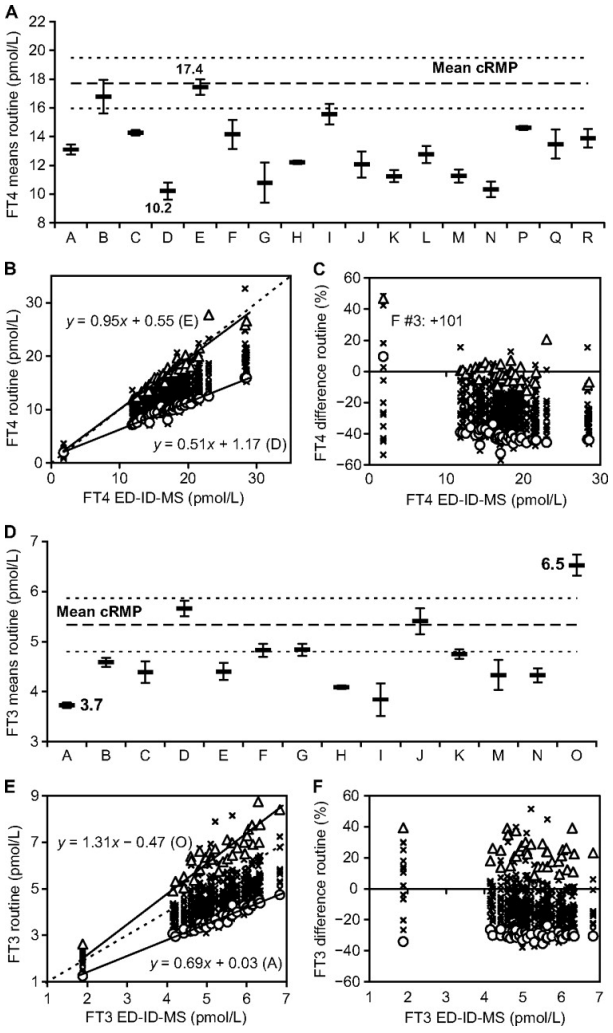


Figure 2. Between Assay Comparison of FT4 and FT3 Measurements in Healthy Euthyroid

Subjects. A=FT4 and D=DT3: assay means versus the mean by the RMPs. Different assays are coded A-O on the x axis, manufacturer codes used to designate assays were different for FT4 and FT3 assays. The dotted lines represent mean +/- 10% of the RMP ED-ID-MS). B=FT4 and E=FT3: scatter plot (x=mean of the RMP vs. y= mean of 6 singlicate results per assay. Line of equality indicated by dotted line. The results for the most deviating assays are indicated by circles and triangles; all other assays are indicated with the same symbol, X. C=FT4 and F=FT3: percent-difference plot indicating the strongest negatively (circles) and positively (triangles) biased assays [50].

Ultrafiltration Methods

A number of studies have used ultrafiltration to remove protein-bound T4 prior to LC-MS/MS measurement of FT4 in the ultrafiltrate [14,17,18,23,55,131,138-142]. Direct FT4 measurements employing ultrafiltration are sometimes higher than those made by equilibrium dialysis, because ultrafiltration avoids dilution effects [140]. Furthermore, ultrafiltration is not influenced by dialyzable inhibitors of T4-protein binding that can be present in conditions such as non-thyroidal illness (NTI) [130]. However, ultrafiltration can be prone to errors when there is a failure to completely exclude protein-bound hormone and/or adsorption of hormone onto the filters, glassware and tubing [127]. In addition, ultrafiltration is temperature sensitive and ultrafiltration performed at ambient temperature (25°C) will report FT4 results that are 67 percent lower than ultrafiltration performed at 37°C [133,158]. However, FT4 concentrations measured by ID-LC-MS/MS following either ultrafiltration at 37°C or equilibrium dialysis usually correlate [159].

Gel Absorption Methods**.**

Some early direct FT4 methods used Sephadex LH-20 columns to separate free from bound hormone before eluting the free T4 from the column for measurement by a sensitive RIA. However, because of a variety of technical issues, assays based on this methodologic approach are not currently used [75].

Indirect FT4 and FT3 Estimate Tests

Two-Test Index Methods (FT4I and FT3I)

Free hormone indexes (FT4I and FT3I) are unitless mathematical calculations made by correcting the total hormone test result for the binding protein, primarily TBG, concentration. These indexes require two separate tests and have been used to estimate free hormone concentrations for more than 40 years [118]. The first test involves the measurement of total hormone (TT4 or TT3) ,whereas the second test assesses the binding protein concentration using either (i) a direct TBG immunoassay, (ii) a Thyroid Hormone Binding Ratio (THBR) or "Uptake" test or (iii) an isotopic determination of the free hormone fraction [118,160].

TBG Immunoassays

There is conflicting data concerning whether indexes employing THBR in preference to direct TBG are diagnostically superior [161]. Free hormone indexes calculated using direct TBG measurement (TT4/TBG) may offer improved diagnostic accuracy over THBR when the total hormone concentration is abnormally high (i.e. hyperthyroidism), or when drug therapies interfere with THBR tests [101,162-165]. Regardless, the TT4/TBG index is not totally independent of the TBG concentration, nor does it correct for Albumin

or Transthyretin binding protein abnormalities (Table 1) [120].

Thyroid Hormone Binding Ratio (THBR) / "Uptake" Tests

The first "T3 uptake" tests developed in the 1950s employed the partitioning of T3-I^{131} tracer between the plasma proteins in the specimen and an inert scavenger (red cell membranes, talc, charcoal, ion-exchange resin or antibody) [119,166,167]. The "uptake" of T3 tracer onto the scavenger provided an indirect, reciprocal estimate of the TBG concentration of the specimen. Initially, T3 uptake tests were reported as percent uptakes (free/total tracer). Typically, sera with normal TBG concentrations had approximately 30 percent of the T3 tracer taken up by the scavenger. During the 1970s methods were refined by replacing I^{131}-T3 tracers by I^{125}-T3, calculating uptakes based on the ratio between absorbent and total minus absorbent counts, and expressing results expressed as a ratio with normal sera having an assigned value of 1.00 [160,167]. Historically, the use of T3 as opposed to T4 tracer was made for practical reasons relating to the ten-fold lower the affinity of TBG for T3 versus T4, facilitating a higher percentage of T3 tracer being taken up by the scavenger and allowing lower isotopic counting times. Because current methods use non-isotopic proprietary T4 or T3 "analogs", counting time is no longer an issue and current tests may use a "T4 uptake" approach - which may be more appropriate for correcting for T4-binding protein effects. Differences between T3 and T4 "uptakes" have not been extensively studied [168]. Although all THBR tests are to some degree TBG dependent, the calculated FT4I and FT3I usually provides an adequate correction for mild TBG abnormalities (i.e. pregnancy and estrogen therapy) [104,122,169-171], although they may fail to correct for grossly abnormal binding proteins [94] in euthyroid patients with congenital TBG extremes [120,122,172], Familial Dysalbuminemic Hyperthyroxinemia (FDH) [75,92,173-176], thyroid hormone autoantibodies [95,97,177,178], non-thyroidal illness (NTI) [120,128,179,180] or medications that directly or indirectly influence thyroid hormone binding to plasma proteins [75,99,120,164,181,182].

Isotopic Index Methods

The first free hormone tests developed in the 1960s were indexes calculated from the product of the free hormone fraction, measured isotopically by dialysis, and TT4 measured by PBI and later RIA [135,183,184]. These early isotopic detection systems were technically demanding and included paper chromatography, electrophoresis, magnesium chloride precipitation and column chromatography [135,153,185-187]. The free fraction index approach was later extended to ultrafiltration and symmetric dialysis, the latter measuring the rate of transfer of isotopically-labeled hormone across a membrane separating two chambers containing the same undiluted specimen [92,138,140,184,188-190]. Ultrafiltration and symmetric dialysis had the advantage of eliminating dilution effects that influenced tracer dialysis values [129,191]. However, free hormone indexes calculated using an isotopic free fraction were not completely independent of the TBG concentration and furthermore were influenced by tracer purity and the buffer matrix employed [137,192].

Clinical Utility of Two-Test Index Methods (FT4I and FT3I

Some favored the two-test FT4I approach for evaluating the thyroid status of patients with abnormal binding protein states like pregnancy and NTI [104,193]. Continued use of the FT4I remains controversial [194]. However, until FT4 immunoassays are re-standardized to remove biases [50,52,53], FT4I remains a useful confirmatory test when

binding proteins are abnormal and when diagnosing central hypothyroidism [195].

Free Thyroid Hormone Immunoassay Methods (FT4 and FT3)

Most free hormone testing is made using FT4 and FT3 immunoassays [87]. These immunoassays are based on "one-step", "labeled antibody" or "two-step" principles, as described below [75,118,196]. For more than twenty years controversy has surrounded the standardization and diagnostic accuracy of these methods, especially in pathophysiologic conditions associated with the binding protein abnormalities such as pregnancy [22,104], or due to polymorphisms, drug interactions, high free fatty acid (FFA) levels or thyroid binding inhibitors such as those present in NTI [25,53,75,92,119,120, 126-128,130,147,150,196-200]. Studies showing correlations between FT4 immunoassay values and both TBG and albumin concentrations, as well as weak inverse FT4/TSH log/linear relationships [17,18,23,126], have emphasized the need to evaluate each method with clinical specimens containing abnormal binding proteins. Currently, most FT4 and FT3 immunoassays display significant negative or positive biases that exceed the intra-individual biological variability (Figure 2) [50,52,53]. The IFCC C-STFT is actively working with the IVD industry to recalibrate their free hormone immunoassays against the RMP [15,50,53,60]. However, although recalibration to the RMP has been shown to greatly reduce between-method biases [50,52,53], implementation of a global re-calibration effort has been delayed by practical, educational and regulatory complexity.

One-Step, FT4 and FT3 Methods

The “one-step” approach uses a proprietary labeled hormone analog, designed for minimal interaction with thyroid hormone binding proteins, that competes with hormone in the specimen for a solid-phase anti-hormone antibody in a classic competitive immunoassay format [22,75,118,119,201,202]. After washing away unbound constituents, the free hormone concentration should be inversely proportional to the labeled analog bound to the solid support. Although conceptually attractive, the diagnostic utility of the one-step approach has been shown to be critically dependent on the degree that the analog is "inert" with respect to binding protein abnormalities [17,18,23,118,119,147,180,200,203-208].

Labeled Antibody FT4 and FT3 Methods

Labeled antibody methods are "one-step" methods that use labeled-antibody in preference to a labeled hormone analog. The free hormone in the specimen competes with solid-phase hormone for the labeled antibody and is quantified as a function of the fractional occupancy of hormone-antibody binding sites in the reaction mixture [22,75,118,120,202,209]. The labeled antibody approach is used as the basis for a number of automated immunoassay platforms because it is easy to automate and considered less binding-protein dependent than the labeled analog approach, because the solid phase hormone does not compete with endogenous free hormone for hormone binding proteins [22,87,118,210,211].

Two-Step, Back-Titration FT4 and FT3 Methods

The two-step approach was first developed by Ekins and colleagues in the late 1970s [75,119,128,202]. Two-step methods typically employ immobilized T4 or T3 antibody (for FT4 and FT3 immunoassays, respectively) to sequester a small proportion of total hormone from a diluted serum specimen without disturbing the original free to protein-bound equilibrium [75,118]. After removing unbound serum constituents by washing, a

labeled probe (^{125}I T4, or more recently a macromolecular T4 conjugate) is added to quantify unoccupied antibody-binding sites that are inversely related to the free hormone concentration - a procedure that has been referred to as "back-titration [118].

Clinical Utility of FT4 and FT3 Measurements

Most FT4 methods give diagnostically reliable results when binding proteins are near-normal, provided that a method-specific reference range is employed [53]. However, both TT3 and FT3 immunoassay methods tend to be inaccurate in the low range [86,212] and have no value for diagnosing or monitoring treatment for hypothyroidism [70,213], although T3 measurement can be useful for diagnosing or confirming unusual cases of hyperthyroidism.

Ambulatory Patients

Free hormone tests (FT4 or FT3) are used in preference to total hormone (TT4 or TT3) measurements in order to improve diagnostic accuracy for detecting hypo- and hyperthyroidism in patients with abnormal thyroid hormone binding proteins (Table 1). FT4 is typically employed as a second-line test for confirming primary thyroid dysfunction detected by an abnormal TSH ,but is the first-line test when thyroid status is unstable (early phase of treating hypo- or hyperthyroidism), in the presence of pituitary/hypothalamic disease when TSH is unreliable, or when patients are taking drugs such as dopamine or glucocorticoids that are known to affect TSH secretion [24,100,101,165,214-219].

Mild "subclinical" thyroid dysfunction is characterized by TSH/FT4 discordances (abnormal TSH/normal FT4). This reflects the intrinsic complex nature of the inverse log/linear TSH/FT4 relationship [24,220,226] - a relationship that is modified by age and gender [227,228]. Thus, small changes in FT4, even within normal limits, are expected to produce a mild degree of TSH abnormality - between 0.05 and 0.3 mIU/L (for subclinical hyperthyroidism) and 5 and 10 mIU/L (for subclinical hypothyroidism). An unexpected TSH/FT4 discordance, if confirmed, should prompt an investigation for interference with FT4, TSH or both tests [229,230]. FT4 interference can result from severe binding protein abnormalities such as congenital TBG excess or deficiency [75,94,122,159,231,232], dysalbuminemias [92,233-236], thyroid hormone autoantibodies [95,97,98,177,178,230,237] or drug interferences [75,99,120].

Hospitalized Patients with Nonthyroidal Illnesses (NTI)

The diagnostic performance of current FT4 methods has not been evaluated in hospitalized patients with NTI where binding protein inhibitors and drug therapies can negatively impact the reliability of both thyroid hormone and TSH testing [24,75,126,130,180,218,238,239]. Three categories of hospitalized patients deserve special attention: a) patients with NTI without known thyroid dysfunction who have a high or low T4 status; b) patients with primary hypothyroidism and concurrent NTI and, c) patients with hyperthyroidism and concurrent NTI [238,240,241]. Because the diagnostic reliability of FT4 testing is still questionable in sick hospitalized patients, a combination of both T4 (FT4 or TT4) and TSH may be needed to assess thyroid status in this setting [24,53,180,242]. In most clinical situations where FT4 and TSH results are discordant, the TSH test is the most diagnostically reliable, provided that the patient does not have pituitary failure or is receiving medications such as glucocorticoids and dopamine that directly inhibit TSH secretion [101,165,218]. Repetitive TSH testing may be helpful in resolving the cause of an abnormal FT4, because the TSH

abnormalities of NTI are typically transient whereas the TSH abnormality will persist if due to underlying thyroid dysfunction [243-246]. It may be useful to test for TPOAb as a marker for underlying thyroid autoimmunity

FT4 and FT3 reference ranges

Current reference ranges for FT4 and FT3 immunoassays are method-dependent because of calibration biases [50,52,53] (Figure 2). This calibration problem negatively impacts the clinical utility of FT3 and FT4 tests because it precludes establishing universal reference ranges that would apply across methods.

Pediatric FT4 and FT3 Reference Ranges

The determination of normal reference limits for pediatric age-groups is especially challenging, given the limited number of studies involving sufficient numbers of healthy children [247-249]. Most studies report that serum TSH peaks after birth and steadily declines throughout childhood to reach adult levels at puberty. Likewise, FT3 declines across the pediatric age groups during childhood and approaches the adult range at puberty, whereas FT4 levels for infants less than a year old are higher than for children 1 to 18 years old who have FT4 similar to that observed for adults [247-252].

Pregnancy FT4 Reference Ranges

As with non-pregnant patients, TSH is the first-line test to use for assessing thyroid status during pregnancy [253]. However, FT4 measurement is needed for monitoring anti-thyroid drug treatment of hyperthyroid pregnant patients who have undetectable TSH. The question whether an isolated low FT4 during pregnancy is a maternal or fetal risk factor, remains controversial [254-259]. However, a number of studies suggest that low FT4 may be a risk factor for gestational diabetes and fetal complications [260-264]. Non-pregnant FT4 reference ranges do not apply to pregnancy since FT4 progressively declines as gestation progresses, necessitating the use of trimester-specific reference ranges [104,113,265-271]. Currently it is not possible to propose universal trimester-specific FT4 reference ranges given current between-method differences [50,53,271] (Figure 2) compounded by differences related to the ethnicity [193,270,272-275], iodine intake [276-278], smoking [279] and BMI [269,270,280-283] between study cohorts. Establishing institution-specific trimester-specific reference ranges from the 2.5 to 97.5 percentiles of least 400 pregnant patients from each trimester [270] is not practical for most institutions. The feasibility of establishing universal trimester-specific reference ranges will improve after the proposed re-standardization of FT4 methods against the RMP [53]. However, binding protein effects will remain and population-specific factors will still have to be considered.

Interferences with Total and Free Thyroid Hormone Tests

Only the physician can suspect interference with a test result and request that the laboratory perform interference checks! This is because the hallmark of interference is discordance between the test result and the clinical presentation of the patient. Failure to recognize interferences can have adverse clinical consequences [229,284-289].

Laboratory checks for interference include showing discordance between different manufacturers methods [290-293], re-measurement of analyte after adding blocking agents

[293-297] and performing linearity studies or precipitating immunoglobulin with polyethylene glycol (PEG) [229,290,291,293,294,298-300]. A change in analyte concentration in response to one of these maneuvers suggests interference, but a lack of effect does not rule out interference. Interferences can be classified as either (a) non-analyte-specific or (b) analyte-specific [301,302].

Non-Analyte-Specific Interferences

Protein Interferences

Immunoassays can be affected by interferences from both paraproteins [303-305] and abnormal immunoglobulins [306,307].

Congenital TBG excess or deficiency.

Free hormone immunoassays and free T4 index tests may be susceptible to interference from grossly abnormal TBG concentrations, such as those seen in congenital TBG excess or deficiency states [75,94,122,159,231,232].

Pregnancy.

Estrogen stimulation causes TBG concentrations to progressively rise to plateau 2.5-fold higher than pre-pregnancy values by mid-gestation [193,308,309]. As a consequence, both TT4 and TT3 increase to approximately 1.5-fold of pre-pregnancy values by mid-gestation [113,310]. Despite the rise in total hormone, both FT4 and FT3 decline to a method-related degree during gestation [104,265-269]. It should be noted that lower FT4 levels would be expected during pregnancy from a consideration of the law of mass action as applied to T4-binding protein interactions [310]. However, the degree of FT4 decline during pregnancy is variable and method-dependent due to standardization differences (Figure 2) and in some cases method sensitivity to the declining albumin concentrations typical of late gestation [18,193,311].

Familial Dysalbuminemic and Transthyretin-Associated Hyperthyroxinemias.

Autosomal dominant mutations in the Albumin or Transthyretin (prealbumin) [312] gene can result in altered protein structures with enhanced affinity for thyroxine and/or triiodothyronine. These abnormal proteins can interfere with FT4 and/or FT3 measurements and result in inappropriately high FT4 and/or FT3 immunoassay values [92,173,237,312]. Familial Dysalbuminemic Hyperthyroxinemia (FDH) is a rare condition with a prevalence of ~1.8 % in the Hispanic population [313]. It arises from a number of genetic variants, with the R218H being the most common, some variants result in extremely high TT4, whereas other mutations (i.e. L66P) affect mainly T3 [233]. Affected individuals are euthyroid and have normal TSH and FT4 when measured by direct techniques such as equilibrium dialysis [92]. Unfortunately, most FT4 estimate tests (immunoassays and indexes) report falsely high values for FDH patients that may prompt inappropriate treatment for presumed hyperthyroidism if the condition is not recognized [92].

Heterophile Antibodies (HAbs)

Heterophile antibodies (HAb) are human poly-specific antibodies targeted against animal antigens, the most common being human anti-mouse antibodies (HAMA) [293,302,314,315]. Alternatively, HAb can target human antigens [302] such as rheumatoid factor (RF), an immunoglobulin commonly associated with autoimmune conditions that is widely considered a heterophile antibody [316]. RF has been shown to interfere with free and total thyroid hormone tests [87] as well as TSH [317] and Tg [318]. HAbs have a prevalence of 30-40 percent [319-321] and have the potential to

interfere with a broad range of methods that use IMA principles [290,300,306,322]. In recent years assay manufacturers have increased the immunoglobulin blocker reagents added to their tests and this has reduced interference from 2 to 5 percent [290,297,323]. However, interference is still seen in approximately one percent of patients who have high enough HAb concentrations to overcome the assay blocker [296,298,322,324]. HAMA interference mostly affects non-competitive immunometric assays (IMA) that employ monoclonal antibodies of murine origin [325]. Assays based on the competitive format that employ high affinity anti-antigen polyclonal antibody reagents, are rarely affected [296,319]. HAb has the potential to interfere with both free [178,321,326-328] and total [178,326,327] thyroid hormone tests, as well as THBR [327], TSH [289,294,300,328-330] and Thyroglobulin (Tg) [295,296,323,324,331,332], TgAb [333] and calcitonin (CT) [300,334-337] methods. Interference from HAb or HAMA typically causes falsely high results for one or more analytes. Less commonly falsely low test results may be seen [332]. The test marketed by one manufacturer can be severely affected, whereas the test from a different manufacturer may appear unaffected. This is why the first step for investigating for interference is re-measurement of the analyte in a different manufacturers method. It should be noted that patients receiving recent vaccines, blood transfusions or monoclonal antibodies (given for treatment or scintigraphy), as well as veterinarians and those coming into contact with animals, are especially prone to test interferences caused by induced HAb and HAMA [298,338].

Anti-Reagent Antibodies

Interference can be caused by antibodies against assay reagents. For example, there are a number of reports of anti-Rhuthenium antibodies interfering with TSH, FT4 and FT3 by [339-343]. In Streptavidin-Biotin based assays interference can result from antibodies targeting either Streptavidin [344] or biotin reagents [345]. Alternatively, high dose biotin ingestion has been known to produce interference with thyroid and other tests in an analyte-specific, platform-specific manner [346-350].

Analyte-Specific Interferences

Analyte-specific interferences typically result from autoantibodies targeting the analyte. Depending on the analyte and test formulation, autoantibody interferences typically cause falsely-high test results, but can cause falsely-low test results, as in the case of Tg autoantibodies . It should be noted that transplacental passage both heterophile antibodies or anti-analyte autoantibodies (i.e. TSHAb or T4Ab) have the potential to interfere with neonatal screening tests [351-354]. Specifically, maternal TSH autoantibodies can cross the placenta and may cause a falsely high TSH screening test in the newborn mimicking congenital hypothyroidism, whereas maternal T4 autoantibodies could cause falsely high neonatal T4 masking the presence of congenital hypothyroidism [230,353].

T4 and T3 Autoantibodies (T4Ab/T3Ab)

T4 and T3 autoantibodies can falsely elevate total hormone, free hormone or THBR measurements depending on the method employed [95,97,98,177,178,230,237]. The prevalence of thyroid hormone autoantibodies approximates 2 percent in the general population but may be as much as 30 percent in patients with autoimmune thyroid disease or other autoimmune conditions [316,355-358]. However, despite their high prevalence, significant interference caused by thyroid autoantibodies is not common and depends on the qualitative characteristics of the autoantibody present (i.e. its affinity for the test reagents). Further, different methods exhibit such interferences to a greater or lesser degree [95,97]. Because autoantibody interference is difficult for the laboratory to

detect proactively, it is the physician who should first suspect interference characterized by unexpected discordance between the clinical presentation of the patient and the test result(s) [96, 178].

SERUM TSH (THYROID STIMULATING HORMONE/THYROTROPIN) MEASUREMENT

Over the last four decades the dramatic improvements in TSH assay sensitivity and specificity have revolutionized thyroid testing and firmly established TSH as the first-line test for ambulatory patients not receiving drugs known to alter TSH secretion [24,70,71,120,216,218,359]. Serum TSH has become the therapeutic target for levothyroxine (L-T4) replacement therapy for hypothyroidism and suppression therapy for differentiated thyroid cancer [72]. The diagnostic superiority of TSH versus FT4 measurement arises from the inverse, predominantly log/linear, TSH/FT4 relationship, that is modified to some extent by factors such as age, sex, active smoking and TPOAb status [7,24,221-228].

TSH Assays

TSH assay "quality" has historically been defined by clinical sensitivity – the ability to discriminate between hyperthyroid and euthyroid TSH values [24,360-364]. The first generation of RIA methods had a detection limit approximating 1.0 mIU/L [365-367] that limited their clinical utility to diagnosing primary hypothyroidism [368-370] and necessitated the use of TRH stimulation to diagnose hyperthyroidism that was characterized by an absent TRH-stimulated TSH response [371-376]. With the advent of immunometric assay (IMA) methodology that uses a combination of poly- and/or monoclonal antibodies targeting different TSH epitope(s) in a "sandwich" format [377-379], a ten-fold improvement in TSH assay sensitivity (~ 0.1 mIU/L) was achieved when using isotopic (I^{125}) signals [380]. This level of sensitivity facilitated the determination of the lower TSH reference limit (as 0.3-0.4 mIU/L), and the detection of overt hyperthyroidism without the need for TRH stimulation [7,374-376,380-386], but was still insufficient for distinguishing between differing degrees of hyperthyroidism (i.e. subclinical versus overt). Sensitization continued until a third-generation of TSH IMAs, using non-isotopic signals, were developed that could achieve a sensitivity of 0.01 mIU/L [7,8,374,387-389]. Initially different non-isotopic signals were used that gave rise to a lexicon of terminology to distinguish between assays: immunoenzymometric assays (IEMA) used enzyme signals; immunofluorometric assays (IFMA) used fluorophors as signals, immunochemiluminometric assays (ICMA) used chemiluminescent molecules as signals and immunobioluminometric assays (IBMA) used bioluminescent signal molecules [8,390]. Current TSH methods are automated ICMAs [87] that all achieve third-generation functional sensitivity (FS = ≤0.01 mIU/L) - a sensitivity the FS level that has subsequently become the standard of care [7,8,52,53,388,391-396].

Functional Sensitivity (FS) - determines the lowest reportable assay limit

During the period of active TSH assay improvement, different non-isotopic IMAs made competing claims for sensitivity. Methods were described as: "sensitive", "highly sensitive", "ultrasensitive" or "supersensitive" - marketing terms that had no scientific definition. This confusion led to a debate concerning what was the most clinically relevant parameter to use to determine the lowest reliable reportable TSH value for clinical practice [8,397-403]. Functional sensitivity (FS), defined as the lowest analyte concentration measured with 20 percent coefficient of variation [24] is now recognized as the parameter that best represents the between-run precision for measuring low analyte concentrations in clinical practice

[24,395,404]. FS is used to define the lower clinical reporting limit for not only for TSH assays, but also Tg and TgAb measurements, for which assay sensitivity is critical [8,24,397,404,405]. Protocols used for establishing FS specify that precision be determined in human serum, not quality control materials based on artificial protein matrices, since immunoassays tend to be matrix-sensitive [406,407]. The time-span used for determining precision is also analyte-specific and should reflect the frequency of testing employed in clinical practice - 6 to 8 weeks for TSH, but 6 to 12 months for the Tg and TgAb assays when used as tumor markers for monitoring differentiated thyroid cancer (DTC). This time-span is important because low-end, between-run assay precision erodes over time as a result of a myriad of variables, reagent lot-to-lot variability being a key variable [9,408-410]. Note that the FS parameter is more stringent than other biochemical sensitivity parameters such as limit of detection (LOD - a within-run parameter) and limit of quantitation (LOQ - a between-run parameter without stipulations regarding matrix and time-span for determining precision) [404,411]. A ten-fold difference in FS has been used to define each more sensitive "generation" of TSH [397] or Tg [32,404,412,413] method. Thus, TSH RIA methods with FS approximating 1.0 mIU/L were designated "first generation", TSH IMA methods with functional sensitivity approximating 0.1 mIU/L were designated "second generation", and TSH IMAs with FS approximating 0.01 mIU/L are designated "third generation" assays [8,57,395,397,405,414]. Analogous to TSH, Tg assays [Section 6A] with FS approximating 1 µg/L are designated "first generation", whereas Tg IMAs with FS approximating 0.10 µg/L meet the criteria for a "second generation" method [32,58,296,395,404,413,415,416].

TSH Biologic Variability

As compared with between-person variability, TSH intra-individual variability is relatively narrow (20-25 percent) in both non-pregnant and pregnant subjects, as compared with between-person variability [29,222,417,418]. In fact, the serum TSH of euthyroid volunteers was found to vary only ~0.5 mIU/L when tested every month over a span of one year [417]. Twin studies suggest that there are genetic factors that determine hypothalamic-pituitary-thyroid setpoints [419-421]. These studies report that the inheritable contribution to the serum TSH level approximates 65 percent [420,422]. This genetic influence appears, in part, to involve single nucleotide polymorphisms in thyroid hormone pathway genes such as the phosphodiesterase gene (PDE8B) [423-425], polymorphisms causing gain [426-433] or loss [434-436] of function TSH receptors [423,437,438] and the type II deiodinase enzyme polymorphisms [423,439]. Undoubtedly, such polymorphisms account for some of the euthyroid outliers that skew TSH reference range calculations [423,434,440].

Figure 3. TSH Between-Method Variability

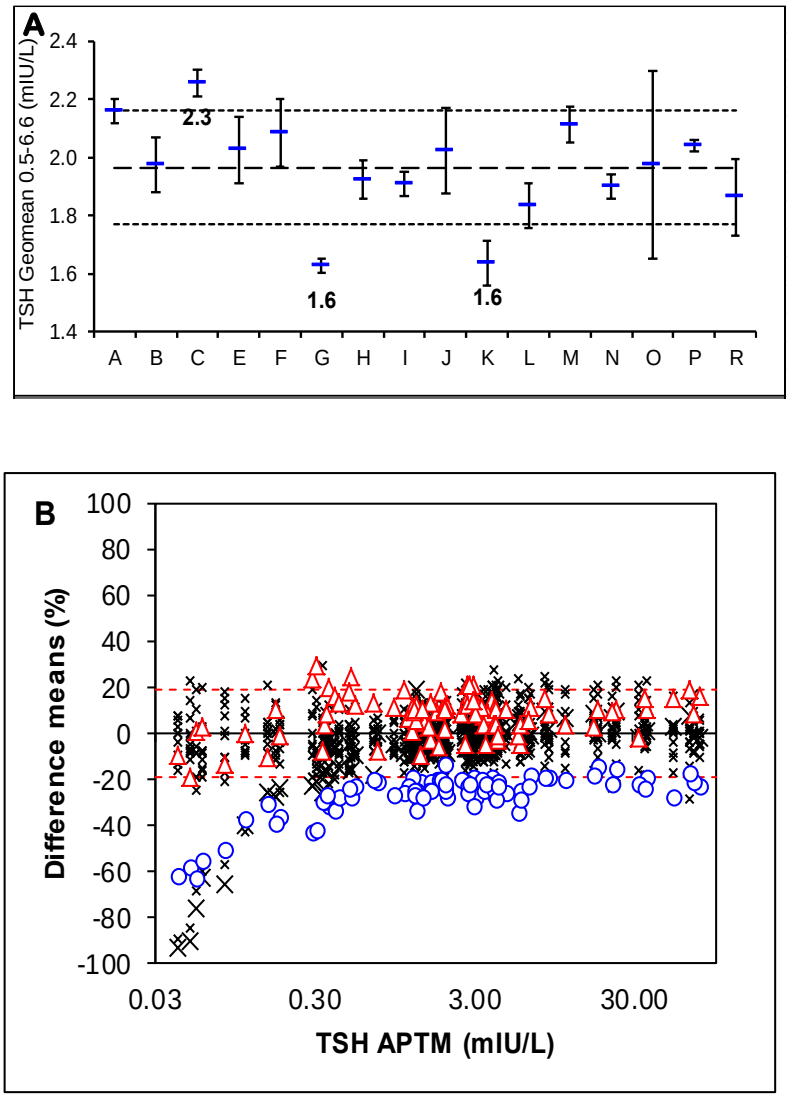


Figure 3. A*. Geometric mean of the TSH results for the range 0.5– 6.6 mIU/L, (x axis, different assays; dotted lines, overall mean and 10% error). In the plots, the 1-sided 95% CIs of the means are shown (note: the wide interval of assay O is due to results from only 2 runs with a high between-run variation and df = 1 by the Satterthwaite approximation). For the assays outside the 10% limit, the mean value is listed.* ***B.*** *Plot showing the %-difference between TSH methods. The most discrepant assays are shown by triangles and circles. Other assays are shown with the same symbol (x) [29,52].*

The narrow TSH within-person variability and low (< 0.6) index of individuality (IoI) [222,417, 418,441-443] limits the clinical utility of using the TSH population-based reference range to detect thyroid dysfunction in an individual patient [222,418,443,444]. When evaluating patients with marginally (confirmed) low (0.1–0.4 mIU/L) or high (4–10 mIU/L) TSH abnormalities, it is more important to consider the degree of TSH abnormality relative to

patient-specific risk factors for cardiovascular disease rather than the degree of the abnormality relative to the TSH reference range [69,445,446].

TSH Reference Ranges - General Considerations

IFCC C-STFT comparison studies (Figure 3) report significant biases between different TSH methods. Currently this prevents establishing universal population or trimester-specific TSH reference ranges that would apply across methods [52,447]. These method biases also impact the frequency of detecting subclinical hypothyroidism [61,448]. Since TSH is a complex glycoprotein, no reference measurement procedure (RMP) is available, or will likely be feasible in the future. However, a harmonization approach [59,60], where methods are recalibrated to the "all method mean", has been shown to have the potential to effectively eliminate current between-method TSH differences that are most pronounced at pathophysiologic levels [29,449]. The IFCC C-STFT is actively working with the IVD industry to encourage manufacturers to harmonize their methods. A reduction of between-method variability could eliminate the need to establish population and trimester-specific TSH reference ranges for each method - a practice that is costly and inconvenient given the large numbers of rigorously screened participants that are necessary to establish reliable 2.5th to 97.5th percentiles for a population [450]. However, even after harmonization minimizes inter-method differences, it remains to be determined to what extent universal ranges would be impacted by other factors such as age [451], ethnicity [396,452] and iodine intake [453]. It may be that a reference range established in one geographic location may not be representative of a different locale or population. After harmonization of TSH methods the advantages of consolidating data from different studies and establishing universal reference limits is clearly apparent.

The TSH Population Reference Range

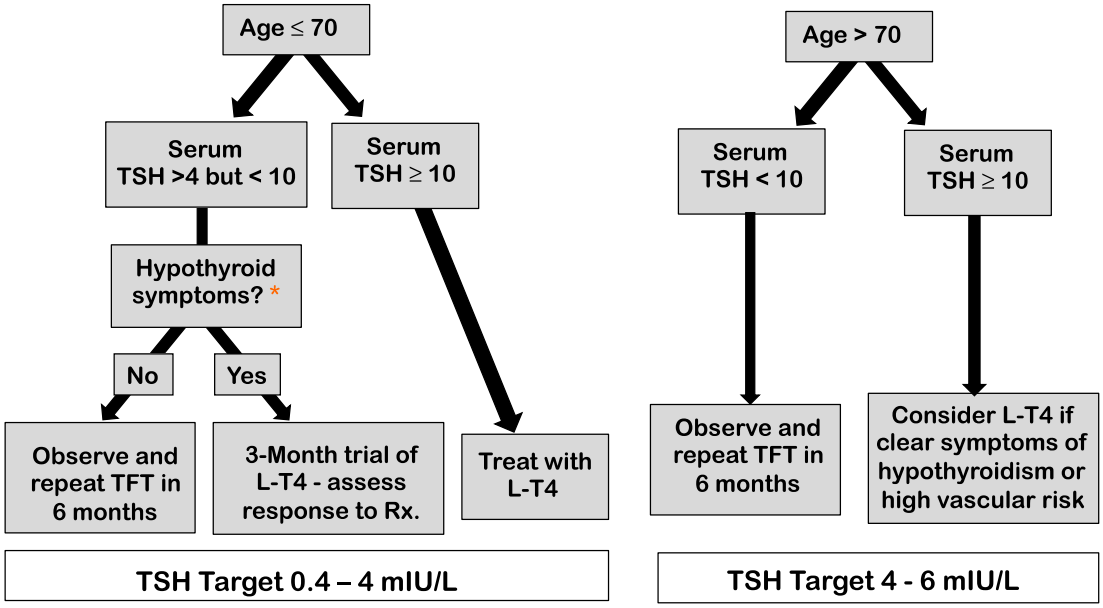
The complex log/linear TSH/FT4 relationship [7,24,221-228] dictates that TSH will be the first abnormality to appear with the development of mild (subclinical) hypo- or hyperthyroidism. It follows that the setting of the TSH reference limits critically influences the frequency of diagnosing subclinical thyroid disease [69,445,448,454].

Guidelines recommend that *"TSH reference intervals should be established from the 95 percent confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers who have: (a) no detectable thyroid autoantibodies, TPOAb or TgAb (measured by sensitive immunoassay); (b) no personal or family history of thyroid dysfunction; (c) no visible or palpable goiter and, (c) who are taking no medications except estrogen"* [24,450].

Multiple factors influence population TSH reference limits, especially the upper (97.5th percentile) limit. Different methods report different ranges for the same population as a result of between-methods biases (Figure 3) [396,448,451,455]. A key factor affecting the upper limit is the stringency used for eliminating individuals with thyroid autoimmunity (thyroid autoantibody positive [456]) from the population [452,456-461]. Other factors relate to population demographics such as sex [452], ethnicity [452,462-464], iodine intake [465], BMI [466-477] and smoking status [462,478,479]. Age is a major factor the influences the TSH upper limit [460,463,480-482] leading to the suggestion that age-specific TSH reference limits should be used (Figure 4) [69,451,480]. However, the relationship between TSH and age is complex. Most studies in iodine sufficient populations have shown an increase in TSH with age [440,452,460,483], whereas other studies have reported no change or a decreased TSH with aging [457,484,485]. This

conflicting data could merely represent population differences - with a rising TSH with age reflecting an increasing prevalence of thyroid autoimmunity in iodine-sufficient populations [452], whereas in iodine deficient populations, increasing autonomy of nodular goiter can result in decreased TSH with aging [486-488]. Some studies have reported that a mild TSH elevation in elderly individuals may convey a survival benefit [481,489-492], whereas other studies dispute this [493,494]. However, TSH is a labile hormone and studies cannot assume that a TSH abnormality found in a single determination is representative of thyroid status in the long-term [495,496].

Figure 4. Guidelines for Diagnosing and Managing Subclinical Hypothyroidism



- ***Depending on patient-specific circumstances: Consider treating individuals with goiter, dyslipidaemia, diabetes, TPOAb-positivity and preconception planning.***

*Figure 4. Suggested management algorithm from reference # 69 Initial management of persistent subclinical hypothyroidism in non-pregnant adults: persistent subclinical hypothyroidism describes patients with elevated serum TSH and within reference range serum FT 4 on two occasions separated by at least 3 months. This algorithm is meant as a guide and clinicians are expected to use their discretion and judgment in interpreting the age threshold around 70 years. * Depending on circumstances, individuals with goiter, dyslipidaemia, and diabetes may also be considered for treatment, along with those with planning pregnancy in the near future.*

TSH is a heterogeneous glycoprotein [497,498], and TRH-mediated changes in TSH glycosylation [499] have the potential to influence immunoactivity [500,501]. A number of pathophysiologic circumstances are known to alter TSH glycosylation [498,500,502-504]. The demonstration that harmonization of TSH methods successfully mininizes between-method differences [52,53] suggests that under normal conditions current TSH IMAs appear to be "glycosylation blind", and detect different TSH glycoforms in an equimolar fashion [52,53,501]. However, future studies need to include sera from

conditions where TRH dysregulation may lead to abnormal TSH glycosylation and bioactivity, such as pituitary dysfunction, NTI and aging [215,239,246,498,505-509].

Pediatric TSH Reference Ranges

The adult TSH population reference range does not apply to neonates or children. Serum TSH values are generally higher in neonates and then gradually decline until the adult range is reached after puberty [250-252, 485, 510-514]. This necessitates using age-specific TSH reference ranges for diagnosing thyroid dysfunction in these different pediatric age groups.

Subclinical Thyroid Dysfunction

Subclinical Hyperthyroidism (SCHY).

The lower (2.5th percentile) TSH reference limit approximates 0.3-0.4 mIU/L, and is fairly independent of the method used [445,452,484,485,515-520]. Subclinical hyperthyroidism (SCHY), is defined as a low but detectable TSH (0.01 –-0.3 mIU/L range) without a FT4 abnormality. The prevalence of endogenous SCHY is low (0.7%) in iodine-sufficient populations [452], but is higher in patients reporting thyroid disease as an iatrogenic consequence of L-T4 replacement therapy [521-523]. SCHY is a risk factor for osteoporosis and increased fracture risk [474,524-526] as well as atrial fibrillation and cardiovascular disease [445,474,527], especially in older patient patients.

Subclinical Hypothyroidism (SCHO).

Subclinical hypothyroidism is defined as a TSH above the upper (97.5th percentile) TSH reference limit without a FT4 abnormality [69,448,454,460,516,528-530]. However, since the setting of the TSH upper limit remains controversial, the prevalence of SCHO is highly variable - 4 to 8.5 % [452,521], rising to 15% in older populations [446,456]. In most cases, SCHO is associated with TPOAb positivity, indicative of an autoimmune etiology [452,456]. The clinical consequences of SCHO relate to the degree of TSH elevation [531]. Most guidelines recommend L-T4 treatment of SCHO when is TSH is above 10 mIU/L [68,69] but below 10 mIU/L recommend L-T4 treatment based on patient-specific risk factors (Figure 4) [69]. There is active debate concerning the efficacy of treating SCHO to prevent progression [532-535], or improve renal [536,537], cardiovascular [474,524,531,538-543], or lipid [544-546] abnormalities that can be associated with SCHO [69,547].

Thyroid Dysfunction and Pregnancy

It is well documented that overt hypo- or hyperthyroidism is associated with both maternal and fetal complications [548-550]. However, the impact of maternal subclinical thyroid dysfunction remains controversial [253]. No maternal or fetal complications appear associated with subclinical hyperthyroidism during pregnancy [258,551]. First trimester "gestational hyperthyroidism" is typically transient and hCG-related, as described above. In contrast, short-term and long-term outcome studies of maternal subclinical hypothyroidism [550] are complicated by heterogeneity among studies arising from a myriad of factors influencing TSH cutoffs, such as gestational stage, TSH method used, maternal TPOAb status, and current and pre-pregnancy iodine intake [277,454]. Using gestational age-specific reference intervals the frequency of SCHO in first

trimester pregnancy approximates 2-5 percent [552-556]. A number of studies have reported that subclinical hypothyroidism is associated with increased frequency of maternal and fetal complications, especially when TPOAb is positive [557-559]. Maternal complications have included miscarriage [474,548,560-562], preeclamsia [548,563], placental abruption [552], preterm delivery [552,562,564] and post-partum thyroiditis [565]. Fetal complications have included intrauterine growth retardation and low birth weight [258,548,566-568] and possible impaired neuropsychological development [550,569,570]. It remains controversial whether L-T4 treatment of SCHO in early gestation decreases risk of complications [559,562,564,571].

Trimester-Specific TSH Reference Ranges.

As with non-pregnant patients, TSH is the first-line test used for assessing thyroid status during pregnancy when gestation-related TSH changes occur [66,67,253,254,555,556,572]. In the first trimester, there is a transient rise in FT4 caused by high hCG concentrations stimulating the TSH receptor - because hCG shares some homology with TSH [254,308,309,573,574]. The degree of TSH suppression is inversely related to the hCG concentration and can be quite profound in patients with hyperemesis who have especially high hCG [271,575-577]. As gestation progresses, TSH tends to return towards pre-pregnancy levels [271]. Recent studies from different geographic areas with diverse iodine intakes have using different TSH methods have reported higher trimester-specific TSH upper limits than recommended by previous guidelines [253,269,271,454,556,578-580]. In response, the American Thyroid Association has recently revised their pregnancy guidelines [66,74] to replace trimester-specific reference limits by a universal upper TSH limit of 4.0 mIU/L, when TPOAb is negative and local reference range data is not available. However, at this time between-method biases (Figure 3) clearly preclude proposing universal TSH or FT4 reference ranges that would apply to all methods and all populations [52,53,267,271,447]. It is critical that the IVD manufacturers respond to the urging of the IFCC C-STFT and harmonize their TSH methods to increase the feasibility of establishing TSH universal reference limits for pregnancy [52,53]. Requiring each institution to establish their own trimester-specific reference ranges for thyroid tests is impractical, given the costs, logistics and ethical considerations involved in recruiting the more than 400 disease-free pregnant women needed to establish reliable ranges for each trimester [270]. Only after methods are re-standardized (FT4) or harmonized (TSH), will it be feasible to propose trimester-specific reference ranges that would apply across methods. However, such ranges would still be influenced by differences in ethnicity [280] and iodine intake, especially pre-pregnancy iodine intake that influences thyroidal iodine stores [277]. There is also a current need to reevaluate optimal TPOAb cutoffs needed to exclude those individuals with thyroid autoimmunity whose inclusion skews TSH upper limits [271,280,454,574,581,582].

Clinical Utility of TSH Measurement

Ambulatory Patients

In the outpatient setting the reliability of TSH testing is not influenced by the time of day of the blood draw, because the diurnal TSH peak occurs between midnight and 0400 [583-586]. Third-generation TSH assays (FS ~0.01 mIU/L) have now become the standard of care because they can reliably detect the full spectrum of thyroid dysfunction from overt

hyperthyroidism to overt hypothyroidism, provided that hypothalamic-pituitary function is intact and thyroid status is stable [24,57,216,242,359,414,587,588]. TSH is also used for optimizing L-T4 therapy - a drug with a very narrow therapeutic index [359,589,590]. Because TSH secretion is slow to respond to changes in thyroxine status there is no need to withhold the L-T4 dose on the day of the blood test [24]. In addition, targeting the degree of TSH suppression relative to recurrence risk plays a critical role in the management of thyroid cancer [72,591-593].

Hospitalized Patients with Nonthyroidal Ilnesses (NTI)

Routine thyroid testing in the hospital setting is not recommended because thyroid test abnormalities are frequently seen in euthyroid sick patients [238,594]. Non-thyroidal illness, sometimes called the "sick euthyroid syndrome" is associated with alterations in hypothalamic/pituitary function and thyroid hormone peripheral metabolism often exacerbated by drug influences [100,218,239,245,595]. T3 levels typically fall early in the illness followed by a fall in T4 as the severity of illness increases. [24,244,595-597]. As thyroid hormone levels fall TSH typically remains unchanged, or may be low early in the illness, especially in response to drug therapies such as dopamine or glucocorticoid [100,101,218]. During the recovery phase, TSH frequently rebounds above the reference range [243]. However, high TSH may also be seen associated with psychiatric illness [598]. It is important to distinguish the generally mild, transient TSH alterations typical of NTI from the more profound and persistent TSH changes associated with hyper- or hypothyroidism [24,238,244].

Misleading TSH Measurements

TSH can be diagnostically misleading either because of (a) biological or (b) technical factors. from heterophile antibodies (HAbs) or endogenous TSH autoantibodies are the most common causes of a falsely high TSH [299,329,599].

Biologic factors causing TSH diagnostic dilemmas

Unstable thyroid funct***ion***
TSH can be misleading when there is unstable thyroid status - such as in the early phase of treating hyper- or hyperthyroidism or non-compliance with L-T4 therapy -when there is a lag in the resetting of pituitary TSH to reflect a new thyroid status [600]. During such periods of instability TSH will be misleading and FT4 will be the more diagnostically reliable test.

Pituitary/Hypothalamic Dysfunction
Pituitary dysfunction is rare in ambulatory patients [509]. TSH measurement is unreliable in cases of both central hypothyroidism and central hyperthyroidism caused by TSH-secreting adenomas [215,217,219,508].

Central Hypothyroidism (CH***)***
Central hypothyroidism (CH) is rare (1/1000 as prevalent as primary hypothyroidism, 1/160,000 detected by neonatal screening) [509, 601]. CH can arise from disease at either the pituitary or hypothalamic level, or both [509]. A major limitation of using a TSH-centered screening strategy is that this strategy will miss a diagnosis of CH, because the TSH isoforms secreted in CH are abnormally glycosylated and bio-

inactive, yet will be detected as paradoxically normal TSH by current IMA methods despite the presence of clinical hypothyroidism [215, 217, 602]. The clinical diagnosis of CH can be confirmed biochemically as a low FT4/normal-low TSH discordance. Serum FT4 should be used to optimize L-T4 replacement therapy. In the absence of clinical suspicion, investigations for pituitary dysfunction should only be initiated after ruling-out technical interference.

TSH-secreting pituitary adenomas

TSHomas are characterized by near-normal TSH despite clinical hyperthyroidism [603]. Since this is a rare (0.7%) type of pituitary adenoma, technical interference causing paradoxically high TSH, such as a TSH autoantibody should be excluded before initiating inconvenient and unnecessary pituitary imaging or dynamic (T3 suppression or TRH stimulation) diagnostic testing. TSHomas are characterized by discordance between the clinical presentation and a paradoxically non-suppressed TSH despite high thyroid hormone levels and clinical hyperthyroidism [604]. This clinical/biochemical discordance reflects adenoma secretion of TSH isoforms with enhanced biologic activity that cannot be distinguished from bioactive TSH by IMA methods. Failure to diagnose the pituitary as the cause of the hyperthyroidism can lead to inappropriate thyroid ablation. The treatment of choice is surgery but in cases of surgical failure somatostatin analog treatment has been found effective [604]. Note that the biochemical profile (high thyroid hormones and non-suppressed TSH) is similar to that seen with thyroid hormone resistance syndromes [605]. When pituitary imaging is equivocal, genetic testing may be necessary to distinguish between these two conditions.

Resistance to Thyroid Hormone (RT***H)***

Resistance to thyroid hormone is biochemically characterized by high thyroid hormone (FT4 +/- T3) levels and a non-suppressed, sometimes slightly elevated TSH without signs and symptoms of thyroid hormone excess [606]. Early cases of resistance to thyroid hormone were shown to result from mutations in the thyroid hormone receptor *B* [607]. More recently the definition of RTH has been broadened to include other causes of thyroid hormone resistance - mutations in the thyroid hormone cell membrane transporter MCT8, and a range of genetic thyroid hormone metabolism defects (SBP2) [608]. These resistance syndromes display a spectrum of clinical and biochemical profiles may need to be identified by specialized genetic testing.

Activating or Inactivating TSH Receptor Mutations

Non-autoimmune hyperthyroidism resulting from an activating mutation of the TSH receptor (TSHR) is rare [426-433]. A spectrum of loss-of-function TSHR mutations (TSH resistance) causing clinical and subclinical hypothyroidism despite high thyroid hormone levels, have also been described [434-436]. Because TSHR mutations are a rare cause of TSH/FT4 discordances, technical interferences should first be excluded before considering a TSHR mutation as the cause of these discordant biochemical profiles.

Technical Factors causing TSH Diagnostic Dilemmas

Causes of technical interferences with TSH measurement are similar to those discussed for thyroid hormone tests.

Non Analyte -Specific Interferences

Heterophile Antibodies (HAbs) can cause falsely high TSH IMA tests [289,294,300,328-330, 609]. The HAb in some patient's sera interfere strongly with some manufacturers tests but appear inert in others [609]. This is why re-measurement in a different manufacturers assay should be the first test for interference. A fall in TSH in response to blocker-tube treatment is typically used to confirm HAb interference

Anti-Reagent Antibody Interferences.
As discussed for free hormone tests,,,,,, some patients have antibodies that target test reagents (such Rhuthenium) that cause interference with TSH and/or free hormone tests. It should be noted that the anti-Rhuthenium antibodies of different patients may affect different analytes to different degrees [339-342].

Tests employing Streptavidin-Biotin reagents are prone to interferences from antibodies targeting either Streptavidin [344] or biotin reagents [345]. Alternatively, high dose biotin ingestion has been known to produce interference with thyroid and other tests in an analyte-specific, platform-specific manner [346-350].

Analyte-specific interferences typically result from autoantibodies targeting the analyte. Depending on the analyte and test formulation, autoantibody interferences typically cause falsely-high test results, but can cause falsely-low test results, as in the case of Tg autoantibodies. It should be noted that transplacental passage both heterophile antibodies or anti-analyte autoantibodies (i.e. TSHAb or T4Ab) have the potential to interfere with neonatal screening tests [351-354]. Specifically, maternal TSH autoantibodies can cross the placenta and may cause a falsely high TSH screening test in the newborn mimicking congenital hypothyroidism, whereas maternal T4 autoantibodies could cause falsely high neonatal T4 masking the presence of congenital hypothyroidism [230, 353].

TSH Autoantibodies (TSHAb)/"Macro TSH".

Analytically suspicious TSH measurements are not uncommon [290] and have been reported in up to five percent of specimens subjected to rigorous screening [294]. In recent years there have been a number of reports of TSHAb, often referred to as "macro" TSH, causing spuriously high TSH results in a range of different methods [610,611]. The prevalence of TSHAb approximates 0.8 percent, but can as high as ~1.6 percent in patients with subclinical hypothyroidism. Showing a lowering of TSH in response to a polyethylene glycol (PEG) precipitation of immunoglobulins is the most convenient test for TSHAb [599,611]. Alternatively, column chromatography can show TSH immunoactivity in a high molecular weight peak representing a bioinactive TSH-immunglobulin complex [599,611].

TSH Variants.

TSH variants are a rare cause of interference [612]. Nine different TSH beta variants have been identified to date [613]. These mutant TSH molecules may have altered immunoactivity and be detected by some TSH IMA methods but not others [612]. The bioactivity of these TSH mutants is variable and can range from normal to bio-inert [613], resulting in discordances between the TSH concentration and clinical status [612] and/or discordant TSH/FT4 relationships [613]. These TSH genetic variants are one of the

causes of congenital hypothyroidism [614-616].

THYROID SPECIFIC AUTOANTIBODIES (TRAB , TPOAB AND TGAB)

Tests for antibodies targeting thyroid-specific antigens such as thyroid peroxidase (TPO), thyroglobulin (Tg) and TSH receptors (TSHR) are used as markers for autoimmune thyroid conditions [37,617]. Over the last four decades, thyroid antibody test methodologies have evolved from semi-quantitative agglutination, complement fixation techniques and whole animal bioassays to specific ligand assays using recombinant antigens or cell culture systems transfected with the human TSH receptor [37,618621]. Unfortunately, the diagnostic and prognostic value of these tests has been hampered by methodologic differences as well as difficulties with assay standardization [622]. Although most thyroid autoantibody testing is currently made on automated immunoassay platforms, methods vary in sensitivity, specificity and the numeric values they report because of standardization issues [44,582,620,623]. Thyroid autoantibody testing can be useful for diagnosing or monitoring treatment for a number of clinical conditions, however these tests should be selectively employed as adjunctive tests to other diagnostic testing procedures.

TSH Receptor Autoantibodies (TRAb)

The TSH receptor (TSHR) serves as a major autoantigen [624,625]. Thyroid gland stimulation occurs when TSH binds to TSHR on thyrocyte plasma membranes and activates the cAMP and phospholipase C signaling pathways [625]. The TSH receptor belongs to the G protein-coupled class of transmembrane receptors. It undergoes complex posttranslational processing in which the ectodomain of the receptor is cleaved to release a subunit into the circulation [624]. A TSH-like thyroid stimulator found uniquely in the serum of Graves' disease patients was first described using a guinea pig bioassay system in 1956 [626]. Later, using a mouse thyroid bioassay system this serum factor displayed a prolonged stimulatory effect as compared to TSH and hence was termed to be a "long-acting thyroid stimulator" or LATS [627,628]. Much later, the LATS factor was recognized not to be a TSH-like protein but an antibody that was capable of stimulating the TSH receptor causing Graves' hyperthyroidism [629]. TSH receptor antibodies have also become implicated in the pathogenesis of Graves' opthalmopathy [629-632]. TRAbs are heterogeneous (polyclonal) and fall into two general classes both of which can be associated with autoimmune thyroid disorders – (a) thyroid stimulating autoantibodies (TSAb) that mimic that the actions of TSH and cause Graves' hyperthyroidism and (b), blocking antibodies (TBAb) that block TSH binding to its receptor and can cause hypothyroidism [37,48,621,625,629,633,634]. Although TSH, TSAb and TBAb appear to bind to different sites on the TSH receptor ectoderm, TSAb and TBAb have similar affinities and often overlapping epitope specificities [635]. In some cases of Graves' hyperthyroidism, TBAb have been detected in association with TSAb [636,637] and the dominance of one over the other can change over time in response to treatment [638]. Because both TSAb and TBAb can be present in the same patient, the relative concentrations and receptor binding characteristics of these two classes of TRAb may influence the severity of Graves' hyperthyroidism and the response to antithyroid drug therapy or pregnancy [624,636,639-643]. For completeness, it should also be mentioned that a third class of "neutral" TRAb has also been described, of which the functional significance has yet to be determined [641,644].

Two different methodologic approaches have been used to quantify TSH receptor antibodies

[40,620,633,645]: (i) TSH receptor tests (TRAb assays) also called TBII or TSH Binding Inhibition Immunoglobulin assays, and (ii) Bioassays that use whole cells transfected with human or chimeric TSH receptors that produce a biologic response (cAMP or bioreporter gene) when TSAb or TBAb are present in a serum specimen. In recent years automated immunometric assays using recombinant human TSHR constructs have been shown to have high sensitivity for reporting positive results in Graves' disease sera [620,646]. However, assay sensitivity varies among current receptor versus bioassay methods [43]

Bioassay methods (TSAb/TBAb)

The first TSH receptor assays used surgical human thyroid specimens, mouse or guinea pig thyroid cells, or rat FRTL-5 cell lines to detect TSH receptor antibodies. These methods typically required pre-extraction of immunoglobulins from the serum specimen [626,633,647-652]. Later, TRAb bioassays used cells with endogenously expressed or stably transfected human TSH receptors and could use unextracted serum specimens [653-655]. Current TRAb bioassays are functional assays that use intact (typically CHO) cells transfected with human or chimeric TSH receptors, which when exposed to serum containing TSH receptor antibodies use cAMP or a reporter gene (luciferase) as a biological marker for any stimulating or blocking activity in a serum [40,42,620,648,651,653,656]. Bioassays are more technically demanding than the more commonly used receptor assays because they use viable cells. However, these functional assays can be modified to detect TBAb that may coexist with TSAb in the same sera and make interpretation difficult [40,657]. The most recent development is for 2nd generation assays to use a chimeric human/rat LH TSHR to effectively eliminate the influence of blocking antibodies. This new approach has shown excellent sensitivity and specificity for diagnosing Graves' hyperthyroidism and clinical utility for monitoring the effects of anti-thyroid drug therapy [42].

TSH Receptor (TRAb)/TSH Binding Inhibitory Immunoglobulin (TBII) Methods

TRAb methods detect serum immunoglobulins that bind TSHR but do not functionally discriminate stimulating from blocking antibodies. TRAb methods are based on standard competitive or noncompetitive principles. First generation methods were liquid-based whereby immunoglobulins in the serum inhibited the binding of ^{125}I-labeled TSH or enzyme-labeled TSH to a TSH receptor preparation [40,658]. These methods used TSH receptors of human, guinea-pig or porcine origin [658]. After 1990, a second-generation of both isotopic and non-isotopic methods were developed that used and immobilized porcine or recombinant human TSH receptors [40,659-661]. These second-generation methods were shown to have significantly more sensitivity for detecting Graves' thyroid stimulating immunoglobulins than first-generation tests [620]. In 2003 a third-generation of non-isotopic methods were developed that were based on serum immunoglobulins competing for immobilized TSHR preparation (recombinant human or porcine TSHR) with a monoclonal antibody (M22) [37,40,42,620,648,656,660,662-666]. 3rd generation assays have also shown a good correlation and comparable overall diagnostic sensitivity with bioassay methods [620,636,648,667,669]. Current third-generation tests have now been automated on several immunoassay platforms [620]. However, between-method variability remains high and interassay precision often suboptimal (CVs > 10 %) despite the use of the same international reference preparation for calibration [622,670]. This fact makes it difficult to compare values using different methods and indicates that further efforts focused on additional assay improvements are needed [37,622,671].

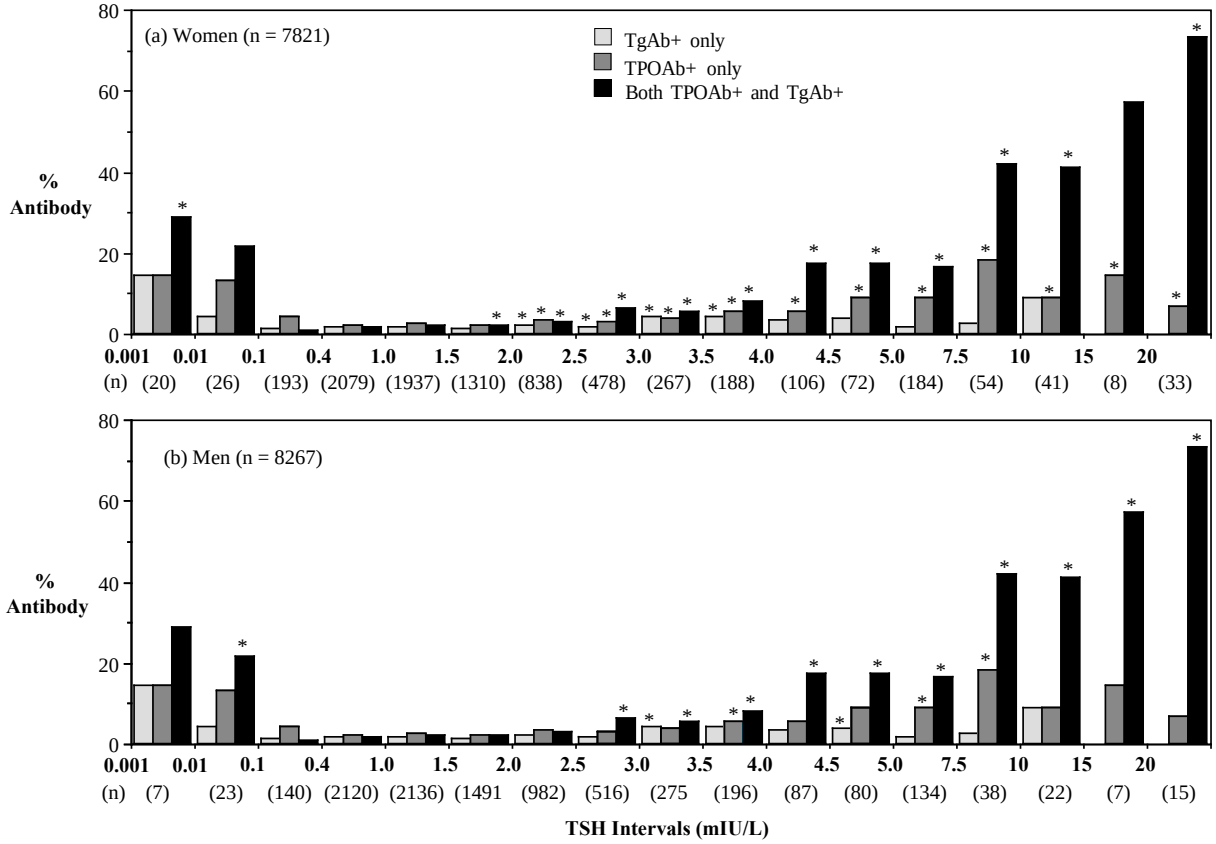
Clinical Use of TRAb Tests

Over the last ten years automated IMA methods have dramatically lowered the cost and increased the availability of TRAb testing [43,646,672]. Automated TRAb IMAs are not functional tests and do not distinguish between stimulating and blocking TRAbs. However this distinction is usually unnecessary, since it is evident from clinical evidence of hyper- or hypothyroid features. Also, both TSHR stimulating and blocking antibodies may be detected simultaneously in the same patient and cause diagnostic confusion [42,673]. Because the sensitivity and specificity of current third-generation TRAb tests is over 98 percent, TRAb testing can be useful for determining the etiology of hyperthyroidism [620,672], as an independent risk factor for Graves' opthalmopathy [632] and may be useful for monitor responses to therapy [620,674,675]. TRAb measured prior to radioiodine therapy for Graves' hyperthyroidism can also help predict the risk for exacerbating opthalmopathy [630,676-680]. There is conflicting data concerning the value of using TRAb to predict the response to antithyroid drug treatment or risk of relapse [42,637,661,667,681-685]. An important application of TRAb testing is to detect high TRAb concentrations in pregnant patients with a history of autoimmune thyroid disease or active or previously treated Graves' hyperthyroidism, in whom transplacental passage of stimulating or blocking TRAb can cause neonatal hyper- or hypothyroidism, respectively [40,67,620,645,686-689]. Because the expression of thyroid dysfunction may be different in the mother and infant, automated IMA methods have the advantage of being able to detect both stimulating and blocking antibodies [690]. It is currently recommended [74] that TRAb be measured in the first trimester in all pregnant patients with active Graves' hyperthyroidism or who have received prior ablative (radioiodine or surgery) therapy for Graves' disease in whom TRAb can remain high even after patients have been rendered hypothyroid and are being maintained on L-T4 replacement therapy. When TRAb is high in the first trimester additional TRAb testing is recommended at weeks 18-22 and 30-34 [24,37,67,74,636,687,691].

Thyroid Peroxidase Autoantibodies (TPOAb)

TPO is a large, dimeric, membrane-associated, globular glycoprotein that is expressed on the apical surface of thyrocytes. TPO autoantibodies (TPOAb) found in sera typically have high affinities for an immunodominant region of the intact TPO molecule. When present, these autoantibodies vary in titre and IgG subclass and display complement-fixing properties [692]. Studies have shown that epitope fingerprints are genetically conserved suggesting a possible functional importance [693]. However, it is still unclear whether the epitope profile correlates with the presence of, or potential for, the development of thyroid dysfunction with which TPOAb presence is most commonly associated [692,694,697].

Figure 5. NHANES III Study Data: Prevalence of Thyroid Autoantibodies Relative to TSH



Prevalence of thyroid antibodies across TSH intervals in women (A) and men (B). The abscissa TSH values correspond to the upper and lower limits of the intervals spanning each set of bars. Asterisks denote a significant difference in prevalence from the TSH range with lowest antibody prevalence, 0.1 and 1.5 mIU/liter for women and 0.1 and 2.0 mIU/liter for men [456].

TPOAb antibodies were initially detected as antibodies against thyroid microsomes (antimicrosomal antibody, AMA) using semi-quantitative complement fixation and tanned erythrocyte hemaagglutination techniques [698-700]. Recent studies have identified the principal antigen in the AMA tests as the thyroid peroxidase (TPO) enzyme, a 100 kD glycosylated protein present in thyroid microsomes [701, 702]. Manual agglutination tests have now been replaced by automated, more specific TPOAb immunoassay or immunometric assay methods that use purified or recombinant TPO [24,37,619,703-710]. Despite calibration against the same International Reference Preparation (MRC 66/387), there is considerable inter-method variability of current TPOAb assays (correlation coefficients 0.65 and 0.87) that precludes the numeric comparison of serum TPOAb values reported by different tests [37,618,619,706,709,710]. It appears that both the methodologic principles of the test and the purity of the TPO reagent used may influence the sensitivity, specificity and reference range of the method [37,619]. The variability in sensitivity limits and the reference ranges of different methods has led to different interpretations regarding the normalcy of having a detectable TPOAb [37,582,710].

TPOAb Clinical Significance

Estimates of TPOAb prevalence depend on the sensitivity and specificity of the method employed [582,710,711]. In addition, ethnic and/or geographic factors (such as iodine intake) influence the TPOAb prevalence in population studies [487]. For example, TPOAb prevalence is significantly higher (~11 percent) in dietary iodine-sufficient countries like the United States and Japan as compared with iodine deficient areas in Europe (~ 6 percent) [452,515,712]. The prevalence of TPOAb is higher in women of all age groups and ethnicities, presumably reflecting the higher propensity for autoimmunity as compared with men [452,712]. Approximately 70-80 % of patients with Graves' disease and virtually all patients with Hashimoto's or post-partum thyroiditis have TPOAb detected [619,706,709,711,713]. TPOAb has, in fact, been implicated as a cytotoxic agent in the destructive thyroiditic process [697,714717]. However, TPOAb prevalence is also significantly higher in various non-thyroidal autoimmune disorders in which no apparent thyroid dysfunction is evident [718-720]. Aging is associated with an increasing prevalence of TPOAb that parallels the increasing prevalence of both subclinical (mild) and clinical hypothyroidism [452]. In fact, the NHANES III survey reported that TPOAb prevalence increases with age and approaches 15-20 percent in elderly females in the iodine-sufficient United States [452]. This same study found that the odds ratio for hypothyroidism was strongly associated with the presence of TPOAb but not TgAb, suggesting that only TPOAb has an autoimmune etiology [452]. Although the presence of TgAb alone did not appear to be associated with hypothyroidism or TSH elevations, the combination of TPOAb and TgAb versus TPOAb alone may be more pathologically significant (Figure 5), although further studies would be needed to confirm this [452,456,459,697]. It is now apparent that the presence of TPOAb in the serum of apparently euthyroid individuals (TSH within reference range) appears to be a risk factor for future development of overt hypothyroidism that subsequently becomes evident at the rate of approximately 2 percent per year in such populations [46,532,692,693].

In this context, it is reasonable to assume that TPOAb measurement may serve as a useful prognostic indicator for future thyroid dysfunction [46,721]. However, it is noteworthy that the detection of TPOAb does not always precede the development of thyroid dysfunction. A recent study suggests that a hypoechoic ultrasound pattern can be seen before a biochemical TPOAb abnormality is detected [458,487]. Further, some individuals with unequivocal TSH elevations, presumably resulting from autoimmune destructive disease of the thyroid, do not have TPOAb detected [456]. Presumably, this paradoxical absence of TPOAb in some patients with elevated TSH likely reflects the suboptimal sensitivity and/or specificity of current TPOAb tests or a non-autoimmune cause of thyroid failure (i.e. atrophic thyroiditis) [452,456,710,722].

Although changes in autoantibody concentrations often occur with treatment or reflect a change in disease activity, serial TPOAb measurements are not recommended for monitoring treatment for autoimmune thyroid diseases [359,619,723]. This is not surprising since treatment of these disorders addresses the consequence (thyroid dysfunction) and not the cause (autoimmunity) of the disease. However, where it may have an important clinical application is to employ the presence of serum TPOAb as a risk factor for developing thyroid dysfunction in patients receiving Amiodarone, Interferon-alpha, Interleukin-2 or Lithium therapies which all appear to act as triggers for initiating autoimmune thyroid dysfunction in susceptible (especially TPOAb-positive) individuals [24,101,724-730].

During pregnancy the presence of TPOAb has been linked to reproductive complications

such as miscarriage, infertility, IVF failure, fetal death, pre-eclampisa, pre-term delivery and post-partum thyroiditis and depression [66,67,564,731-742]. However, if this association represents cause or effect has yet to be been resolved.

Thyroglobulin Autoantibodies (TgAb)

Thyroglobulin autoantibodies predominantly belong to the immunoglobulin G (IgG) class, are not complement fixing and are generally conformational [743]. Serum TgAb were the first thyroid antibody to be detected in patients with autoimmune thyroid disorders using tanned red cell hemagglutination techniques [699]. Subsequently, methodologies for detecting TgAb have evolved in parallel with those for TPOAb measurement from semi-quantitative techniques, to more sensitive ELISA and RIA methods and most recently non-isotopic competitive or non-competitive immunoassays [10,37,44,706,710,713,744-747]. Unfortunately, the inter-method variability of these TgAb assays is even greater than that of TPOAb tests discussed above [10,37,44,745-747]. Additionally, high levels of thyroglobulin in the serum have the potential to influence TgAb measurements [747-750]. Between-method variability is influenced by the purity and the epitope specificity of the Tg reagent, as well as the patient-specific epitope specificity of the TgAb in the serum [751,752]. As with TPOAb methods, TgAb tests have highly variable sensitivity limits and cut-off values for "TgAb positivity", despite the use of the same International Reference Preparation (MRC 65/93) (Figure 6) [10, 44, 745-747, 753]. It should be noted that the manufacturer-recommended cutoffs are set for diagnosing thyroid autoimmunity and are too high for detecting levels of TgAb that interfere with Tg measurements - the much lower assay FS limit (Figure 6) is the recommended cutoff to define TgAb-positivity for DTC monitoring [24]. Although there are reports that low levels of TgAb may be present in normal euthyroid individuals, it is unclear whether this represents assay noise due to matrix effects or "natural" antibodies [744,754]. Further complicating this question are studies suggesting that there may be qualitative differences in TgAb epitope specificities expressed by normal individuals versus patients with either differentiated thyroid cancers (DTC) or autoimmune thyroid disorders [744,752,755]. These differences in test sensitivity and specificity negatively impact the reliability of determining the TgAb status (positive versus negative) of specimens prior to Tg testing.

Clinical Utility of TgAb Tests

Autoantibodies against Tg are encountered in autoimmune thyroid conditions, usually in association with TPOAb [46,452,746, 756]. However, the NHANES III survey found that only three percent of subjects with no risk factors for thyroid disease had serum TgAb present without detectable TPOAb (Figure 5) [452,456]. Further, in these subjects there was no association observed between the isolated presence of TgAb and TSH abnormalities [452,456]. This suggests that it may be unnecessary to measure both TPOAb and TgAb for a routine evaluation of thyroid autoimmunity [37,46,456]. In fact, when autoimmune thyroid disease is present, there is some evidence that assessing the combination of TPOAb and TgAb has greater diagnostic utility than the TPOAb measurement alone (Figure 5) [46,456,459,757].

TgAb measurement is primarily used as an adjunctive test to serum Tg measurement when monitoring patients with differentiated thyroid cancers (DTC) [72,593]. The role of TgAb testing is two-fold: 1) to authenticate that a Tg measurement is not compromised by TgAb interference, 2) as an independent surrogate tumor-marker in the ~20 percent of patients with circulating TgAb. Current guidelines recommend that all sera be prescreened for TgAb by a sensitive immunoassay method prior to serum Tg testing, because there appears to be

no threshold TgAb concentration that precludes TgAb interference with Tg measurements [9,10,24,44,593,713,746,758]. Immunoassay methods detect TgAb in approximately 25 percent of patients presenting with DTC [44,713,759-761]. The prevalence of TgAb is typically higher in patients with papillary versus follicular tumors and is frequently associated with the presence of lymph node metastases [746,759,761, 62]. Perhaps of even greater importance is the observation that serially determined TgAb concentrations may also serve as an independent parameter for detecting changes in tumor mass in patients with an established diagnosis of DTC [Figure 6Ad(ii)] [761-766]. For example, after TgAb-positive patients are rendered disease-free by surgery, TgAb concentrations typically progressively decline during the first few post-operative years and typically become undetectable after a median of three years of follow-up [761,762,766]. In contrast, a rise in, or de novo appearance of, TgAb is often the first indication of tumor recurrence [713,761,762]. However, when using serial TgAb measurements as a surrogate marker for changes in tumor burden it is essential to use the same TgAb method, because of the large between-method differences observed with this assay (Figure 6) [9,10,44,713,745,747,753].

Figure 6 TgAb Method Comparison

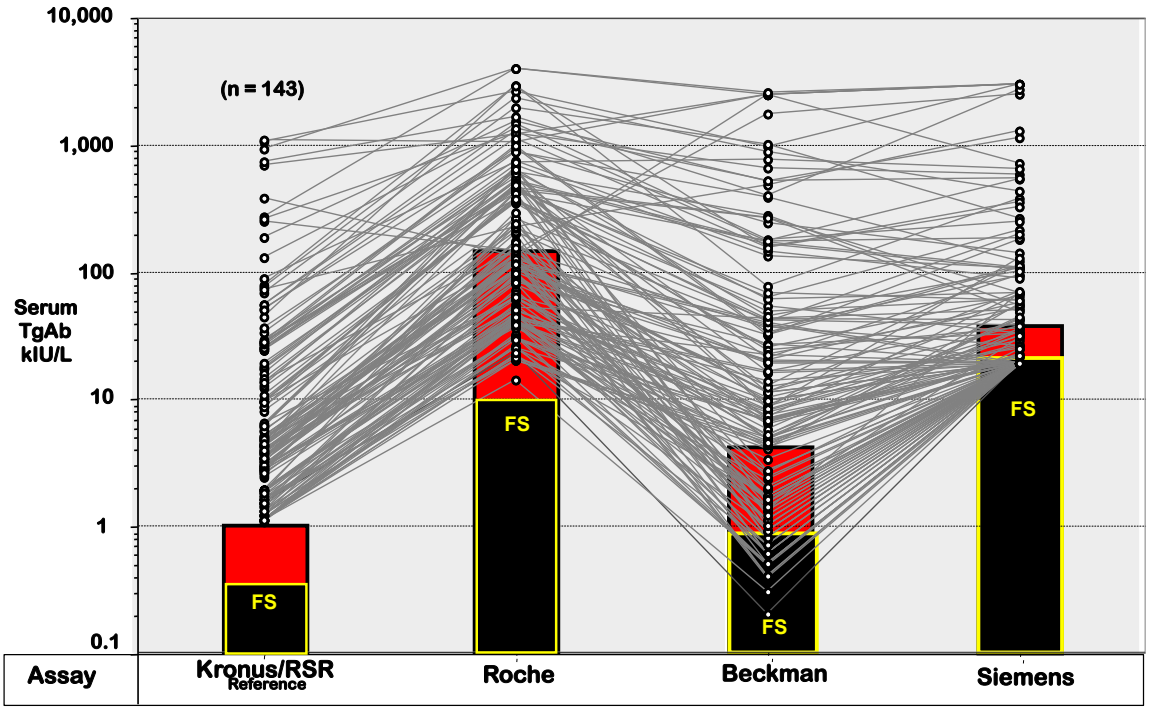


Figure 6. TgAb Method Comparison. 143 DTC sera with TgAb above the MCO for the reference method (Kronus/RSR) and evidence of TgAb interference with Tg measurement (Tg-IMA/Tg-RIA ratios < 80 percent) were measured by three different TgAb methods. The red bars show the manufacturer recommended cutoff for TgAb-positivity for each method. The black bar denotes the functional sensitivity (FS) of each method [44].

THYROGLOBULIN (TG)

Thyroglobulin plays a central role in a wide variety of pathophysiologic thyroid conditions,

including acting as an autoantigen for thyroid autoimmunity [617,743,767]. Serum Tg levels can serve as a marker for iodine status of a population [768-771], whereas dyshormongenesis resulting from genetic defects in Tg biosynthesis is a cause of congenital hypothyroidism [24,772-775]. Because Tg has a thyroid-tissue specific origin, a Tg measurement can aid in determining the etiology of congenital hypothyroidism (athyreosis versus dyshormonogenesis) [776,777]. Likewise, a paradoxically low serum Tg can be used to distinguish factitious hyperthyroidism from the high Tg expected with endogenous hyperthyroidism [778-780]. This chapter focuses on the primary clinical use of Tg measurement - a tumor-marker test for post-operative monitoring of patients with follicular-derived (differentiated) thyroid cancer (DTC) [32,72,404,781-788]. (Table 3)

Most Tg testing is currently made by rapid, automated immunometric assays (IMA) with second-generation functional sensitivity (^{2G}-Tg-IMA, FS≤ 0.1 µg/L). Assays with this level of FS obviate the need for recombinant human TSH (rhTSH) stimulation [[11,32,72,416,784,789-793]. The major limitation of IMA methodology is its propensity for TgAb interference causing falsely low/undetectable serum Tg-IMA that can mask disease [10,31,45,58,760,790,794-798]. Currently, most laboratories in the United States first establish the TgAb status of the specimen (negative or positive) in order to restrict Tg-IMA testing to TgAb-negative sera, whereas TgAb-positive specimens are reflexed for testing by Tg methodologies believed to be less prone to interferences, such as RIA [30,32,796] or LC-MS/MS [31,799-801].

Technical Limitations of Tg Methods

Thyroglobulin measurement remains technically challenging [788]. Five methodologic problems impair the clinical utility of this test: (a) between-method biases; (b) suboptimal functional sensitivity; (c) suboptimal between-run precision over the typical clinical interval used to monitor DTC patients (6-12 months); (d) "hook" problems (some IMA methods), and interferences caused by both (e) Heterophile antibodies (HAb) and (f) Tg autoantibodies (TgAb).

Tg Assay Functional Sensitivity

As discussed for TSH, assay functional sensitivity (FS) represents the lowest analyte value that can be reliably detected under clinical practice conditions. For Tg assays FS is defined by the lowest Tg concentration that can be measured in human serum with 20 percent coefficient of variation (CV) in runs made over a 6-12 month period using at least two different lots of reagents and two instrument calibrations [24,58,72,404,802]. These stipulations are needed because assay precision erodes over time and the clinical interval for serum Tg monitoring of DTC patients is typically long (6-12 months) [9,408,803]. For Tg assays it is critical to use FS as the lowest reporting limit in preference to a LOQ calculation (20 percent CV), because LOQ does not stipulate a relevant time-span for assessing precision [24,405,407,804,805]. Another stipulation of the FS protocol [24] is to assess precision using the appropriate test matrix (human serum) in preference to a commercial QC preparation, because instruments and methods are matrix-sensitive [407]. Since Tg-IMA testing is typically restricted to TgAb-negative sera, precision estimates should be made in TgAb-negative human serum pools [407]. In contrast, Tg-RIA and Tg-LC-MS/MS testing is typically reserved for sera containing TgAb, necessitating precision estimation in TgAb-positive human serum pools.

As with TSH [220,397], there has been a progressive improvement in the FS of Tg methods that has led to the adoption of a generational approach to Tg assay nomenclature.

Currently, some Tg-IMAs, all Tg-RIAs and all Tg-LC-MS/MS methodologies still only have first-generation functional sensitivity (FS = 0.5-1.0 µg/L) [4,10,32,33,58]. Over the last ten years second-generation immunometric assays ($^{2G-}$Tg-IMA), characterized by an order of magnitude greater functional sensitivity (FS 0.05-0.10 µg/L), have become available. $^{2G-}$Tg-IMA testing is now considered the standard of care in the absence of TgAb [31-33,58,72,296,783,806-808]. When disease is absent the basal serum $^{2G-}$Tg-IMA is typically below 0.5 µg/L, even without RAI treatment [809,810]. It follows that the inferior FS (~1 µg/L) of first-generation assays can barely distinguish subnormal values from the Tg levels seen when an intact thyroid gland is present (~2-40 µg/L), and are clearly too insensitive to detect recurrences in thyroidectomized patients unless recombinant human TSH (rhTSH) stimulation is employed [296,593,758,782,811,812]. Now that $^{2G-}$IMA-Tg testing has become the standard of care [72], there is no longer a need for routine rhTSH stimulation to boost the Tg level to values detectable by first-generation tests, because basal (TSH suppressed) Tg correlates with rhTSH-stimulated Tg measured by $^{2G-}$Tg-IMA [10,11,32,58,72,296,413, 416,789,791-793,806,807,813-816]. Studies have shown that a basal $^{2G-}$Tg-IMA below 0.1 µg/L predicts a negative rhTSH test (rhTSH-stimulated Tg <2.0 µg/L) with a high degree of confidence [72,296,791,792,817]. Even so, the use of a 2nd generation Tg assay does not eliminate the need for periodic ultrasound examinations, because many histologically confirmed lymph nodes metastases are inefficient Tg secretors and may be associated with an undetectable serum Tg, even when measured by $^{2G-}$Tg-IMA [807,818-821].

Figure 7. Serum Tg Measurements in DTC Patients with Structural Disease

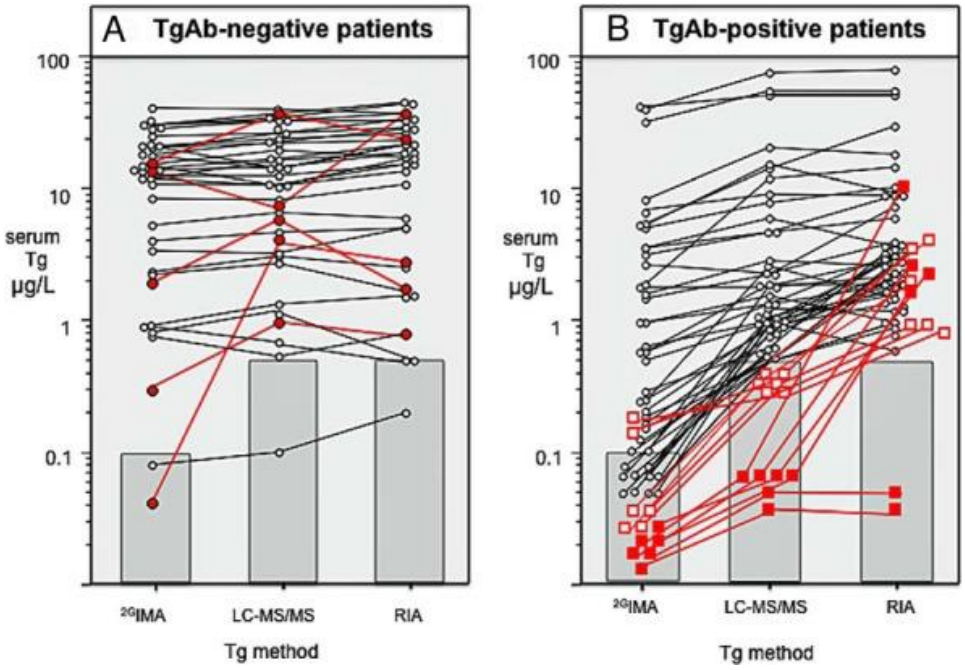


Figure 7. Panel A shows the comparison of serum Tg values reported for 37 TgAb-negative DTC patients with persistent/recurrent DTC measured by a $^{2G-}$Tg-IMA (Beckman), Tg-LC-MS/MS (Mayo Medical Labs) and the USC Tg-RIA method. Sera with Tg values below the FS limit of the method are shown in the shaded areas, Although each method was standardized against CRM-457, the sera marked in red displayed > 30% difference in Tg values that reflected different method specificities for detecting tumor-derived Tg molecules -

differences with the potential to disrupt clinical management following a change in Tg method. Panel B shows the method comparison for 52 TgAb-positive DTC patients with structural disease. Sera with unequivocally undetectable Tg-LC-MS/MS values (no peak) are shown by solid red squares, whereas sera with marginally detectable Tg-LC-MS/MS values in the 0.3 to 0.5 µg/L range are shown by open red squares [31].

Between-Method Biases

Although most Tg methods claim to be standardized against the Certified Reference Preparation CRM-457 [9,822,823] there can be significant differences between the Tg values reported for the same serum measured by different methods, even in the absence of TgAb (Figure 7A) [10,19,24,32,58,799,824]. Between-method Tg variability is higher than the biologic variability (~16 percent) in euthyroid subjects [442, 803]. In fact, studies have shown that there can be a two-fold difference in Tg values reported for the same serum measured by different methods [32]. Although this reflects standardization and matrix differences to some extent [299,797], for the most part this between-method variability reflects differences in method specificities for detecting heterogeneous serum Tg isoforms [10,825-827]. It should be noted that because IMA methodology uses monoclonal antibody reagents, IMAs have narrower specificities for detecting Tg heterogeneity than RIA methods that use polyclonal antibodies [9,10,826-829]. Because Tg-IMAs differ in their sensitivity to TgAb interference, between-method Tg variability can also result from using an insensitive TgAb test that reports false-negative TgAb values (Figure 6) [19,44,830].

When TgAb is absent and a $^{2G-}$Tg-IMA method is used consistently, the between-run precision across a 6-12 month timespan (the typical interval for monitoring DTC patients) is less than 10%, yet the between-method variability seen for some TgAb-negative patients (shown in Figure 7A by red lines) can be greater than 30 percent [31]. These differences likely reflect different method specificities for detecting heterogeneous serum Tg isoforms. Clearly this magnitude of between-method difference has the potential to disrupt serial Tg monitoring and could negatively impact clinical management should a change in Tg method be made without re-baselining the Tg level [10,24,58,72,805]. In recognition of the differences between Tg methods, current guidelines stress the critical importance of using the same Tg method (and preferably the same laboratory) to monitor the serum Tg trend in DTC patients [72].

High-Dose Hook Effect

Tumor marker tests employing IMA methods can be prone to so-called "high-dose hook effects", whereby very high antigen concentrations can overwhelm the binding capacity of the monoclonal antibody reagents leading to a falsely normal/low value [9,831-834]. This phenomenon reduces the ability of the endogenous analyte to form a bridge between the capture and signal monoclonals resulting in an inappropriately low signal [9,831,835,836]. Manufacturers have largely overcome hook problems by adopting a two-step procedure, whereby a wash step is used to remove unbound antigen after the first incubation of specimen with the capture monoclonal antibody before introducing the labeled monoclonal followed by a second incubation when signal binds captured antigen [790,832]. When using any particular IMA method, it is primarily the laboratory's responsibility to determine whether a hook effect is likely to generate falsely normal or low values.

Approaches for detecting and overcoming hook effects occurring with IMA methods are:
- Routinely run each specimen at two dilutions. For example, the value obtained with a 1/5

or 1/10 dilution of the test serum would, if a hook effect were present, be higher than that obtained with an undiluted sample.

- To carry out appropriate dilution studies to rule out a possible hook effect when an unexpectedly low serum Tg value is encountered for a patient with known metastatic disease. In such cases, consultation with the physician may provide valuable information regarding this issue.
- To perform a Tg recovery test. If there is a hook effect present, the recovery of added antigen (Tg) will produce an inappropriately low result.

Interferences with Tg Measurement

Heterophile Antibody (HAb) Interferences

As discussed for FT4 and TSH, HAb, including human anti-mouse antibodies (HAMA) and Rheumatoid Factor (RF), interferes selectively with IMA but not RIA or Tg-LC-MS/MS methodologies [295,296,318,323,324,331,332,761,837]. HAb interferences are thought to reflect the binding of human immunoglobulins in the serum specimen to the murine-derived monoclonal antibody IMA reagents. The rabbit polyclonal antibodies (PAb) used for Tg-RIA methods are not susceptible to this problem. In most cases HAb interferences are characterized by a false-positive Tg-IMA result [323,324,331,784], although falsely-low Tg-IMA results have also been reported [332].

Tg Autoantibody (TgAb) Interferences

TgAb interference with Tg measurement remains the major problem that limits the clinical utility of Tg testing. TgAb has the potential to undermine the clinical reliability of Tg measurements by both in-vitro mechanisms (epitope masking/low recoveries) [10,760,796,838, 839] and/or in-vivo mechanisms (enhanced TgAb-mediated Tg clearance) [677,840-842], irrespective of the Tg methodology used. There appears to be no threshold TgAb concentration that precludes TgAb interference [9,10,24,31,44,72,746,796,830]. High TgAb concentrations do not necessarily interfere, whereas low TgAb may profoundly interfere [9,31,44,761,795,796,830,839,843-846]. The Tg recovery approach is not reliable for detecting TgAb interference [10,752,839].

In-vitro Mechanisms of TgAb Interference.

TgAb interferes with Tg testing in a qualitative, quantitative and method-dependent manner [44,761,796,838,847,848]. The potential for in vitro interference is multifactorial and depends not only on the assay methodology (IMA, RIA or LC-MS/MS), but also the concentration and epitope specificity of the patient's TgAb [10,761,844]. RIA methodology appears to quantify total Tg (free Tg + TgAb-bound Tg) whereas IMA primarily detects only the free Tg moiety - Tg molecules whose epitopes are not masked by TgAb complexing. Steric masking of Tg epitopes is the reason why TgAb interference with IMA methodology is always unidirectional (underestimation), and why a low Tg-IMA/Tg-RIA ratio has been used to indicate TgAb interference [31,44,713,797,849,850]. The new Tg-LC-MS/MS methodology uses trypsin digestion of Tg-TgAb complexes to liberate a Tg proteotypic peptide. This conceptually attractive approach was primarily developed to overcome TgAb interference with IMA and thereby eliminate falsely low/undetectable Tg-IMA results that can mask disease. However, recent studies have reported a high percentage (>40%) of TgAb-positive DTC patients with structural disease who have paradoxically undetectable Tg-LC-MS/MS [31,799-801]. The reason why LC-MS/MS fails to detect Tg despite disease when TgAb is present needs further

study. Possibilities to investigate include, tumor Tg polymorphisms that prevent the production of the Tg-specific tryptic peptide [21], suboptimal trypsinization of Tg-TgAb complexes, or Tg levels that are truly below detection because of increased clearance of Tg-TgAb complexes by the hepatic asialoglycoprotein receptor [677,840-842].

In-vivo Mechanisms of TgAb Interference.

A number of studies over past decades have suggested that the presence of TgAb enhances Tg metabolic clearance. In the 1967 Weigle et al showed enhanced clearance of endogenously ^{131}I-labeled Tg in rabbits, after inducing TgAb by immunizing the animals with an immunogenic Tg preparation (840). Human studies of Tg and TgAb acute responses to sub-total thyroidectomy have also suggested that TgAb may increase Tg metabolic clearance (851). Changes (rise or fall) in TgAb versus Tg-RIA concentrations are typically concordant and appropriate for clinical status, whereas the direction of change of Tg-IMA is typically discordant with not only TgAb but also Tg-RIA and clinical status (31,32,44,713,798). In general, the change in TgAb concentrations tends to be steeper than for Tg-RIA (713), as would be consistent with TgAb-mediated Tg clearance. It may be that some TgAbs act as "sweeper" antibodies that facilitate clearance of antigen (842,852-854).

Figure 9. Influence of Changing TgAb status on Tg-IMA & Tg-RIA)

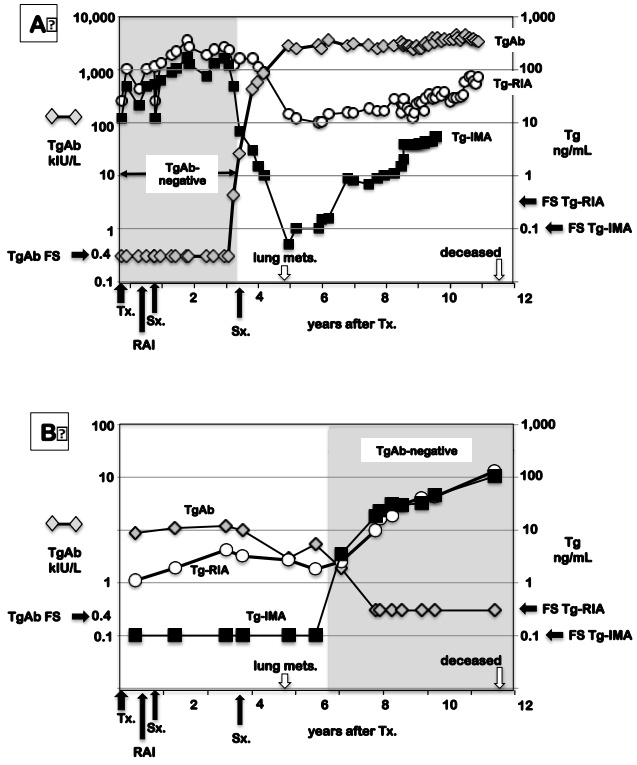


Figure 9 Serial TgAb, Tg-RIA and Tg-IMA concentrations in two DTC patients who underwent a change in TgAb status (panel A, negative to positive) or (panel B, positive to

negative) before death from structural DTC. Panel A: When TgAb appeared de novo 2.5 years after initial treatment (thyroidectomy, Tx + RAI) for PTC a progressive fall in Tg-IMA to undetectable levels occurred together with an approximate 90 percent fall in Tg-RIA. Thereafter as disease exacerbated, TgAb remained elevated and Tg-IMA rose to parallel Tg-RIA but at an 80 percent lower concentration. Panel B. This patient was TgAb-positive at the time of initial Tx+RAI treatment at which time Tg-RIA was detectable and Tg-IMA was undetectable. Despite extensive disease, TgAb became undetectable 5 years after initial treatment. This change in TgAb status was associated with a rapid rise in Tg-IMA to parallel a steep increase in Tg-RIA with a doubling time <1 year before demise.

Figure 9 provides insights on the influence of TgAb on Tg measurements. These two DTC patients who eventually died of structural disease, illustrate how changes in TgAb status (Panel A-TgAb-negative to TgAb-positive versus Panel B- TgAb-positive to TgAb-negative) can produce Tg method discordances. These patients also serve to illustrate how disparate TgAb versus Tg responses can be associated with a poor prognosis and emphasize why a Tg measurement cannot be interpreted without knowing the TgAb status of the specimen (72). The de novo appearance of TgAb in the patient shown in Figure 9A either reflects a change in tumor-derived heterogeneity (secretion of a more immunogenic Tg molecule), or immune system recognition of tumor-derived Tg. In the patient shown in Figure 9B, TgAb was lost despite exacerbation of disease. This TgAb loss could be a response to the decrease in normally iodinated Tg antigen as normal remnant tissue was destroyed by RAI, at the same time as poorly iodinated (less immunogenic) tumor-derived Tg was rising with exacerbation of disease.

TgAb interference with Tg-RIA.

Tg-RIA methodology is based on Tg antigen (from serum or added ^{125}I-Tg tracer) competing for a low concentration of polyclonal (rabbit) Tg antibody (PAb). After incubation, the Tg-PAb complex is precipitated and the serum antigen concentration quantified as an inverse relationship to the ^{125}I-Tg in the precipitate. The first Tg-RIAs developed in the 1970s were very insensitive (~2 µg/L) (4,855). Over subsequent decades some Tg-RIAs have achieved first-generation functional sensitivity (FS = 0.5 µg/L) by using a 48-hour pre-incubation before adding a high specific activity ^{125}I-Tg tracer (856,857). The use of a high affinity PAb (858) coupled with a species-specific second antibody minimizes TgAb interference. Resistance to TgAb interference is evidenced by appropriately normal Tg-RIA values for TgAb-positive euthyroid controls (10) and detectable Tg-RIA for TgAb-positive DTC patients with structural disease (Figures 7B and 8) (31). The clinical performance of this Tg-RIA contrasts with IMA methods that report paradoxically undetectable serum Tg for some TgAb-positive normal euthyroid subjects (10) as well as TgAb-positive Graves' hyperthyroid patients (794) TgAb-positive patients with structural disease (Figures 7B and 8) (10). It should be noted that the propensity of TgAb to interfere with Tg-RIA determinations and cause underestimation (859) or overestimation (847,860) depends on not only the assay formulation but also patient-specific interactions between the endogenous Tg and TgAb in the specimen and the exogenous RIA reagents (848).

TgAb interference with Tg-IMA.

Non-competitive IMA methodology is based on a two-site reaction that involves antigen capture by a solid-phase monoclonal antibody (MAb) followed by addition of a labeled MAb that targets different epitopes of the captured antigen (377). TgAb interferes with IMA methodology by steric inhibition. Specifically, when the Tg epitope(s) necessary for binding to the IMA monoclonals are blocked by TgAb complexing, the 2-site reaction

cannot take place and the test antigen is reported as falsely low or undetectable. This mechanism involving epitope masking is supported by recovery studies (data not shown). Clinically, the Tg-IMA underestimation caused by TgAb interference is evident from paradoxically low/undetectable Tg-IMA seen for TgAb-positive normal controls (10), patients with Graves' hyperthyroidism (794) and DTC patients with active disease (Figures 7B and 8) (9,10,44,45,752,755,846,861-863). High Tg concentrations can overwhelm the TgAb binding capacity rendering Tg-IMA concentrations detectable and lessening the degree of interference (31,44). It follows that as Tg concentrations rise, more Tg is free, the influence of TgAb lessens and the discordance between Tg-IMA and Tg-RIA disappears (Figure 9) (31,44). Although some IMA methods have claimed to overcome TgAb interference by using monoclonal antibodies directed against specific epitopes not involved in thyroid autoimmunity (790,864), this approach does not overcome TgAb interferences in clinical practice, possibly because less restricted TgAb epitopes are more often associated with thyroid carcinoma than with autoimmune thyroid conditions (746,752,755,862,865).

TgAb Interference with Tg LC-MS/MS.

The new LC-MS/MS methods measure Tg as a Tg-specific peptide(s) generated after trypsinization of serum containing Tg-TgAb complexes (16,21,790,866). Currently LC-MS/MS methods only have first-generation functional sensitivity (FS ~ 0.5 µg/L) (19,20,799). Tg-LC-MS/MS methodology has been shown free from HAb/HAMA interferences (837), and is being promoted as being free from TgAb interference (19,20,799). However, the reliability of using LC-MS/MS to detect Tg in the presence of TgAb is currently questionable. A number of studies have reported that over 40 percent of TgAb-positive patients with structural disease have paradoxically undetectable Tg-LC-MS/MS values (31,799-801). The most recent study concluded that Tg-LC-MS/MS offers no diagnostic advantage over $^{2G-}$Tg-IMA when TgAb is present (801). This study also confirmed earlier observations (867) that the higher the TgAb concentration, the more likely that Tg-LC-MS/MS would be undetectable despite disease (801). An inverse relationship between TgAb concentration and Tg-LC-MS/MS detectability would be expected if the presence of TgAb enhanced Tg clearance in vivo (see above).

Use of the TgAb Trend as a Surrogate DTC Tumor-Marker (Table 2)

IIt is now generally recognized that the serum TgAb concentration can be used as a surrogate tumor-marker for TgAb-positive DTC patients in whom the reliability of Tg testing is compromised by TgAb interference [Figures 9 and 10] (24,32,45,72,743,761-764,766,796,868-874). Following successful surgery (± RAI treatment), TgAb concentrations typically decline progressively over subsequent months, and may become undetectable during the first few post-operative years as a result of reduced Tg antigen stimulation of the immune system (32,44,72,762-766,870,875). The time needed for TgAb to become undetectable is inversely related to TgAb concentration around the time of initial treatment (32). It should be noted that in the early post-operative period a significant percentage (~5%) of TgAb-negative patients develop transient de novo TgAb-positivity, presumably a response to Tg antigen released by surgical trauma (876). Such TgAb-negative to TgAb-positive conversions is one reason why Guidelines mandate that TgAb be measured with every Tg test (45,72). Transient rises in TgAb may be seen in response to the acute release of Tg following thyroid surgery (877,878), fine needle aspiration biopsy (879,880) or more chronically (months) in response to radiolytic damage following RAI treatment (759,761,881-884). Patients exhibiting a TgAb decline of more than 50 percent by the end of the first post-operative year have been shown to

have a low recurrence risk (762,874,876,885,886). In contrast, patients with persistent/recurrent disease may exhibit only a marginal TgAb decline, or have stable or rising TgAb (760,762,764,796,868,874). In fact, a rise, or de novo appearance of, TgAb, is an indication of persistent/recurrent disease (Figure 9A) (9,10,32,44,745,747,753,762-764,796,850,873,887). Because TgAb tests differ in sensitivity and specificity (44,45,745,753,888,889) (Figure 6), it is essential that serum TgAb concentrations be measured using the same manufacturers method and preferably the same laboratory (10,44,45,72,710,745,747,753,796,888,890,891).

Table 3--Clinical Significance of Changes in TgAb Concentrations:

1. Approximately 25 percent of DTC patients have TgAb detected before or within three months of surgery [713,760]. TgAb prevalence in DTC patients is double that of the general population [452,713].
2. Pre-operative TgAb-positivity is a risk factor for PTC in nodules with indeterminate cytology [892-895].
3. The post-operative trend in TgAb (measured with the same method and preferably by the same laboratory) can be a useful surrogate tumor marker. A declining TgAb trend is a good prognostic sign, whereas a stable or rising TgAb may indicate persistent/recurrent disease [24,32,45,72,743,762-764,766,796,868-870,872-874].
4. After successful treatment for DTC, TgAb (and Tg-RIA) concentrations typically fall more than 50% in first post-operative year and continue to fall in subsequent months-years, often becoming undetectable within a median time of four years [32,760,761,876].
5. With successful treatment of disease, serum Tg-RIA typically becomes undetectable (< 0.5 µg/L) before TgAb [32,896].
6. The time needed for a TgAb-positive patient to become TgAb-negative in response to successful treatment is proportional to the initial TgAb concentration, perhaps
7. Approximately 10 percent of TgAb-negative DTC patients develop TgAb-positivity during post-operative monitoring [850], necessitating TgAb measurement with every Tg test [45,72].
8. Most (75 %) TgAb-negative to TgAb-positive conversions are transient (months) and occur in response to the release of Tg antigen by surgical trauma [677,877], fine-needle biopsy [880] or RAI treatment [759,761,881-884].
9. Approximately 3 percent of TgAb-negative DTC patients exhibit a de novo TgAb appearance more than one year following thyroidectomy without an initiating factor (surgery, biopsy or RAI treatment). Such TgAb-negative to TgAb-positive conversions are often associated with the presence representing the long-lived memory of plasma cells [32,896,897]. of metastatic disease, such as illustrated in Figure 9A [763,887].
10. The de novo appearance of TgAb is typically associated with a rapid fall in Tg-IMA, often to undetectability, as a result of TgAb interference (Figure 9A). TgAb interference is less apparent when Tg-IMA is high before a TgAb appearance, because a high Tg concentration can saturate TgAb binding sites and reduce interference [31,44].
11. When serum Tg (RIA or IMA) persists after TgAb disappearance (~3% of cases) risk for disease remains (Figure 9B).

The Use of Serum Tg for Monitoring Patients with DTC

Over the past decade, the incidence of DTC has substantially risen partly as a result of detecting small thyroid nodules and micropapillary cancers (72,898-900) by ultrasound and

other anatomic imaging modalities used for nonthyroidal purposes (901-904). Although most DTC patients are rendered disease-free by their initial surgery, overall approximately 15 percent of patients experience recurrences and approximately 5 percent die from disease-related complications (790,905-908). A risk-stratified approach to diagnosis and treatment is now recommended by current guidelines (72,785,787,908). In most cases, persistent/recurrent disease is detected within the first five post-operative years, although recurrences can occur decades after initial surgery, necessitating life-long monitoring for recurrence (906,907). Since most patients have a low pre-test probability for disease, protocols for follow-up need a high negative predictive value (NPV) to eliminate unnecessary testing, as well as a high positive predictive value (PPV) for identifying patients with persistent/recurrent disease. Serum Tg testing is generally recognized more sensitive for detecting disease than diagnostic ^{131}I whole body scanning (909-912). It is recommended that biochemical testing (serum Tg+TgAb) be used in conjunction with periodic ultrasound (72,787,912,913). The persistent technical limitations of Tg and TgAb measurements necessitate close physician-laboratory cooperation.

The majority (~75%) of DTC patients have no Tg antibodies detected (713). In the absence of TgAb, four factors primarily influence the interpretation of serum Tg concentrations: (1) the mass of thyroid tissue present (normal tissue + tumor); (2) The intrinsic ability the tumor to secrete Tg; (3) the presence of any inflammation of, or injury to, thyroid tissue, such as following fine needle aspiration biopsy, surgery, RAI therapy or thyroiditis; and (4) the degree of TSH receptor stimulation by TSH, hCG or TSAb (24). In the presence of TgAb, interference with Tg measurement remains a problem necessitating a shift in focus from monitoring serum Tg as the primary tumor-marker, to monitoring the serum TgAb concentration as a surrogate tumor-marker.

Serum Tg Reference Ranges

The serum Tg reference range in adults approximates 3-40 µg/L (24,914). Serum Tg is higher in newborn infants but falls to the adult range after two years of age (915,916). However, because most Tg testing is made following surgery (thyroidectomy or lobectomy) for DTC, the Tg reference range is only relevant in the preoperative period. Tg methods can report up to 2-fold differences in numeric values for the same serum specimen (32). Between-method variability reflects differences in assay standardization and specificity for recognizing different serum Tg isoforms (10,58,825-827). When evaluating a thyroidectomized patient, the reference range of the assay should be adjusted for thyroid mass (thyroidectomy versus lobectomy) as well as the TSH status of the patient (24,882).

When using a $^{2G-}$Tg-IMA method standardized directly against the International Reference Preparation CRM-457, Tg should be detectable in all sera from TgAb-negative normal euthyroid subjects. Although the intra-individual serum Tg variability is relatively narrow (CV ~15%) (442,825), the Tg population reference range for TgAb-negative euthyroid subjects is broad, (~ 3 to 40 µg/L) (30,58,817,914). It follows that 1 gram of normal thyroid tissue results in ~1.0 µg/L Tg in the circulation under euthyroid TSH conditions (24,917,918). Following a lobectomy, euthyroid patients should be evaluated using a mass-adjusted reference range (1.5 - 20 µg/L). The range should be lowered a further 50 percent (0.75 - 10 µg/L) during TSH-suppression (24,882). After thyroidectomy, the typical 1-2 gram thyroid remnant (790,919) would be expected to produce a serum Tg below 2 µg/L (with a non-elevated TSH) (809,810). By this same reasoning, truly athyreotic patients would be expected to have no Tg detected irrespective of their TSH status (24).

Pre-operative Tg Measurement

An elevated Tg is a non-specific indicator of thyroid pathology and cannot be used to diagnose malignancy. However, a number of studies have reported that a Tg elevation, detected decades before a DTC diagnosis, is a risk factor for thyroid malignancy (920-926). This suggests that most thyroid cancers secrete Tg protein to an equal or greater degree than normal thyroid tissue, underscoring the importance of Tg as a DTC tumor marker (927). Approximately 50 percent of DTC patients have an elevated preoperative serum Tg, the highest serum Tg concentrations are seen in Follicular > Hurthle > Papillary (927). Up to one-third of tumors may be poor Tg secretors relative to tumor mass, especially tumors containing the BRAF mutation associated with reduced expression of Tg protein (928). Although current guidelines do not recommend routine pre-operative serum Tg measurement (72,782), some believe that a preoperative serum Tg (drawn before or more than two weeks after FNA) can provide information regarding the tumor's intrinsic ability to secrete Tg and thus aid with the interpretation of postoperative Tg changes (929,930). For example, knowing that a tumor is an inefficient Tg secretor could prompt a physician to focus more on anatomic imaging and less on postoperative Tg monitoring (928,931).

Post-operative Tg measurement - First Post-Operative Year

Because TSH exerts such a strong influence on serum Tg concentrations it is important to promptly initiate thyroid hormone therapy after surgery to establish a stable post-operative Tg baseline to begin biochemical monitoring (882). When surgery is followed by RAI treatment it may take time (months) to establish a stable Tg baseline because the Tg rises in response to TSH-stimulation may be augmented by Tg release from radiolytic damage. Short-term rhTSH stimulation is expected to produce an approximate 10-fold serum Tg elevation (412), whereas chronic endogenous TSH stimulation following thyroid hormone withdrawal results in an approximate 20-fold serum Tg rise (811). Serum Tg measurements performed as early as 6 to 8 weeks after thyroidectomy have been shown to have prognostic value - the higher the serum Tg the greater the risk of persistent/recurrent disease (813,895,932-940). Since the half-life of Tg in the circulation approximates 3 days (941), the acute Tg release resulting from the surgical injury and healing of surgical margins should largely resolve within the first six months, provided that post-operative thyroid hormone therapy prevents TSH from rising. Patients who receive RAI for remnant ablation may exhibit a slow Tg decline over subsequent years, presumably reflecting the long-term radiolytic destruction of remnant tissue (942,943).

The Tg secretion expected from the ~1 gram of normal remnant tissue left after thyroidectomy (790,919), is expected to result in a serum Tg concentration ~1.0 µg/L under non-elevated TSH conditions (24). It should be noted that many thyroidectomized patients have a low serum Tg (0.10 – 0.99 µg/L) detected by ^{2G}-Tg-IMA. A recent study found that in the first six months following thyroidectomy (without RAI treatment) disease-free PTC patients had a serum Tg nadir < 0.5 µg/L when TSH was maintained below 0.5 mIU/L (32,809,810). This is consistent with earlier studies using receiver operator curve (ROC) analysis that found a 6-week serum Tg of <1.0 µg/L, when measured during TSH suppression had a 98 percent negative predictive value (NPV) (although the positive predictive value (PPV) was only 43 percent) (940).

Long-term Tg monitoring (without TSH stimulation)

The higher the post-operative serum Tg measured without TSH stimulation, the greater the

risk for persistent/recurrent disease (813,932-940). If a stable TSH is maintained (≤0.5 mIU/L) (32,810), changes in the serum Tg will reflect changes in tumor mass. Under these conditions a rising Tg would be suspicious for tumor recurrence whereas a declining Tg levels suggests the absence or regression of disease. Now that sensitive $^{2G-}$Tg-IMA methods have become the standard of care, the *trend* in serum Tg, measured without TSH stimulation, is a more reliable indicator for disease status than using a fixed Tg cutoff value to assess disease (32,72,413,785,806,911,940,944-949). It is the degree of Tg elevation, not merely a "detectable" Tg, that is the risk factor for disease, since Tg “detectability” is merely determined by the assay FS (58,783,807,810,816). As with other tumor-markers such as Calcitonin, the Tg doubling time, measured without TSH stimulation, can be used as a prognostic marker that has an inverse relationship to mortality (809,949-956). However, between-method variability necessitates that the serum Tg trend be established using the same method, and preferably the same laboratory. One approach used to mitigate between-run imprecision and improve the reliability of establishing the Tg trend has been to measure the current specimen concurrently (in the same run) with an archived specimen from the patient, thereby eliminating run-to-run variability and increasing the confidence for detecting small changes in serum Tg (9,804).

Serum Tg responses to TSH Stimulation

The degree of tumor differentiation determines the presence and density of TSH receptors that in large part determine the magnitude of the serum Tg response to TSH stimulation (928,931,957,958). The serum Tg rise in response to endogenous TSH (thyroid hormone withdrawal) is twice that seen with short-term rhTSH stimulation (~20-fold versus ~10-fold, respectively) (593,758,811,819,959). RhTSH administration was adopted as a standardized approach for stimulating serum Tg into the measureable range of the insensitive first-generation tests (296,593,758,782,811,812). A consensus rhTSH-stimulated serum Tg cut-off of ≥2.0 µg/L, measured 72 hours after the second dose of rhTSH, was found to be a risk factor for disease (758,811). A "positive" rhTSH response had a higher NPV (>95 percent) than basal (unstimulated) Tg measured by an insensitive first-generation test (813,818,819,911,937,940,945,946,948,960,961). However, a negative rhTSH test did not guarantee the absence of tumor (811,819,960). Furthermore, the reliability of adopting a fixed numeric rhTSH-Tg cut-off value for a positive response is problematic, given that different methods can report different numeric Tg values for the same specimen (Figure 7) (10,58). Other variables include differences in the dose of rhTSH delivered relative to absorption from the injection site as well as the surface area and age of the patient (962-965). One critical variable is the TSH sensitivity of tumor tissues, with poorly differentiated tumors having blunted TSH-mediated Tg responses (928,958,966). When using a more sensitive $^{2G-}$Tg-IMA, an undetectable basal Tg (<0.10 µg/L) had a comparable NPV to rhTSH stimulation, and was rarely associated with a "positive" rhTSH-stimulated response (>2.0 µg/L) (58,296,416,792,806,807,814,816,967,968). This relationship would be expected, given the strong relationship between basal Tg and rhTSH-stimulated Tg values (296,816). As $^{2G-}$Tg-IMA methods have become the standard of care, it became apparent the rhTSH-stimulated Tg value provides no additional information over and above a basal Tg measured by 2nd generation assay (58,72,296,416,792,807,814,816,967).

One important use of rhTSH-stimulated Tg testing remains - that as a test for HAb/HAMA/RF interferences. Specifically when the Tg-IMA value appears clinically inappropriate (usually high), an absent rhTSH-stimulated Tg response suggests interference, and a blocker tube test is indicated (296). An alternative reason for an absent/blunted rhTSH-stimulated response is the presence of TgAb (816). A blunted rhTSH-

stimulated Tg response might be expected if TgAb enhanced the clearance of Tg-TgAb complexes (794,840,842,851).

Tg Measurement in FNA Needle Washouts (FNA-Tg)

Because Tg protein is tissue-specific, the detection of Tg in non-thyroidal tissues or fluids (such as pleural fluid) indicates the presence of metastatic thyroid cancer (779). Struma ovarii is the only (rare) condition in which the Tg in the circulation does not originate from the thyroid (969,970). Cystic thyroid nodules are commonly encountered in clinical practice, the large majority arising from follicular epithelium and the minority from parathyroid epithelium. A high concentration of Tg or parathyroid hormone (PTH) measured in the cyst fluid provides a reliable indicator of the tissue origin of the cyst (thyroid versus parathyroid, respectively), information critical for surgical decision-making (779,971). Lymph node metastases are found in up to 50 percent of patients with papillary cancers but only 20 percent of follicular cancers (972-975). High-resolution ultrasound has now become an important component of the protocols used for postoperative surveillance for recurrence (72,593,758). Although ultrasound characteristics are helpful for distinguishing benign reactive lymph nodes from those suspicious for malignancy, the finding of Tg in the needle washout of a lymph node biopsy has higher diagnostic accuracy than the ultrasound appearance (976-988). An FNA needle washout is now widely accepted as a useful adjunctive test for improving the diagnostic sensitivity of the cytological evaluation of a suspicious lymph node or thyroid mass (976-981,983,986,987,989). The current protocol for obtaining FNA-Tg samples recommends rinsing the biopsy needle in 1.0 mL of saline and sending this specimen to the laboratory for Tg analysis. In thyroidectomized patients a common cutoff value for a "positive" FNA-Tg result is 1.0 µg/L (980,987,990), however this cutoff can vary by assay and Institution (986,991). For investigations of suspicious lymph nodes in patients with an intact thyroid, a higher FNA-Tg cutoff value (~35-40 µg/L) is recommended (978,982). There is still controversy whether TgAb interferes with FNA-Tg analyses (979,992,993). It should be noted that when the serum TgAb concentration is very high and there is serum contamination of the FNA wash, the expected ~40-fold dilution in the wash fluid may be insufficient to lower TgAb below detection, and there is potential for TgAb to interfere with the FNA-Tg IMA test causing a falsely low/undetectable FNA-Tg result. The FNA needle wash-out procedure can also be used to detect Calcitonin in neck masses of patients with primary and metastatic medullary thyroid cancer (971,994-996), and FNA-PTH determinations may be useful for identifying lymph nodes arising from parathyroid tissue (971).

THYROID SPECIFIC MRNAS USED AS THYROID TUMOR MARKERS

Reverse transcription-polymerase chain reaction (RT-PCR) has been used to detect thyroid specific mRNAs (Tg, TSHR, TPO and NIS) in the peripheral blood of patients with DTC (918,997-999). Initial studies suggested that circulating Tg mRNA might be employed as a useful tumor marker for thyroid cancer, especially in TgAb-positive patients in whom Tg measurements were subject to assay interference (1000,1001). More recently, this approach has been applied to the detection of NIS, TPO and TSH receptor (TSHR) mRNA (1001-1005). Although some studies have suggested that thyroid specific mRNA measurements could be useful for cancer diagnosis and detecting recurrent disease, most studies have concluded that they offer no advantages over sensitive serum Tg measurements (918,1001,1006,1007). Further, the recent report of false positive Tg mRNA results in patients with congenital athyreosis (1008) suggests that Tg mRNA can arise as an assay artifact originating from non-thyroid tissues, or illegitimate transcription (1009,1010).

Conversely, false negative Tg mRNA results have also been observed in patients with documented metastatic disease (1011-1013). Although Tg, TSHR, NIS and TPO are generally considered “thyroid specific” proteins, mRNAs for these antigens have been detected in a number of non-thyroidal tissues such as lymphocytes, leukocytes, kidney, hepatocytes, brown fat and skin (625,1014-1019). Additional sources of variability in mRNA analyses relate to the use of primers that detect splice variants, sample-handling techniques that introduce variability, and difficulties in quantifying the mRNA detected (1006,1011). There is now a general consensus is that thyroid specific mRNA measurements presently lack the optimal specificity and practicality to be useful tumor markers (918,1001,1006). Finally, the growing number of reports of functional TSH receptors and Tg mRNA present in non-thyroidal tissues further suggests that these mRNA measurements will have limited clinical utility in the management of DTC in the future (625,1017-1019).

REFERENCES

1. Benotti J, Benotti N. Protein-bound iodine, total iodine and butanol extractable iodine by partial automation. Clin Chem 1963; 9:408-416
2. Chopra IJ. A radioimmunoassay for measurement of thyroxine in unextracted serum. J Clin Endocrinol Metab 1972; 34:938-947
3. Chopra IJ, Ho RS, Lam R. An improved radioimmunoassay of triiodothyronine in serum: Its application to clinical and physiological studies. J Lab Clin Med 1972; 80:729-?
4. Van Herle AJ, Uller RP, Matthews NL, Brown J. Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest 1973; 52:1320-1327
5. Nicoloff JT, Spencer CA. Clinical review 12: The use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab 1990; 71:553-558
6. Ekins R. Measurement of free hormones in blood. Endocrine Rev 1990; 11:5-46
7. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, Gray D, Nicoloff JT. Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab 1990; 70:453-460
8. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. Clinical Chemistry 1996; 42:141-145
9. Spencer CA, Takeuchi M, Kazarosyan M. Current Status and Performance Goals for Serum Thyroglobulin Assays. Clin Chem 1996; 42:164-173
10. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, Lopresti JS. Clinical Impact of Thyroglobulin (Tg) and Tg autoantibody Method Differences on the Management of patients with Differentiated Thyroid Carcinomas. J Clin Endocrinol Metab 2005; 90:5566-5575
11. Giovanella L, Treglia G, Sadeghi R, Trimboli P, Ceriani L, Verburg FA. Unstimulated high-sensitive thyroglobulin in follow-up of differentiated thyroid cancer patients: a meta-analysis. J Clin Endocrinol Metab 2014; 99:440-447
12. Thienpont LM, De Brabandere VI, Stockl D, De Leenheer AP. Development of a new method for the determination of thyroxine in serum based on isotope dilution gas chromatography mass spectrometry. Biological mass spectrometry 1994; 23:475-482
13. Thienpont LM, Fierens C, De Leenheer AP, Przywara L. Isotope dilution-gas chromatography/mass spectrometry and liquid chromatography/electrospray ionization-tandem mass spectrometry for the determination of triiodo-L-thyronine in serum. Rapid communications in mass spectrometry : RCM 1999; 13:1924-1931
14. Kahric-Janicic N, Soldin SJ, Soldin OP, West T, Gu J, Jonklaas J. Tandem mass spectrometry improves the accuracy of free thyroxine measurements during pregnancy. Thyroid 2007; 17:303-311

15. Thienpont LM, Beastall G, Christofides ND, Faix JD, Ieiri T, Jarrige V, Miller WG, Miller R, Nelson JC, Ronin C, Ross HA, Rottmann M, Thijssen JH, Toussaint B. Proposal of a candidate international conventional reference measurement procedure for free thyroxine in serum. Clin Chem Lab Med 2007; 45:934-936
16. Hoofnagle AN, Becker JO, Wener MH, Heinecke JW. Quantification of thyroglobulin, a low-abundance serum protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. Clin Chem 2008; 54:1796-1804
17. Jonklaas J, Kahric-Janicic N, Soldin OP, Soldin SJ. Correlations of free thyroid hormones measured by tandem mass spectrometry and immunoassay with thyroid-stimulating hormone across 4 patient populations. Clin Chem 2009; 55:1380-1388
18. van Deventer HE, Mendu DR, Remaley AT, Soldin SJ. Inverse log-linear relationship between thyroid-stimulating hormone and free thyroxine measured by direct analog immunoassay and tandem mass spectrometry. Clin Chem 2011; 57:122-127
19. Clarke NJ, Zhang Y, Reitz RE. A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med 2012; 60:1157-1163
20. Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem 2013; 59:982-990
21. Hoofnagle AN, Roth MY. Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry. J Clin Endocrinol Metab 2013; 98:1343-1352
22. Thienpont LM, Van Uytfanghe K, Poppe K, Velkeniers B. Determination of free thyroid hormones. Best Pract Res Clin Endocrinol Metab 2013; 27:689-700
23. Gounden V, Jonklaas J, Soldin SJ. A pilot study: subclinical hypothyroidism and free thyroid hormone measurement by immunoassay and mass spectrometry. Clin Chim Acta 2014; 430:121-124
24. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 2003; 13:3-126
25. Dufour DR. Laboratory tests of thyroid function: uses and limitations. Endocr Metab Clin North Am 2007; 36:155-169
26. Thienpont LM, Van Uytfanghe K, Marriot 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; 51:161-168
27. Yue B, Rockwood AL, Sandrock T, La'ulu SL, Kushnir MM, Meikle AW. Free thyroid hormones in serum by direct equilibrium dialysis and online solid-phase extraction--liquid chromatography/tandem mass spectrometry. Clin Chem 2008; 54:642-651
28. Jonklaas J, Sathasivam A, Wang H, Gu J, Burman KD, Soldin SJ. Total and free thyroxine and triiodothyronine: measurement discrepancies, particularly in inpatients. Clin Biochem 2014; 47:1272-1278
29. Thienpont LM, Van Uytfanghe K, Beastall G, Faix JD, Ieiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B. Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 1: thyroid-stimulating hormone. Clin Chem 2010; 56:902-911
30. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab 2005; 90:5566-5575
31. Spencer C, Petrovic I, Fatemi S, LoPresti J. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric

assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab 2014; 99:4589-4599
32. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies. Curr Opin Endocrinol Diabetes Obes 2014; 21:394-404
33. Giovanella L, Clark P, Chiovato L, Duntas LH, Elisei R, Feldt-Rasmussen U, Leenhardt L, Luster M, Schalin-Jantti C, Schott M, Seregni E, Rimmele H, Smit JW, Verburg FA. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. Eur J Endocrinol 2014; 171:R33-46
34. Robbins J, Cheng SY, Gershengorn MC, Glinoer D, Cahnmann HJ, Edelnoch H. Thyroxine transport proteins of plasma. Molecular properties and biosynthesis. Recent Prog Horm Res 1978; 34:477-519
35. Bartalena L, Robbins J. Thyroid hormone transport proteins. Clin Lab Med 1993; 13:583-598
36. Schussler GC. The thyroxine-binding proteins. Thyroid 2000; 10:141-149
37. Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin and thyrotropin receptor. Clin Chem 1996; 42:160-163
38. Kamijo K. TSH-receptor antibody measurement in patients with various thyrotoxicosis and Hashimoto's thyroiditis: a comparison of two two-step assays, coated plate ELISA using porcine TSH-receptor and coated tube radioassay using human recombinant TSH-receptor. Endocr J 2003; 50:113-116
39. Kamijo K. TSH-receptor antibodies determined by the first, second and third generation assays and thyroid-stimulating antibody in pregnant patients with Graves' disease. Endocr J 2007; 54:619-624
40. Ajjan RA, Weetman AP. Techniques to quantify TSH receptor antibodies. Nat Clin Pract Endocrinol Metab 2008; 4:461-468
41. Kamijo K, Murayama H, Uzu T, Togashi K, Olivo PD, Kahaly GJ. Similar clinical performance of a novel chimeric thyroid-stimulating hormone receptor bioassay and an automated thyroid-stimulating hormone receptor binding assay in Graves' disease. Thyroid 2011; 21:1295-1299
42. Giuliani C, Cerrone D, Harii N, Thornton M, Kohn LD, Dagia NM, Bucci I, Carpentieri M, Di Nenno B, Di Blasio A, Vitti P, Monaco F, Napolitano G. A TSHR-LH/CGR chimera that measures functional thyroid-stimulating autoantibodies (TSAb) can predict remission or recurrence in Graves' patients undergoing antithyroid drug (ATD) treatment. J Clin Endocrinol Metab 2012; 97:1080-1087
43. Diana T, Wuster C, Kanitz M, Kahaly GJ. Highly variable sensitivity of five binding and two bio-assays for TSH-receptor antibodies. J Endocrinol Invest 2016; 39:1159-1165
44. Spencer C, Petrovic I, Fatemi S. Current thyroglobulin autoantibody (TgAb) assays often fail to detect interfering TgAb that can result in the reporting of falsely low/undetectable serum Tg IMA values for patients with differentiated thyroid cancer. J Clin Endocrinol Metab 2011; 96:1283-1291
45. Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, Rimmele H, Seregni E, Smit JW, Theimer C, Giovanella L. Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. Thyroid 2013; 23:1211-1225
46. Hutfless S, Matos P, Talor MV, Caturegli P, Rose NR. Significance of prediagnostic thyroid antibodies in women with autoimmune thyroid disease. J Clin Endocrinol Metab 2011; 96:E1466-1471
47. Balucan FS, Morshed SA, Davies TF. Thyroid autoantibodies in pregnancy: their

role, regulation and clinical relevance. J Thyroid Res 2013; 2013:182472
48. Morshed SA, Davies TF. Graves' Disease Mechanisms: The Role of Stimulating, Blocking, and Cleavage Region TSH Receptor Antibodies. Horm Metab Res 2015; 47:727-734
49. Thienpont LM, Van Uytfanghe K, Van Houcke S. Standardization activities in the field of thyroid function tests: a status report. Clin Chem Lab Med 2010; 48:1577-1583
50. Thienpont LM, Van Uytfanghe K, Beastall G, Faix JD, Ieiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B. Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 2: free thyroxine and free triiodothyronine. Clin Chem 2010; 56:912-920
51. Van Houcke SK, Van Uytfanghe K, Shimizu E, Tani W, Umemoto M, Thienpont LM. IFCC international conventional reference procedure for the measurement of free thyroxine in serum: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group for Standardization of Thyroid Function Tests (WG-STFT)(1). Clin Chem Lab Med 2011; 49:1275-1281
52. Thienpont LM, Van Uytfanghe K, Van Houcke S, Das B, Faix JD, MacKenzie F, Quinn FA, Rottmann M, Van den Bruel A. A Progress Report of the IFCC Committee for Standardization of Thyroid Function Tests. Eur Thyroid J 2014; 3:109-116
53. Faix JD, Miller WG. Progress in standardizing and harmonizing thyroid function tests. Am J Clin Nutr 2016; 104 Suppl 3:913s-917s
54. Thienpont LM, Beastall G, Christofides ND, Faix JD, Ieiri T, Miller WG, Miller R, Nelson JC, Ross HA, Ronin C, Rottmann M, Thijssen JH, Toussaint B. Measurement of free thyroxine in laboratory medicine--proposal of measurand definition. Clin Chem Lab Med 2007; 45:563-564
55. Soldin OP, Soldin SJ. Thyroid hormone testing by tandem mass spectrometry. Clin Biochem 2011; 44:89-94
56. Steele BW, Wang E, Klee GG, Thienpont LM, Soldin SJ, Sokoll LJ, Winter WE, Fuhrman SA, Elin RJ. Analytic bias of thyroid function tests: analysis of a College of American Pathologists fresh frozen serum pool by 3900 clinical laboratories. Arch Pathol Lab Med 2005; 129:310-317
57. Beckett G, MacKenzie F. Thyroid guidelines - are thyroid-stimulating hormone assays fit for purpose? Ann Clin Biochem 2007; 44:203-208
58. Schlumberger M, Hitzel A, Toubert ME, Corone C, Troalen F, Schlageter MH, Claustrat F, Koscielny S, Taieb D, Toubeau M, Bonichon F, Borson-Chazot F, Leenhardt L, Schvartz C, Dejax C, Brenot-Rossi I, Torlontano M, Tenenbaum F, Bardet S, Bussiere F, Girard JJ, Morel O, Schneegans O, Schlienger JL, Prost A, So D, Archambeaud F, Ricard M, Benhamou E. Comparison of seven serum thyroglobulin assays in the follow-up of papillary and follicular thyroid cancer patients. J Clin Endocrinol Metab 2007; 92:2487-2495
59. Stockl D, Van Uytfanghe K, Van Aelst S, Thienpont LM. A statistical basis for harmonization of thyroid stimulating hormone immunoassays using a robust factor analysis model. Clin Chem Lab Med 2014; 52:965-972
60. Vesper HW, Myers GL, Miller WG. Current practices and challenges in the standardization and harmonization of clinical laboratory tests. Am J Clin Nutr 2016; 104 Suppl 3:907s-912s
61. Gharib H, Tuttle RM, Baskin HJ, Fish LH, Singer PA, McDermott MT. Subclinical thyroid dysfunction: A joint statement on management from the American Association of Clinical Endocrinologiss, the American Thyroid Association and the Endocrine Society. J Clin Endocrinol Metab 2005; 90
62. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 2006; 154:787-803

63. Negro R, Beck-Peccoz P, Chiovato L, Garofalo P, Guglielmi R, Papini E, Tonacchera M, Vermiglio F, Vitti P, Zini M, Pinchera A. Hyperthyroidism and pregnancy. An Italian Thyroid Association (AIT) and Italian Association of Clinical Endocrinologists (AME) joint statement for clinical practice. J Endocrinol Invest 2011; 34:225-231
64. Bahn RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, Laurberg P, McDougall IR, Montori VM, Rivkees SA, Ross DS, Sosa JA, Stan MN. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid 2011; 21:593-646
65. Takami H, Ito Y, Okamoto T, Yoshida A. Therapeutic strategy for differentiated thyroid carcinoma in Japan based on a newly established guideline managed by Japanese Society of Thyroid Surgeons and Japanese Association of Endocrine Surgeons. World J Surg 2011; 35:111-121
66. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S, Wiersinga W. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 2011; 21:1081-1125
67. De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, Eastman CJ, Lazarus JH, Luton D, Mandel SJ, Mestman J, Rovet J, Sullivan S. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2012; 97:2543-2565
68. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid 2012; 22:1200-1235
69. Pearce S.H.S, Brabant G, Duntas L.H, Monzani F, Peeters R.P, Razvi S, Wemeau J. 2013 ETA Guideline: Management of Subclinical Hypothyroidism. Eur Thyroid J 2013; 2:215-228
70. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid 2014; 24:1670-1751
71. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees S, Samuels M, Sosa JA, Stan MN, Walter M. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and other causes of Thyrotoxicosis. Thyroid 2016;
72. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016; 26:1-133
73. Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedus L, Paschke R, Valcavi R, Vitti P. AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS, AMERICAN COLLEGE OF ENDOCRINOLOGY, AND ASSOCIAZIONE MEDICI ENDOCRINOLOGI MEDICAL GUIDELINES FOR CLINICAL PRACTICE FOR THE DIAGNOSIS AND MANAGEMENT OF THYROID NODULES--2016 UPDATE. Endocr Pract 2016; 22:622-639
74. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman W, Laurberg P, Lazarus JH, Mandel SJ, Peeters R, Sullivan S. 2016 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease during Pregnancy and the Postpartum. Thyroid 2017;

75. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. Endocrinol Metab Clin North Am 2001; 30:265-289
76. Murphy BE, Pattee CJ, Gold A. Clinical evaluation of a new method for the determination of serum thyroxine. J Clin Endocrinol Metab 1966; 26:247-256
77. Islam KN, Ihara M, Dong J, Kasagi N, Mori T, Ueda H. Micro open-sandwich ELISA to rapidly evaluate thyroid hormone concentration from serum samples. Bioanalysis 2010; 2:1683-1687
78. Soldin OP, Tractenberg RE, Soldin SJ. Differences between measurements of T4 and T3 in pregnant and nonpregnant women using isotope dilution tandem mass spectrometry and immunoassays: are there clinical implications? Clin Chim Acta 2004; 347:61-69
79. Soukhova N, Soldin OP, Soldin SJ. Isotope dilution tandem mass spectrometric method for T4/T3. Clin Chim Acta 2004; 343:185-190
80. Thienpont LM, Van Uytfanghe K, Beastall G, Faix JD, Ieiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B. Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 3: total thyroxine and total triiodothyronine. Clin Chem 2010; 56:921-929
81. Matsuda M, Sakata S, Komaki T, Nakamura s, Kojima N, Takuno H, Miura K. Effect of 8-anilino-1-napthalene sulfonic acid (ANS) on the interaction between thyroid hormone and anti-thyroid hormone antibodies. Clin Chim Acta 1989; 185:139-146
82. Chopra IJ, Taing P, Mikus L. Direct determination of free triiodothyronine (T3) in undiluted serum by equilibrium dialysis/radioimmunoassay (RIA). Thyroid 1996; 6:255-259
83. Zucchelli GC, Pilo A, Chiesa MR, Piro MA. Progress report on a national quality-control survey of triiodothyronine and thyroxin assay. Clin Chem 1984; 30:395-398
84. Klee GG. Clinical usage recommendations and analytic performance goals for total and free triiodothyronine measurements. Clin Chem 1996; 42:155-159
85. Karapitta CD, Sotiroudis TG, Papadimitriou A, Xenakis A. Homogeneous enzyme immunoassay for triiodothyronine in serum. Clin Chem 2001; 47:569-574
86. Welsh KJ, Soldin SJ. DIAGNOSIS OF ENDOCRINE DISEASE: How reliable are free thyroid and total T3 hormone assays? Eur J Endocrinol 2016; 175:R255-r263
87. Martel J, Despres N, Ahnadi CE, Lachance JF, Monticello JE, Fink G, Ardemagni A, Banfi G, Tovey J, Dykes P, John R, Jeffery J, Grant AM. Comparative multicentre study of a panel of thyroid tests using different automated immunoassay platforms and specimens at high risk of antibody interference. Clin Chem Lab Med 2000; 38:785-793
88. 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; 51:2303-2311
89. Zhou Q, Li S, Li X, Wang W, Wang Z. Comparability of five analytical systems for the determination of triiodothyronine, thyroxine and thyroid-stimulating hormone. Clin Chem Lab Med 2006; 44:1363-1366
90. Van Houcke SK, Stepman HC, Thienpont LM, Fiers T, Stove V, Couck P, Anckaert E, Gorus F. Long-term stability of laboratory tests and practical implications for quality management. Clin Chem Lab Med 2013; 51:1227-1231
91. Abduljabbar M, Al Shahri A, Afifi A. Is umbilical cord blood total thyroxin measurement effective in newborn screening for hypothyroidism? J Med Screen 2009; 16:119-123
92. Cartwright D, O'Shea P, Rajanayagam O, Agostini M, Barker P, Moran C, Macchia E, Pinchera A, John R, Agha A, Ross HA, Chatterjee VK, Halsall DJ. Familial dysalbuminemic hyperthyroxinemia: a persistent diagnostic challenge. Clin Chem 2009; 55:1044-1046

93. Ross HA, de Rijke YB, Sweep FC. Spuriously high free thyroxine values in familial dysalbuminemic hyperthyroxinemia. Clin Chem 2011; 57:524-525
94. Pappa T, Ferrara AM, Refetoff S. Inherited defects of thyroxine-binding proteins. Best Pract Res Clin Endocrinol Metab 2015; 29:735-747
95. Zouwail SA, O'Toole AM, Clark PM, Begley JP. Influence of thyroid hormone autoantibodies on 7 thyroid hormone assays. Clin Chem 2008; 54:927-928
96. Giovanella L, Dorizzi RM, Keller F. A hypothyroid patient with increased free thyroid hormones. Clin Chem Lab Med 2008; 46:1650-1651
97. Massart C, Elbadii S, Gibassier J, Coignard V, Rasandratana A. Anti-thyroxine and anti-triiodothyronine antibody interferences in one-step free triiodothyronine and free thyroxine immunoassays. Clin Chim Acta 2009; 401:175-176
98. Loh TP, Leong SM, Loke KY, Deepak DS. Spuriously elevated free thyroxine associated with autoantibodies, a result of laboratory methodology: case report and literature review. Endocr Pract 2014; 20:e134-139
99. Surks MI, Sievert R. Drugs and thyroid function. N Engl J Med 1995; 333:1688-1694
100. Stockigt JR, Lim CF. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. Best Pract Res Clin Endocrinol Metab 2009; 23:753-767
101. Kundra P, Burman KD. The effect of medications on thyroid function tests. The Medical clinics of North America 2012; 96:283-295
102. Burr WA, Evans SE, Lee J, Prince HP, Ramsden DB. The ratio of thyroxine to thyroxine-binding globulin in the assessment of thyroid function. Clin Endocrinol (Oxf) 1979; 11:333-342
103. Glinoer D, Fernandez-Deville M, Ermans AM. Use of direct thyroxine-binding globulin measurement in the evaluation of thyroid function. J Endocrinol Invest 1978; 1:329-335
104. Lee RH, Spencer CA, Mestman JH, Miller EA, Petrovic I, Braverman LE, Goodwin TM. Free T4 immunoassays are flawed during pregnancy. Am J Obstet Gynecol 2009; 200:e1-6
105. Van den Berghe G. Endocrine changes in critically ill patients. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society 1999; 9 Suppl A:77-81
106. Wilson KL, Casey BM, McIntire DD, Cunningham FG. Is total thyroxine better than free thyroxine during pregnancy? Am J Obstet Gynecol 2014; 211:132.e131-136
107. Sriphrapradang C, Bhasipol A. Differentiating Graves' disease from subacute thyroiditis using ratio of serum free triiodothyronine to free thyroxine. Annals of medicine and surgery (2012) 2016; 10:69-72
108. Ito M, Toyoda N, Nomura E, Takamura Y, Amino N, Iwasaka T, Takamatsu J, Miyauchi A, Nishikawa M. Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with 3,5,3'-triiodothyronine-predominant Graves' disease. Eur J Endocrinol 2011; 164:95-100
109. Yoshimura Noh J, Momotani N, Fukada S, Ito K, Miyauchi A, Amino N. Ratio of serum free triiodothyronine to free thyroxine in Graves' hyperthyroidism and thyrotoxicosis caused by painless thyroiditis. Endocr J 2005; 52:537-542
110. Tagami T, Hagiwara H, Kimura T, Usui T, Shimatsu A, Naruse M. The incidence of gestational hyperthyroidism and postpartum thyroiditis in treated patients with Graves' disease. Thyroid 2007; 17:767-772
111. Whitworth AS, Midgley JE, Wilkins TA. A comparison of free T4 and the ratio of total T4 to T4-binding globulin in serum through pregnancy. Clin Endocrinol (Oxf) 1982; 17:307-313
112. Weeke J DL, Granlie K, Eskjaer Jensen S, Kjaerulff E, Laurberg P, Magnusson B,. A longitudinal study of serum TSH, and total and free iodothyronines during normal

pregnancy. Acta Endocrinol (Copenh) 1982; 101:531-537
113. Zhang X, Li C, Mao J, Wang W, Xie X, Peng S, Wang Z, Han C, Zhang X, Wang D, Fan C, Shan Z, Teng W. Gestation-Specific Changes in Maternal Thyroglobulin during Pregnancy and Lactation in an Iodine-Sufficient Region in China: A Longitudinal Study. Clin Endocrinol (Oxf) 2016;
114. Korevaar TI, Chaker L, Medici M, de Rijke YB, Jaddoe VW, Steegers EA, Tiemeier H, Visser TJ, Peeters RP. Maternal total T4 during the first half of pregnancy: physiologic aspects and the risk of adverse outcomes in comparison with free T4. Clin Endocrinol (Oxf) 2016; 85:757-763
115. Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregersen HE, Rasmussen OS, Larsen KR, Eriksen GM, Johannesen PL. Amelioration of some pregnancy-associated variations in thyroid function by iodine supplementation. J Clin Endocrinol Metab 1993; 77:1078-1083
116. Mandel SJ, Spencer CA, Hollowell JG. Are detection and treatment of thyroid insufficiency in pregnancy feasible? Thyroid 2005; 15:44-53
117. Ekins R. Measurement of free hormones in blood. Endocr Rev 1990; 11:5-46
118. Faix JD. Principles and pitfalls of free hormone measurements. Best Pract Res Clin Endocrinol Metab 2013; 27:631-645
119. Midgley JE. Direct and indirect free thyroxine assay methods: theory and practice. Clin Chem 2001; 47:1353-1363
120. Stockigt JR, Lim CF. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. Best Pract Res Clin Endocrinol Metab 2009; 23:753-767
121. Sarne DH, Refetoff S, Nelson JC, Linarelli LG. A new inherited abnormality of thyroxine-binding globulin (TBG-San Diego) with decreased affinity for thyroxine and triiodothyronine. J Clin Endocrinol Metab 1989; 68:114-119
122. Nelson JC, Tomei RT. Dependence of the thyroxin/thyroxin-binding globulin (TBG) ratio and the free thyroxin index on TBG concentrations. Clin Chem 1989; 35:541-544
123. Nelson JC, Wilcox BR, Pandian MR. Dependence of free thyroxine estimates obtained with equilibrium tracer dialysis on the concentration of thyroxine-binding globulin. Clin Chem 1992; 38:1294-1300
124. Nelson JC, Nayak SS, Wilcox RB. Variable underestimates by serum free thyroxine (T4) immunoassays of free T4 concentrations in simple solutions. J Clin Endocrinol Metab 1994; 79:1373-1375
125. Nelson JC, Wilcox RB. Analytical performance of free and total thyroxine assays. Clin Chem 1996; 42:146-154
126. Toldy E, Locsei Z, Szabolcs I, Bezzegh A, Kovács GL. Protein interference in thyroid assays: an in vitro study with in vivo consequences. Clin Chim Acta 2005; 353:93-104
127. Fritz KS, Wilcox RB, Nelson JC. Quantifying spurious free T4 results attributable to thyroxine-binding proteins in serum dialysates and ultrafiltrates. Clin Chem 2007; 53:985-988
128. Fillée C, Cumps J, Ketelslegers JM. Comparison of three free T4 (FT4) and free T3 (FT3) immunoassays in healthy subjects and patients with thyroid diseases and severe non-thyroidal illnesses. Clin Lab 2012; 58:725-736
129. Uchimura H, Nagataki S, Tabuchi T, Mizuno M, Ingbar SH. Measurements of free thyroxine: comparison of per cent of free thyroxine in diluted and undiluted sera. J Clin Endocrinol Metab 1976; 42:561-566
130. Iitaka M, Kawasaki S, Sakurai S, Hara Y, Kuriyama R, Yamanaka K, Kitahama s, Miura S, Kawakami Y, Katayama S. Serum substances that interfere with thyroid hormone assays in patients with chronic renal failure. Clin Endocrinol 1998; 48:739-746
131. Holm SS, Andreasen L, Hansen SH, Faber J, Staun-Olsen P. Influence of adsorption

and deproteination on potential free thyroxine reference methods. Clin Chem 2002; 48:108-114
132. Nelson JC, Weiss RM. The effect of serum dilution on free thyroxine (T4) concentration in the low T4 syndrome of nonthyroidal illness. J Clin Endocrinol Metab 1985; 61:239-246
133. Christofides ND, Midgley JE. Inaccuracies in free thyroid hormone measurement by ultrafiltration and tandem mass spectrometry. Clin Chem 2009; 55:2228-2229; author reply 2229-2230
134. Van Uytfanghe K, Stockl 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; 52:1817-1821
135. Sterling K, Brenner MA. Free thyroxine in human serum : simplified measurement with the aid of magnesium precipitation. J Clin Invest 1966; 45:153-163
136. Ellis SM, Ekins R. Direct measurement by radioimmunoassay of the free thyroid hormone concentrations in serum. Acta Endocrinol (Suppl) 1973; 177:106-
137. Nelson JC, Tomei RT. Direct determination of free thyroxin in undiluted serum by equilibrium dialysis/radioimmunoassay. Clin Chem 1988; 34:1737-1744
138. Wang YS, Hershman JM, Pekary AE. Improved ultrafiltration method for simultaneous measurement of free thyroxin and free triiodothyronine in serum. Clin Chem 1985; 31:517-522
139. Weeke J, Boye N, Orskov H. Ultrafiltration method for direct radioimmunoassay measurement of free thyroxine and free tri-iodothyronine in serum. Scand J Clin Lab Invest 1986; 46:381-389
140. Tikanoja SH, Liewendahl BK. New ultrafiltration method for free thyroxin compared with equilibrium dialysis in patients with thyroid dysfunction and nonthyroidal illness. Clin Chem 1990; 36:800-804
141. Soldin SJ, Soukhova N, Janicic N, Jonklaas J, Soldin OP. The measurement of free thyroxine by isotope dilution tandem mass spectrometry. Clin Chim Acta 2005; 358:113-118
142. Fritz KS, Wilcox RB, Nelson JC. A direct free thyroxine (T4) immunoassay with the characteristics of a total T4 immunoassay. Clin Chem 2007; 53:911-915
143. Romelli PB, Pennisi F, Vancheri L. Measurement of free thyroid hormones in serum by column adsorption chromatography and radioimmunoassay. J Endocrinol Invest 1979; 2:25-40
144. Ross HA, Benraad TJ. Is free thyroxine accurately measurable at room temperature? Clin Chem 1992; 38:880-886
145. Wilcox RB, Nelson JC, Tomei RT. Heterogeneity in affinities of serum proteins for thyroxine among patients with non-thyroidal illness as indicated by the serum free thyroxine response to serum dilution. Eur J Endocrinol 1994; 131:9-13
146. Wang R, Nelson JC, Weiss RM, Wilcox RB. Accuracy of free thyroxine measurements across natural ranges of thyroxine binding to serum proteins. Thyroid 2000; 10:31-39
147. Csako G, Zweig MH, Benson C, Ruddel M. On the albumin-dependence of measurements of free thyroxin. II. Patients with non-thyroidal illness. Clin Chem 1987; 33:87-92
148. Witherspoon LR, el Shami AS, Shuler SE, Neely H, Sonnemaker R, Gilbert SS, Alyea K. Chemically blocked analog assays for free thyronines. II. Use of equilibrium dialysis to optimize the displacement by chemical blockers of T4 analog and T3 analog from albumin while avoiding displacement of T4 and T3 from thyroxin-binding globulin. Clin Chem 1988; 34:17-23
149. Witherspoon LR, el Shami AS, Shuler SE, Neely H, Sonnemaker R, Gilbert SS, Alyea K. Chemically blocked analog assays for free thyronines. I. The effect of chemical

blockers on T4 analog and T4 binding by albumin and by thyroxin-binding globulin. Clin Chem 1988; 34:9-16
150. Csako G, Zweig MH, Glickman J, Ruddel M, Kestner J. Direct and indirect techniques for free thyroxin compared in patients with nonthyroidal illness. II. Effect of prealbumin, albumin, and thyroxin-binding globulin. Clin Chem 1989; 35:1655-1662
151. Weeke J, Orskov H. Ultrasensitive radioimmunoassay for direct determination of free triiodothyronine concentration in serum. Scand J Clin Lab Invest 1975; 35:237-244
152. Ekins RP. Ligand assays: from electrophoresis to miniaturized microarrays. Clin Chem 1998; 44:2015-2030
153. Levinson SS, Rieder SV. Parameters affecting a rapid method in which Sephadex is used to determine the percentage of free thyroxine in serum. Clin Chem 1974; 20:1568-1572
154. Holm SS, Hansen SH, Faber J, Staun-Olsen P. Reference methods for the measurement of free thyroid hormones in blood: evaluation of potential reference methods for free thyroxine. Clin Biochem 2004; 37:85-93
155. Hopley CJ, Stokes P, Webb KS, Baynham M. The analysis of thyroxine in human serum by an 'exact matching' isotope dilution method with liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry : RCM 2004; 18:1033-1038
156. Jonklaas J, Kahric-Janicic N, Soldin OP, Soldin SJ. Correlations of free thyroid hormones measured by tandem mass spectrometry and immunoassay with thyroid-stimulating hormone across 4 patient populations. Clin Chem 2009; 55:1380-1388
157. Soldin OP, Soldin SJ. Thyroid hormone testing by tandem mass spectrometry. Clin Biochem 2011; 44:89-94
158. Soldin OP, Jang M, Guo T, Soldin SJ. Pediatric reference intervals for free thyroxine and free triiodothyronine. Thyroid 2009; 19:699-702
159. Tractenberg RE, Jonklaas J, Soldin SJ. Agreement of immunoassay and tandem mass spectrometry in the analysis of cortisol and free t4: interpretation and implications for clinicians. Int J Anal Chem 2010; 2010
160. Larsen PR, Alexander NM, Chopra IJ, Hay ID, Hershman JM, Kaplan MM, Mariash CN, Nicoloff JT, Oppenheimer JH, Solomon DH, Surks MI. Revised nomenclature for tests of thyroid hormones and thyroid-related proteins in serum. (Committee on Nomenclature of the American Thyroid Association). Clin Chem 1987; 33:2114-2119
161. Litherland PGH, Bromage NR, Hall RA. Thyroxine binding globulin (TBG) and thyroxine binding prealbumin (TBPA) measurement, compared with the conventional T3 uptake in the diagnosis of thyroid disease. Clin Chim Acta 1982; 122:345-352
162. Burr WA, Ramsden DB, Evans SE, Hogan T, Hoffenberg R. Concentration of thyroxine-binding globulin: value of direct assay. Br Med J 1977; 1:485-488
163. Surks MI, DeFesi CR. Normal serum free thyroid hormone concentrations in patients treated with phenytoin or carbamazepine. A paradox resolved. Jama 1996; 275:1495-1498
164. Stevenson HP, Archbold GP, Johnston P, Young IS, Sheridan B. Misleading serum free thyroxine results during low molecular weight heparin treatment. Clin Chem 1998; 44:1002-1007
165. Berberoğlu M. Drugs and thyroid interaction. Pediatr Endocrinol Rev 2003; 1:251-256
166. Witherspoon LR, Shuler SE, Garcia MM. The triiodothyronine uptake test: an assessment of methods. Clin Chem 1981; 27:1272-1276
167. Witherspoon LR, Shuler SE, Gilbert S. Estimation of thyroxin, triiodothyronine, thyrotropin, free thyroxin, and triiodothyronine uptake by use of magnetic-particle solid phases. Clin Chem 1985; 31:413-419
168. Harpen MD, Lee WNP, Siegel JA, Greenfield MA. Serum binding of triiodothyronine:

theoretical and practical implications for in vitro triiodothyronine uptake. Endocrinol 1982; 110:1732-1739
169. Wilke TJ. Free thyroid hormone index, thyroid hormone/thyroxin-binding globulin ratio, triiodothyronine uptake, and thyroxin-binding globulin compared for diagnostic value regarding thyroid function. Clin Chem 1983; 29:74-79
170. Faix JD, Rosen HN, Velazquez FR. Indirect estimation of thyroid hormone-binding proteins to calculate free thyroxine index: comparison of nonisotopic methods that use labeled thyroxine ("T-uptake"). Clin Chem 1995; 41:41-47
171. Roberts RF, La'ulu SL, Roberts WL. Performance characteristics of seven automated thyroxine and T-uptake methods. Clin Chim Acta 2007; 377:248-255
172. Burr WA, Ramsden DB, Hoffenberg R. Hereditary abnormalities of thyroxine-binding globulin concentration. A study of 19 kindreds with inherited increase or decrease of thyroxine-binding globulin. The Quarterly journal of medicine 1980; 49:295-313
173. Jensen IW, Faber J. Familial dysalbuminemic hyperthyroxinemia: a review. J Royal Soc Med 1988; 81:34-37
174. Tokmakjian S, Haines M, Edmonds M. Free thyroxine measured by the Ciba Corning ACS-180 on samples from patients with familial dysalbuminemic hyperthyroxinemia. Clin Chem 1993; 39:1748-1749
175. Pannain S, Feldman M, Eiholzer U, Weiss RE, Scherberg NH, Refetoff S. Familial dysalbuminemic hyperthyroxinemia in a Swiss family caused by a mutant albumin (R218P) shows an apparent discrepancy between serum concentration and affinity for thyroxine. J Clin Endocrinol Metab 2000; 85:2786-2792
176. Hoshikawa S, Mori K, Kaise N, Nakagawa Y, Ito S, Yoshida K. Artifactually elevated serum-free thyroxine levels measured by equilibrium dialysis in a pregnant woman with familial dysalbuminemic hyperthyroxinemia. Thyroid 2004; 14:155-160
177. Pietras SM, Safer JD. Diagnostic confusion attributable to spurious elevation of both total thyroid hormone and thyroid hormone uptake measurements in the setting of autoantibodies: case report and review of related literature. Endocr Pract 2008; 14:738-742
178. van der Watt G, Haarburger D, Berman P. Euthyroid patient with elevated serum free thyroxine. Clin Chem 2008; 54:1239-1241
179. Surks MI, Hupart KH, Pan C, Shapiro LE. Normal free thyroxine in critical nonthyroidal illnesses measured by ultrafiltration of undiluted serum and equilibrium dialysis. J Clin Endocrinol Metab 1988; 67:1031-1038
180. Sapin R, Schlienger JL, Gasser F, Noel E, Lioure B, Grunenberger F, Goichot B, Grucker D. Intermethod discordant free thyroxine measurements in bone marrow-transplanted patients. Clin Chem 2000; 46:418-422
181. Mendel CM, Frost PH, Kunitake ST, Cavalieri RR. Mechanism of the heparin-induced increase in the concentration of free thyroxine in plasma. J Clin Endocrinol Metab 1987; 65:1259-
182. Hawkins RC. Furosemide interference in newer free thyroxine assays. Clin Chem 1998; 44:2550-2551
183. Schussler GC, Plager JE. Effect of preliminary purification of 131-Thyroxine on the determination of free thyroxine in serum. J Clin Endocrinol 1967; 27:242-250
184. Sophianopoulos J, Jerkunica I, Lee CN, Sgoutas D. An improved ultrafiltration method for free thyroxine and triiodothyronine in serum. Clin Chem 1980; 26:159-162
185. Sterling K, Hededus A. Free thyroxine in human serum. J Clin Invest 1962; 41:1031-1040
186. Oppenheimer JH, Squef R, Surks MI, Hauer H. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoresis techniques. J Clin Invest 1963; 42:1769-?
187. Snyder SM, Cavalieri RR, Ingbar SH. Simultaneous measurement of percentage free

thyroxine and triiodothyronine : comparison of equilibrium dialysis and sephadex chromatography. J Nucl Med 1976; 17:660-664
188. Ross HA. A dialysis rate method for the measurement of free triiodothyronine and steroid hormones in blood. Experimentia 1978; 34:538-539
189. Ross HA, Visser JW, der Kinderen PJ, Tertoolen JF, Thijssen JH. A comparative study of free thyroxine estimations. Ann Clin Biochem 1982; 19:108-113
190. Swinkels LM, Ross HA, Benraad TJ. A symmetric dialysis method for the determination of free testosterone in human plasma. Clin Chim Acta 1987; 165:341-349
191. Nelson JC, Weiss RM. The effects of serum dilution on free thyroxine (T4) concentration in the low T4 syndrome of nonthyroidal illness. J Clin Endocrinol Metab 1985; 61:239-246
192. Witherspoon LR, Shuler SE, Garcia MM, Zollinger LA. Effects of contaminant radioactivity on results of 125I-radioligand assay. Clin Chem 1979; 25:1975-1977
193. Bourcigaux N, Lepoutre-Lussey C, Guéchot J, Donadille B, Faugeron I, Ouzounian S, Christin-Maître S, Bouchard P, Duron F. Thyroid function at the third trimester of pregnancy in a Northern French population. Ann Endocrinol (Paris) 2010; 71:519-524
194. Midgley JE, Hoermann R. Measurement of total rather than free thyroxine in pregnancy: the diagnostic implications. Thyroid 2013; 23:259-261
195. Pantalone KM, Hatipoglu B, Gupta MK, Kennedy L, Hamrahian AH. Measurement of Serum Free Thyroxine Index May Provide Additional Case Detection Compared to Free Thyroxine in the Diagnosis of Central Hypothyroidism. Case reports in endocrinology 2015; 2015:965191
196. Ekins R. Free hormone assays. Nucl Med Commun 1993; 14:676-688
197. Bayer MF. Free thyroxine results are affected by albumin concentration and nonthyroidal illness. Clin Chim Acta 1983; 130:391-396
198. Liewendahl K, Tikanoja S, Helenius T, Valimaki M. Discrepancies between serum free triiodothyronine and free thyroxin as measured by equilibrium dialysis and analog radioimmunoassay in nonthyroidal illnesses. Clin Chem 1984; 30:760-762
199. Midgley JEM, Sheehan CP, Christofides ND, Fry JE, Browning D, Mandel R. Concentrations of free thyroxin and albumin in serum in nonthyroidal illness : assay artefacts and physiological influences. Clin Chem 1990; 36:765-771
200. Wilcox RB, Nelson JC. Counterpoint: legitimate and illegitimate tests of free-analyte assay function: we need to identify the factors that influence free-analyte assay results. Clin Chem 2009; 55:442-444
201. Ekins R. Validity of analog free thyroxin immunoassays. Clin Chem 1987; 33:2137-2152
202. Ekins R. Analytical measurements of free thyroxine. Clinics Lab Med 1993; 13:599-630
203. Bayer MF. Free thyroxine results are affected by albumin concentration and nonthyroidal illness. Clin Chim Acta 1983; 130:391-396
204. Ekins R, Edwards P, Jackson T, Geiscler D. Interpretation of labeled-analog free hormone assay. Clin Chem 1984; 30:491-493
205. Meinhold H, Wenzel KW. Comparative methodological studies with six commercial FT4 kits. NucCompact 1985; 16:317-320
206. Midgley JEM, Moon CR, Wilkins TA. Validity of analog free thyroxin immunoassays. Part II. Clin Chem 1987; 33:2145-2152
207. Zacharopoulou AD, Christofidis I, Kakabakos SE, Koupparis MA. Free thyroxine solid-analog immunoassays. investigation of the albumin effect on the antibody binding to immobilized thyroxine-protein conjugates. J Immunoassay Immunochem 2002; 23:95-105
208. Midgley JE, Christofides ND. Point: legitimate and illegitimate tests of free-analyte assay function. Clin Chem 2009; 55:439-441

209. Piketty ML, d'Herbomez M, Le Guillouzic D, Lebtahi R, Cosson E, Dumont A, Dilouya A, Helal BO. Clinical comparison of three labeled-antibody immunoassays of free triiodothyronine. Clin Chem 1996; 42:933-941
210. Sheehan CP, Christofides ND. One-step, labeled-antibody assay for measuring free thyroxin. II. Performance in a multicenter trial. Clin Chem 1992; 38:19-25
211. Sapin R, d'Herbomez M. Free thyroxine measured by equilibrium dialysis and nine immunoassays in sera with various serum thyroxine-binding capacities. Clin Chem Lab Med 2003; 49:1531-1535
212. Masika LS, Zhao Z, Soldin SJ. Is measurement of TT3 by immunoassay reliable at low concentrations? A comparison of the Roche Cobas 6000 vs. LC-MSMS. Clin Biochem 2016; 49:846-849
213. Livingston M, Birch K, Guy M, Kane J, Heald AH. No role for tri-iodothyronine (T3) testing in the assessment of levothyroxine (T4) over-replacement in hypothyroid patients. British journal of biomedical science 2015; 72:160-163
214. Persani L, Asteria C, Beck-Peccoz P. Dissociation between immunological and biological activities of circulating TSH. Exp Clin Endocrinol 1994; 102:38-48
215. Persani L, Ferretti E, Borgato S, Faglia G, Beck-Peccoz P. Circulating thyrotropin bioactivity in sporadic central hypothyroidism. J Clin Endocrinol Metab 2000; 85:3631-3635
216. Baskin HJ, Cobin RH, Duick DS, Gharib H, Guttler RB, Kaplan MM, Segal RL. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract 2002; 8:457-469
217. Lania A, Persani L, Beck-Peccoz P. Central hypothyroidism. Pituitary 2008; 11:181-186
218. Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab 2009; 23:793-800
219. Roelfsema F, Kok S, Kok P, Pereira AM, Biermasz NR, Smit JW, Frolich M, Keenan DM, Veldhuis JD, Romijn JA. Pituitary-hormone secretion by thyrotropinomas. Pituitary 2009; 12:200-210
220. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, Nicoloff JT. Applications of a new chemiluninometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab 1990; 70:453-460
221. Meier CA, Maisey MN, Lowry A, Müller J, Smith MA. Interindividual differences in the pituitary-thyroid axis influence the interpretation of thyroid function tests. CLIN Endocrinol (Oxf) 1993; 39:101-107
222. Benhadi N, Fliers E, Visser TJ, Reitsma JB, Wiersinga WM. Pilot study on the assessment of the setpoint of the hypothalamus-pituitary-thyroid axis in healthy volunteers. Eur J Endocrinol 2010; 162:323-329
223. Hoermann R, Eckl W, Hoermann C, Larisch R. Complex relationship between free thyroxine and TSH in the regulation of thyroid function. Eur J Endocrinol 2010; 162:1123-1129
224. Clark PM, Holder RL, Haque SM, Hobbs FD, Roberts LM, Franklyn JA. The relationship between serum TSH and free T4 in older people. J Clin Pathol 2012; 65:463-465
225. De Grande LA, Van Uytfanghe K, Thienpont LM. A Fresh Look at the Relationship between TSH and Free Thyroxine in Cross-Sectional Data. Eur Thyroid J 2015; 4:69-70
226. Brown SJ, Bremner AP, Hadlow NC, Feddema P, Leedman PJ, O'Leary PC, Walsh JP. The log TSH-free T4 relationship in a community-based cohort is nonlinear and is influenced by age, smoking and thyroid peroxidase antibody status. Clin Endocrinol (Oxf) 2016;
227. Hadlow NC, Rothacker KM, Wardrop R, Brown SJ, Lim EM, Walsh JP. The

relationship between TSH and free T(4) in a large population is complex and nonlinear and differs by age and sex. J Clin Endocrinol Metab 2013; 98:2936-2943
228. Chaker L, Korevaar TI, Medici M, Uitterlinden AG, Hofman A, Dehghan A, Franco OH, Peeters RP. Thyroid Function Characteristics and Determinants: The Rotterdam Study. Thyroid 2016; 26:1195-1204
229. Jones AM, Honour JW. Unusual results from immunoassays and the role of the clinical endocrinologist. Clin Endocrinol (Oxf) 2006; 64:234-244
230. Teti C, Nazzari E, Galletti MR, Mandolfino MG, Pupo F, Pesce G, Lillo F, Bagnasco M, Benvenga S. Unexpected Elevated Free Thyroid Hormones in Pregnancy. Thyroid 2016; 26:1640-1644
231. Soheilipour F, Fazilaty H, Jesmi F, Gahl WA, Behnam B. First report of inherited thyroxine-binding globulin deficiency in Iran caused by a known de novo mutation in SERPINA7. Molecular genetics and metabolism reports 2016; 8:13-16
232. Jin HY. Thyroxine binding globulin excess detected by neonatal screening. Annals of pediatric endocrinology & metabolism 2016; 21:105-108
233. Greenberg SM, Ferrara AM, Nicholas ES, Dumitrescu AM, Cody V, Weiss RE, Refetoff S. A novel mutation in the Albumin gene (R218S) causing familial dysalbuminemic hyperthyroxinemia in a family of Bangladeshi extraction. Thyroid 2014; 24:945-950
234. Osaki Y, Hayashi Y, Nakagawa Y, Yoshida K, Ozaki H, Fukazawa H. Familial Dysalbuminemic Hyperthyroxinemia in a Japanese Man Caused by a Point Albumin Gene Mutation (R218P). Japanese clinical medicine 2016; 7:9-13
235. Kragh-Hansen U, Minchiotti L, Coletta A, Bienk K, Galliano M, Schiott B, Iwao Y, Ishima Y, Otagiri M. Mutants and molecular dockings reveal that the primary L-thyroxine binding site in human serum albumin is not the one which can cause familial dysalbuminemic hyperthyroxinemia. Biochim Biophys Acta 2016; 1860:648-660
236. Cho YY, Song JS, Park HD, Kim YN, Kim HI, Kim TH, Chung JH, Ki CS, Kim SW. First Report of Familial Dysalbuminemic Hyperthyroxinemia With an ALB Variant. Annals of laboratory medicine 2017; 37:63-65
237. Sapin R, Gasser F, Schlienger JL. Familial dysalbuminemic hyperthyroxinemia and thyroid hormone autoantibodies: interference in current free thyroid hormone assays. Horm Res 1996; 45:139-141
238. Stockigt JR. Guidelines for diagnosis and monitoring of thyroid disease: nonthyroidal illness. Clin Chem 1996; 42:188-192
239. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. Thyroid 2014; 24:1456-1465
240. Kaptein EM, Macintyre SS, Weiner JM, Spencer CA. Free thyroxine estimates in nonthyroidal illness: comparison of eight methods. J Clin Endocrinol Metab 1981; 52:1073-1077
241. Drinka PJ, Nolten WE, Voeks S, Langer E, Carlson IH. Misleading elevation of the free thyroxine index in nursing home residents. Arch Pathol Lab Med 1991; 115:1208-1211
242. Becker DV, Bigos ST, Gaitan E, Morris JC, Rallison ML, Spencer CA, Sugawara M, Middlesworth LV, Wartofsky L. Optimal use of blood tests for assessment of thyroid function. JAMA 1993; 269:2736
243. Hamblin PS, Dyer SA, Mohr VS, Le Grand BA, Lim CF, Tuxen DV, Topliss DJ, Stockigt JR. Relationship between thyrotropin and thyroxine changes during recovery from severe hypothyroxinemia of critical illness. J Clin Endocrinol Metab 1986; 62:717-722
244. Spencer CA, Eigen A, Shen D, Duda M, Qualls S, Weiss S, Nicoloff JT. Specificity of sensitive assays of thyrotropin (TSH) used to screen for thyroid disease in hospitalized patients. Clin Chem 1987; 33:1391-1396
245. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. J Endocrinol 2015; 225:R67-81

246. Moura Neto A, Zantut-Wittmann DE. Abnormalities of Thyroid Hormone Metabolism during Systemic Illness: The Low T3 Syndrome in Different Clinical Settings. International journal of endocrinology 2016; 2016:2157583
247. Lem AJ, de Rijke YB, van Toor H, de Ridder MA, Visser TJ, Hokken-Koelega AC. Serum thyroid hormone levels in healthy children from birth to adulthood and in short children born small for gestational age. J Clin Endocrinol Metab 2012; 97:3170-3178
248. Chaler EA, Fiorenzano R, Chilelli C, Llinares V, Areny G, Herzovich V, Maceiras M, Lazzati JM, Mendioroz M, Rivarola MA, Belgorosky A. Age-specific thyroid hormone and thyrotropin reference intervals for a pediatric and adolescent population. Clin Chem Lab Med 2012; 50:885-890
249. La'ulu SL, Rasmussen KJ, Straseski JA. Pediatric Reference Intervals for Free Thyroxine and Free Triiodothyronine by Equilibrium Dialysis-Liquid Chromatography-Tandem Mass Spectrometry. Journal of clinical research in pediatric endocrinology 2016; 8:26-31
250. Soldin SJ, Cheng LL, Lam LY, Werner A, Le AD, Soldin OP. Comparison of FT4 with log TSH on the Abbott Architect ci8200: Pediatric reference intervals for free thyroxine and thyroid-stimulating hormone. Clin Chim Acta 2010; 411:250-252
251. Verburg FA, Kirchgässner C, Hebestreit H, Steigerwald U, Lentjes EG, Ergezinger K, Grelle I, Reiners C, Luster M. Reference ranges for analytes of thyroid function in children. Horm Metab Res 2011; 43:422-426
252. Loh TP, Sethi SK, Metz MP. Paediatric reference interval and biological variation trends of thyrotropin (TSH) and free thyroxine (T4) in an Asian population. J Clin Pathol 2015; 68:642-647
253. Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J 2014; 3:76-94
254. Chan S, Boelaert K. Optimal management of hypothyroidism, hypothyroxinaemia and euthyroid TPO antibody positivity preconception and in pregnancy. Clin Endocrinol (Oxf) 2015; 82:313-326
255. Furnica RM, Lazarus JH, Gruson D, Daumerie C. Update on a new controversy in endocrinology: isolated maternal hypothyroxinemia. J Endocrinol Invest 2015; 38:117-123
256. Noten AM, Loomans EM, Vrijkotte TG, van de Ven PM, van Trotsenburg AS, Rotteveel J, van Eijsden M, Finken MJ. Maternal hypothyroxinaemia in early pregnancy and school performance in 5-year-old offspring. Eur J Endocrinol 2015; 173:563-571
257. Min H, Dong J, Wang Y, Wang Y, Teng W, Xi Q, Chen J. Maternal Hypothyroxinemia-Induced Neurodevelopmental Impairments in the Progeny. Molecular neurobiology 2016; 53:1613-1624
258. Tong Z, Xiaowen Z, Baomin C, Aihua L, Yingying Z, Weiping T, Zhongyan S. The Effect of Subclinical Maternal Thyroid Dysfunction and Autoimmunity on Intrauterine Growth Restriction: A Systematic Review and Meta-Analysis. Medicine (Baltimore) 2016; 95:e3677
259. Dosiou C, Medici M. MANAGEMENT OF ENDOCRINE DISEASE: Isolated maternal hypothyroxinemia during pregnancy: Knowns and unknowns. Eur J Endocrinol 2016;
260. Agarwal MM, Dhatt GS, Punnose J, Bishawi B, Zayed R. Thyroid function abnormalities and antithyroid antibody prevalence in pregnant women at high risk for gestational diabetes mellitus. Gynecol Endocrinol 2006; 22:261-266
261. Cleary-Goldman J, Malone FD, Lambert-Messerlian G, Sullivan L, Canick J, Porter TF, Luthy D, Gross S, Bianchi DW, D'Alton ME. Maternal thyroid hypofunction and pregnancy outcome. Obstet Gynecol 2008; 112:85-92
262. Oguz A, Tuzun D, Sahin M, Usluogullari AC, Usluogullari B, Celik A, Gul K. Frequency of isolated maternal hypothyroxinemia in women with gestational diabetes mellitus in a moderately iodine-deficient area. Gynecol Endocrinol 2015; 31:792-795

263. Haddow JE, Craig WY, Neveux LM, Palomaki GE, Lambert-Messerlian G, Malone FD, D'Alton ME. Free Thyroxine During Early Pregnancy and Risk for Gestational Diabetes. PLoS One 2016; 11:e0149065
264. Yang S, Shi FT, Leung PC, Huang HF, Fan J. Low Thyroid Hormone in Early Pregnancy Is Associated with an Increased Risk of Gestational Diabetes Mellitus. J Clin Endocrinol Metab 2016:jc20161506
265. Panesar NS, Li CY, Rogers MS. Reference intervals for thyroid hormones in pregnant Chinese women. Ann Clin Biochem 2001; 38:329-332
266. Sapin R, D'Herbomez M, Schlienger JL. Free thyroxine measured with equilibrium dialysis and nine immunoassays decreases in late pregnancy. Clin Lab 2004; 50:581-584
267. Berta E, Samson L, Lenkey A, Erdei A, Cseke B, Jenei K, Major T, Jakab A, Jenei Z, Paragh G, Nagy EV, Bodor M. Evaluation of the thyroid function of healthy pregnant women by five different hormone assays. Pharmazie 2010; 65:436-439
268. Anckaert E, Poppe K, Van Uytfanghe K, Schiettecatte J, Foulon W, Thienpont LM. FT4 immunoassays may display a pattern during pregnancy similar to the equilibrium dialysis ID-LC/tandem MS candidate reference measurement procedure in spite of susceptibility towards binding protein alterations. Clin Chim Acta 2010; 411:1348-1353
269. Männistö T, Surcel HM, Ruokonen A, Vääräsmäki M, Pouta A, Bloigu A, Järvelin MR, Hartikainen AL, Suvanto E. Early pregnancy reference intervals of thyroid hormone concentrations in a thyroid antibody-negative pregnant population. Thyroid 2011; 21:291-298
270. Medici M, Korevaar TI, Visser WE, Visser TJ, Peeters RP. Thyroid function in pregnancy: what is normal? Clin Chem 2015; 61:704-713
271. Laurberg P, Andersen SL, Hindersson P, Nohr EA, Olsen J. Dynamics and Predictors of Serum TSH and fT4 Reference Limits in Early Pregnancy: A Study Within the Danish National Birth Cohort. J Clin Endocrinol Metab 2016; 101:2484-2492
272. Price A, Obel O, Cresswell J, Catch I, Rutter S, Barik S, Heller SR, Weetman AP. Comparison of thyroid function in pregnant and non-pregnant Asian and western Caucasian women. Clin Chim Acta 2001; 308:91-98
273. Dhatt GS, Jayasundaram R, Wareth LA, Nagelkerke N, Jayasundaram K, Darwish EA, Lewis A. Thyrotrophin and free thyroxine trimester-specific reference intervals in a mixed ethnic pregnant population in the United Arab Emirates. Clin Chim Acta 2006; 370:147-151
274. La'ulu SL, Roberts WL. Ethnic differences in first-trimester thyroid reference intervals. Clin Chem 2011; 57:913-915
275. Korevaar TI, Medici M, de Rijke YB, Visser W, de Muinck Keizer-Schrama SM, Jaddoe VW, Hofman A, Ross HA, Visser WE, Hooijkaas H, Steegers EA, Tiemeier H, Bongers-Schokking JJ, Visser TJ, Peeters RP. Ethnic differences in maternal thyroid parameters during pregnancy: the Generation R study. J Clin Endocrinol Metab 2013; 98:3678-3686
276. Antonangeli L, Maccherini D, Cavaliere R, Di Giulio C, Reinhardt B, Pinchera A, Aghini-Lombardi F. Comparison of two different doses of iodide in the prevention of gestational goiter in marginal iodine deficiency: a longitudinal study. Eur J Endocrinol 2002; 147:29-34
277. Moleti M, Di Bella B, Giorgianni G, Mancuso A, De Vivo A, Alibrandi A, Trimarchi F, Vermiglio F. Maternal thyroid function in different conditions of iodine nutrition in pregnant women exposed to mild-moderate iodine deficiency: an observational study. Clin Endocrinol 2011; 74:762-768
278. Shi X, Han C, Li C, Mao J, Wang W, Xie X, Li C, Xu B, Meng T, Du J, Zhang S, Gao Z, Zhang X, Fan C, Shan Z, Teng W. Optimal and safe upper limits of iodine intake for early pregnancy in iodine-sufficient regions: a cross-sectional study of 7190 pregnant women in

China. J Clin Endocrinol Metab 2015; 100:1630-1638
279. Mannisto T, Hartikainen AL, Vaarasmaki M, Bloigu A, Surcel HM, Pouta A, Jarvelin MR, Ruokonen A, Suvanto E. Smoking and early pregnancy thyroid hormone and anti-thyroid antibody levels in euthyroid mothers of the Northern Finland Birth Cohort 1986. Thyroid 2012; 22:944-950
280. Ashoor G, Kametas NA, Akolekar R, Guisado J, Nicolaides KH. Maternal thyroid function at 11-13 weeks of gestation. Fetal Diagn Ther 2010; 27:156-163
281. Pop VJ, Biondi B, Wijnen HA, Kuppens SM, Lvader H. Maternal thyroid parameters, body mass index and subsequent weight gain during pregnancy in healthy euthyroid women. Clin Endocrinol (Oxf) 2013; 79:577-583
282. Bestwick JP, John R, Maina A, Guaraldo V, Joomun M, Wald NJ, Lazarus JH. Thyroid stimulating hormone and free thyroxine in pregnancy: expressing concentrations as multiples of the median (MoMs). Clin Chim Acta 2014; 430:33-37
283. Han C, Li C, Mao J, Wang W, Xie X, Zhou W, Li C, Xu B, Bi L, Meng T, Du J, Zhang S, Gao Z, Zhang X, Yang L, Fan C, Teng W, Shan Z. High Body Mass Index Is an Indicator of Maternal Hypothyroidism, Hypothyroxinemia, and Thyroid-Peroxidase Antibody Positivity during Early Pregnancy. BioMed research international 2015; 2015:351831
284. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. Lancet 2000; 355:712-715
285. Ballieux BE, Weijl NI, Gelderblom H, van Pelt J, Osanto S. False-positive serum human chorionic gonadotropin (HCG) in a male patient with a malignant germ cell tumor of the testis: a case report and review of the literature. The oncologist 2008; 13:1149-1154
286. Henry N, Sebe P, Cussenot O. Inappropriate treatment of prostate cancer caused by heterophilic antibody interference. Nature clinical practice Urology 2009; 6:164-167
287. Georges A, Charrie A, Raynaud S, Lombard C, Corcuff JB. Thyroxin overdose due to rheumatoid factor interferences in thyroid-stimulating hormone assays. Clin Chem Lab Med 2011; 49:873-875
288. Bjerner J, Bolstad N, Piehler A. Belief is only half the truth--or why screening for heterophilic antibody interference in certain assays makes double sense. Ann Clin Biochem 2012; 49:381-386
289. Pishdad GR, Pishdad P, Pishdad R. The effect of glucocorticoid therapy on a falsely raised thyrotropin due to heterophilic antibodies. Thyroid 2013; 23:1657-1658
290. Marks V. False-positive immunoassay results: a multicenter survey of erroneous immunoassay results from assays of 74 analytes in 10 donors from 66 laboratories in seven countries. Clin Chem 2002; 48:2008-2016
291. Ellis MJ, Livesey JH. Techniques for identifying heterophile antibody interference are assay specific: study of seven analytes on two automated immunoassay analyzers. Clin Chem 2005; 51:639-641
292. Lewandowski KC, Dabrowska K, Lewinski A. Case report: When measured free T4 and free T3 may be misleading. Interference with free thyroid hormones measurements on Roche(R) and Siemens(R) platforms. Thyroid research 2012; 5:11
293. Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. Best Pract Res Clin Endocrinol Metab 2013; 27:647-661
294. Emerson JF, Ngo G, Emerson SS. Screening for interference in immunoassays. Clin Chem 2003; 49:1163-1169
295. Massart C, Corcuff JB, Bordenave L. False-positive results corrected by the use of heterophilic antibody-blocking reagent in thyroglobulin immunoassays. Clin Chim Acta 2008; 388:211-213
296. Spencer CA, Fatemi S, Singer P, Nicoloff JT, LoPresti JS. Serum Basal Thyroglobulin Measured by A 2nd Generation Assay Correlates with the Recombinant

Human TSH-Stimulated Thyroglobulin Response in Patients Treated for Differentiated Thyroid Cancer Thyroid 2010; 20:587-595
297. Koshida S, Asanuma K, Kuribayashi K, Goto M, Tsuji N, Kobayashi D, Tanaka M, Watanabe N. Prevalence of human anti-mouse antibodies (HAMAs) in routine examinations. Clin Chim Acta 2010; 411:391-394
298. Ismail AA. On detecting interference from endogenous antibodies in immunoassays by doubling dilutions test. Clin Chem Lab Med 2007; 45:851-854
299. Ross HA, Menheere PP, Thomas CM, Mudde AH, Kouwenberg M, Wolffenbuttel BH. Interference from heterophilic antibodies in seven current TSH assays. Ann Clin Biochem 2008; 45:616-618
300. Gulbahar O, Konca Degertekin C, Akturk M, Yalcin MM, Kalan I, Atikeler GF, Altinova AE, Yetkin I, Arslan M, Toruner F. A Case With Immunoassay Interferences in the Measurement of Multiple Hormones. J Clin Endocrinol Metab 2015; 100:2147-2153
301. Klee GG. Interferences in hormone immunoassays. Clin Lab Med 2004; 24:1-18
302. Sturgeon CM, Viljoen A. Analytical error and interference in immunoassay: minimizing risk. Ann Clin Biochem 2011; 48:418-432
303. King RI, Florkowski CM. How paraproteins can affect laboratory assays: spurious results and biological effects. Pathology 2010; 42:397-401
304. Imperiali M, Jelmini P, Ferraro B, Keller F, della Bruna R, Balerna M, Giovanella L. Interference in thyroid-stimulating hormone determination. Eur J Clin Invest 2010; 40:756-758
305. LeGatt DF, Higgins TN. Paraprotein interference in immunoassays. Ther Drug Monit 2015; 37:417
306. Covinsky M, Laterza O, Pfeifer JD, Farkas-Szallasi T, Scott MG. Lamda antibody to Esherichia coli produces false-positive results in multiple immunometric assays. Clin Chem 2000; 46:1157-1161
307. Luzzi VI, Scott MG, Gronowski AM. Negative thyrotropin assay interference associated with an IgGkappa paraprotein. Clin Chem 2003; 49:709-710
308. Glinoer D, de Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A, Kinthaert J, Lejeune B. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 1990; 71:276-287
309. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocrinol Rev 1997; 18:404-433
310. Ramsden DB, Sheppard MC, Sawers RS, Smith SC, Hoffenberg R. Serum free thyroxine concentrations in normal euthyroid subjects and ones with high serum thyroxine binding globulin concentration. Clin Chim Acta 1983; 130:211-217
311. Guven S, Alver A, Mentese A, Ilhan FC, Calapoglu M, Unsal MA. The novel ischemia marker 'ischemia-modified albumin' is increased in normal pregnancies. Acta Obstet Gynecol Scand 2009; 88:479-482
312. Cameron SJ, Hagedorn JC, Sokoll LJ, Caturegli P, Ladenson PW. Dysprealbuminemic hyperthyroxinemia in a patient with hyperthyroid graves disease. Clin Chem 2005; 51:1065-1069
313. DeCosimo DR, Fang SL, Braverman LE. Prevalence of familial dysalbuminemic hyperthyroxinemia in Hispanics. Ann Intern Med 1987; 107:780-781
314. Kricka LJ. Human anti-animal antibody interference in immunological assays. Clin Chem 1999; 45:942-956
315. Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta 2002; 325:1-15
316. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem 1998; 44:440-454
317. Mongolu S, Armston AE, Mozley E, Nasruddin A. Heterophilic antibody interference

affecting multiple hormone assays: Is it due to rheumatoid factor? Scand J Clin Lab Invest 2016; 76:240-242
318. Astarita G, Gutierrez S, Kogovsek N, Mormandi E, Otero P, Calabrese C, Alcaraz G, Vazquez A, Abalovich M. False positive in the measurement of thyroglobulin induced by rheumatoid factor. Clin Chim Acta 2015; 447:43-46
319. Weber TH, Käpyaho KI, Tanner P. Endogenous interference in immunoassays in clinical chemistry. A review. Scand J Clin Lab Invest 1990; 201:77-82
320. Bjerner J, Nustad K, Norum LF, Olsen KH, Børmer OP. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48:613-621
321. Ghosh S, Howlett M, Boag D, Malik I, Collier A. Interference in free thyroxine immunoassay. Eur J Intern Med 2008; 19:221-222
322. Preissner CM, Dodge LA, O'Kane DJ, Singh RJ, Grebe SK. Prevalence of heterophilic antibody interference in eight automated tumor marker immunoassays. Clin Chem 2005; 51:208-210
323. Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SK. Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. J Clin Endocrinol Metab 2003; 88:3069-3074
324. Verburg FA, Wäschle K, Reiners C, Giovanella L, Lentjes EG. Heterophile Antibodies Rarely Influence the Measurement of Thyroglobulin and Thyroglobulin Antibodies in Differentiated Thyroid Cancer Patients. Horm Metab Res 2010; 42:736-739
325. Bjerner J, Olsen KH, Bormer OP, Nustad K. Human heterophilic antibodies display specificity for murine IgG subclasses. Clin Biochem 2005; 38:465-472
326. Choi WW, Srivatsa S, Ritchie JC. Aberrant thyroid testing results in a clinically euthyroid patient who had received a tumor vaccine. Clin Chem 2005; 51:673-675
327. Monchamp T, Chopra IJ, Wah DT, Butch AW. Falsely elevated thyroid hormone levels due to anti-sheep antibody interference in an automated electrochemiluminescent immunoassay. Thyroid 2007; 17:271-275
328. Chin KP, Pin YC. Heterophile antibody interference with thyroid assay. Intern Med 2008; 47:2033-2037
329. Tan MJ, Tan F, Hawkins R, Cheah WK, Mukherjee JJ. A hyperthyroid patient with measurable thyroid-stimulating hormone concentration - a trap for the unwary. Ann Acad Med Singapore 2006; 35:500-503
330. Ross HA, Menheere PP, Thomas CM, Mudde AH, Kouwenberg M, Wolffenbuttel BH. Interference from heterophilic antibodies in seven current TSH assays. Ann Clin Biochem 2008; 45:616
331. Giovanella L, Ghelfo A. Undetectable serum thyroglobulin due to negative interference of heterophile antibodies in relapsing thyroid carcinoma. Clin Chem 2007; 53:1871-1872
332. Giovanella L, Keller F, Ceriani L, Tozzoli R. Heterophile antibodies may falsely increase or decrease thyroglobulin measurement in patients with differentiated thyroid carcinoma. Clin Chem Lab Med 2009; 47:952-954
333. Petrovic. I, Mandel. S, Fatemi. S, J LoPresti, C Spencer. Heterophile Antibodies (HAb/HAMA) Interfere with Automated TgAb IMA Tests. Thyroid 2016; 26:A120
334. Papapetrou PD, Polymeris A, Karga H, Vaiopoulos G. Heterophilic antibodies causing falsely high serum calcitonin values. J Endocrinol Invest 2006; 29:919-923
335. Kim JM, Chung KW, Kim SW, Choi SH, Min HS, Kim JN, Won WJ, Kim SK, Lee JI, Chung JH, Kim SW. Spurious hypercalcitoninemia in patients with nodular thyroid disease induced by heterophilic antibodies. Head Neck 2010; 32:68-75
336. Giovanella L, Suriano S. Spurious hypercalcitoninemia and heterophilic antibodies in patients with thyroid nodules. Head Neck 2011; 33:95-97
337. Bories PN, Broutin A, Delette A, Labelle G, Popovici T. Comparison of the Elecsys

calcitonin assay with the Immulite 1000 assay. Describing one case with heterophilic antibody interference. Clin Chem Lab Med 2016; 54:e45-47
338. Nakano K, Yasuda K, Shibuya H, Moriyama T, Kahata K, Shimizu C. Transient human anti-mouse antibody generated with immune enhancement in a carbohydrate antigen 19-9 immunoassay after surgical resection of recurrent cancer. Ann Clin Biochem 2016; 53:511-515
339. Sapin R, Agin A, Gasser F. Efficacy of a new blocker against anti-ruthenium antibody interference in the Elecsys free triiodothyronine assay. Clin Chem Lab Med 2007; 45:416-418
340. Ando T, Yasui J, Inokuchi N, Usa T, Ashizawa K, Kamihara S, Eguchi K. Non-specific activities against ruthenium crosslinker as a new cause of assay interference in an electrochemilluminescent immunoassay. Intern Med 2007; 46:1225-1229
341. Buijs MM, Gorgels JP, Endert E. Interference by antiruthenium antibodies in the Roche thyroid-stimulating hormone assay. Ann Clin Biochem 2011; 48:276-281
342. Ohba K, Noh JY, Unno T, Satoh T, Iwahara K, Matsushita A, Sasaki S, Oki Y, Nakamura H. Falsely elevated thyroid hormone levels caused by anti-ruthenium interference in the Elecsys assay resembling the syndrome of inappropriate secretion of thyrotropin. Endocr J 2012; 59:663-667
343. Gessl A, Blueml S, Bieglmayer C, Marculescu R. Anti-ruthenium antibodies mimic macro-TSH in electrochemiluminescent immunoassay. Clin Chem Lab Med 2014; 52:1589-1594
344. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. Arch Pathol Lab Med 2013; 137:1141-1146
345. Vos MJ, Rondeel JM, Mijnhout GS, Endert E. Immunoassay interference caused by heterophilic antibodies interacting with biotin. Clin Chem Lab Med 2016;
346. Kwok JS, Chan IH, Chan MH. Biotin interference on TSH and free thyroid hormone measurement. Pathology 2012; 44:278-280
347. Wijeratne NG, Doery JC, Lu ZX. Positive and negative interference in immunoassays following biotin ingestion: a pharmacokinetic study. Pathology 2012; 44:674-675
348. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious Graves' Disease Due to Biotin Immunoassay Interference-A Case and Review of the Literature. J Clin Endocrinol Metab 2016; 101:3251-3255
349. Pedersen IB, P L. Biochemical Hyperthyroidism in a Newborn Baby Caused by Assay Interaction from Biotin Intake. Eur Thyroid J 2016; 5:212-215
350. Barbesino G. Misdiagnosis of Graves' Disease with Apparent Severe Hyperthyroidism in a Patient Taking Biotin Megadoses. Thyroid 2016; 26:860-863
351. Lazarus JH, John R, Ginsberg J, Hughes IA, Shewring G, Smith BR, Woodhead JS, Hall R. Transient neonatal hyperthyrotrophinaemia: a serum abnormality due to transplacentally acquired antibody to thyroid stimulating hormone. British medical journal (Clinical research ed) 1983; 286:592-594
352. Newman JD, Bergman PB, Doery JC, Balazs ND. Factitious increase in thyrotropin in a neonate caused by a maternally transmitted interfering substance. Clin Chem 2006; 52:541-542
353. Benvenga S, Ordookhani A, Pearce EN, Tonacchera M, Azizi F, Braverman LE. Detection of circulating autoantibodies against thyroid hormones in an infant with permanent congenital hypothyroidism and her twin with transient congenital hypothyroidism: possible contribution of thyroid hormone autoantibodies to neonatal and infant hypothyroidism. J Pediatr Endocrinol Metab 2008; 21:1011-1020
354. Rix M, Laurberg P, Porzig C, Kristensen SR. Elevated thyroid-stimulating hormone level in a euthyroid neonate caused by macro thyrotropin-IgG complex. Acta paediatrica (Oslo, Norway : 1992) 2011; 100:e135-137

355. Sakata S, Matsuda M, Ogawa T, Takuno H, Matsui I, Sarui H, Yasuda K. Prevalence of thyroid hormone autoantibodies in healthy subjects. Clin Endocrinol (Oxf) 1994; 41:365-370
356. Benvenga S, Trimarchi F. Thyroid hormone autoantibodies in Hashimoto's thyroiditis: often transient but also increasingly frequent. Thyroid 2003; 13:995-996; author reply 996
357. Gangemi S, Saitta S, Lombardo G, Patafi M, Benvenga S. Serum thyroid autoantibodies in patients with idiopathic either acute or chronic urticaria. J Endocrinol Invest 2009; 32:107-110
358. Colucci R, Lotti F, Dragoni F, Arunachalam M, Lotti T, Benvenga S, Moretti S. High prevalence of circulating autoantibodies against thyroid hormones in vitiligo and correlation with clinical and historical parameters of patients. The British journal of dermatology 2014; 171:786-798
359. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA. Clinical Practice Guidelines for Hypothyroidism in Adults: Co-sponsored by American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract 2012; 18:1-207
360. Cobb WE, Lamberton RP, Jackson IMD. Use of a rapid, sensitive immunoradiometric assay for Thyrotropin to distinguish normal from hyperthyroid subjects. Clin Chem 1984; 30:1558-1560
361. Piketty ML, Talbot JN, Askienazy S, Milhaud G. Clinical significance of a low concentration of Thyrotropin" five immunometric "kit" assays compared. Clin Chem 1987; 33:1237-1241
362. Spencer CA, Nicoloff JT. Improved radioimmunoassay for human TSH. Clin Chim Acta 1980; 108:415-424
363. Mori T, Imura H, Bito S, Ikekubo K, Inoue S, Hashida S, Ishikawa E, Ogawa H. Clinical usefulness of a highly sensitive enzyme-immunoassay of TSH. Clin Endocrinol 1987; 27:1-10
364. Evans MC. Ten commercial kits compared for assay of Thyrotropin in the normal and thyrotoxic range. Clin Chem 1988; 34:123-127
365. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest 1960; 39:1157-1175
366. Utiger RD. Radioimmunoassay of human plasma thyrotropin. J Clin Invest 1965; 44:1277-1286
367. Odell WD, Wilber JF, Paul WE. Radioimmunoassay of thyrotropin in human serum. J Clin Endocrinol 1965; 25:1179-1188
368. Patel YC, Burger HG, Hudson B. Radioimmunoassay of serum thyrotropin: Sensitivity and Specificity. J Clin Endocrinol 1971; 33:768-774
369. Hall R, Amos J, Ormston BJ. Radioimmunoassay of human serum thyrotropin. Br Med J 1971; 1:582-?
370. Hershman JM. Utility of the radioimmunoassay of serum thyrotropin in man. Ann Intern Med 1971; 74:481-490
371. Haigler ED, Pittman JA, Hershman JM, Baugh CM. Direct evaluation of pituitary Thyrotropin reserve utilizing synthetic Thyrotropin Releasing Hormone. J Clin Endocrinol Metab 1971; 33:573-581
372. Hall R, Ormston BJ, Besser GM, Cryer RJ, McKendrick M. The thyrotropin-releasing hormone test in diseases of the pituitary and hypothalamus. Lancet 1972; i:759-763
373. Evans M, Croxson MS, Wilson TM, Ibbertson HK. The screening of patients with suspected thyrotoxicosis using a sensitive TSH radioimmunoassay. Clin Endocrinol 1985; 22:445-451
374. Spencer C, Schwarzbein D, Guttler R, LoPresti J, Nicoloff J. TRH stimulation test responses employing 3rd. and 4th. generation TSH assay technology. J Clin Endocrinol

Metab 1993; 76:494-499
375. Atmaca H, Tanriverdi F, Gokce C, Unluhizarci K, Kelestimur F. Do we still need the TRH stimulation test? Thyroid 2007; 17:529-533
376. Duntas LH, Emerson CH. On the Fortieth Anniversary of Thyrotropin-Releasing Hormone: The Hormone that Launched a New Era. Thyroid 2009; 19:1299-1301
377. Miles LEM, Hales CN. Labeled antibodies and immunological assay systems. Nature 1968; 219:186-189
378. Kohler G, Milstein C. Continuous culture of fused cells secreting specific antibody of predefined specificity. Nature 1975; 256:495-497
379. Winter G, Milstein C. Man-made antibodies. Nature 1991; 349:293-299
380. Seth J, Kellett HA, Caldwell G, Sweeting VM, Beckett GJ, Gow SM, Toft AD. A sensitive immunoradiometric assay for serum thyroid stimulating hormone: a replacement for the thyrotropin releasing test. Br Med J 1984; 289:1334-1336
381. Bernutz C, Horn K, Konig A, Pickardt CR. Advantages of sensitive assays for Thyrotropin in the diagnosis of thyroid disorders. J Clin Chem Clin Biochem 1985; 23:851-856
382. Wiersinga WM, Endert E, Trip MD, Verhaest-de Jong N. Immunoradiometric assay of Thyrotropin in plasma: its value in predicting response to thyroliberin stimulation and assessing thyroid function in amiodarone-treated patients. Clin Chem 1986; 32:433-436
383. Bassett F, Eastman CJ, Ma G, Maberly GF, Smith HC. Diagnostic value of Thyrotropin concentrations in serum as measured by a sensitive immunoradiometric assay. Clin Chem 1986; 32:461-464
384. Martino E, Bambini G, Bartalena L, Mammoli C, Aghini-Lombardi F, Baschieri L, Pinchera A. Human serum thyrotropin measurement by ultrasensitive immunoradiometric assay as a first-line test in the evaluation of thyroid function. Clin Endocrinol 1986; 24:141-148
385. Thornes HM, McLeod DT, Carr D. Economy and efficiency in routine thyroid-function testing: use of a sensitive immunoradiometric assay for thyrotropin in a General Hospital Laboratory. Clin Chem 1987; 33:1636-1638
386. Klee GG, Hay ID. Assessment of sensitive Thyrotropin assays for an expanded role in thyroid function testing: proposed criteria for analytic performance and clinical utility. J Clin Endocrinol Metab 1987; 64:461-471
387. Kasagi K, Kousaka T, Misaki T, Iwata M, Alam MS, Konishi J. Comparison of serum thyrotrophin concentrations determined by a third generation assay in patients with various types of overt and subclinical thyrotoxicosis. Clin Endocrinol 1999; 50:185-189
388. Taimela E, Tahtela R, Koskinen P, Nuutila P, Forsstrom J, Taimela S, Karonen SL, Valimaki M, Irjala K. Ability of two new thyrotropin (TSH) assays to separate hyperthyroid patients from euthyroid patients with low TSH. Clin Chem 1994; 40:101-105
389. Ross DS, Daniels GH, Gouveia D. The use and limitations of a chemiluminescent thyrotropin assay as a single thyroid function test in an out-patient endocrine clinic. J Clin Endocrinol Metab 1990; 71:764-769
390. Hay ID, Bayer MF, Kaplan MM, Klee GG, Larsen PR, Spencer CA. American Thyroid Association Assessment of Current Free Thyroid Hormone and Thyrotropin Measurements and Guidelines for Future Clinical Assays. Clin Chem 1991; 37:2002 - 2008
391. Spencer CA, Takeuchi M, Kazarosyn M, MacKenzie F, Beckett GJ, Wilkinson E. Interlaboratory/intermethod differences in functional sensitivity of immunometric assays for thyrotropin (TSH): impact on reliability of measurement of subnormal concentration. Clin Chem 1995; 41:367-374
392. Vogeser M, Weigand M, Fraunberger P, Fischer H, Cremer P. Evaluation of the ADVIA Centaur TSH-3 assay. Clin Chem Lab Med 2000; 38:331-334
393. Hendriks HA, Kortlandt W, Verweij WM. Standardized comparison of processing

capacity and efficiency of five new-generation immunoassay analyzers. Clin Chem 2000; 46:105-111
394. Thienpont LM, Van Houcke SK. Traceability to a common standard for protein measurements by immunoassay for in-vitro diagnostic purposes. Clin Chim Acta 2010; 411:2058-2061
395. Owen WE, Gantzer ML, Lyons JM, Rockwood AL, Roberts WL. Functional sensitivity of seven automated thyroid stimulating hormone immunoassays. Clin Chim Acta 2011; 412:2336-2339
396. Sarkar R. TSH Comparison Between Chemiluminescence (Architect) and Electrochemiluminescence (Cobas) Immunoassays: An Indian Population Perspective. Indian journal of clinical biochemistry : IJCB 2014; 29:189-195
397. Nicoloff J SC. Use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab 1990; 71:553-558
398. Fraser CG, Browning MCK. A plea for abandonment of the term "Highly Sensitive" for Thyrotropin assays. Clin Chem 1986; 32:569-570
399. Caldwell G, Gow SM, Sweeting VM, Beckett GJ, Seth J, Toft AD. Value and limitations of a highly sensitive immunoradioimetric assay for Thyrotropin in the study of thyrotroph function. Clin Chem 1987; 33:303-305
400. Hildebrandt L, White GH. Is a "Super-Sensitive" thyrotropin assay ("Magic Lite") of more diagnostic value? Clin Chem 1988; 34:2584-2585
401. Surmont DWA, Alexandre JA. Adaptations to keep a Thyrotropin immunoradiometric assay "supersensitive" with automated pipetting. Clin Chem 1988; 34:370-371
402. Gaines-Das RE, Brettschneider H, Bristow AF. International Federation of Clinical Chemistry. The effects of common matrices for assay standards on the performance of "ultra sensitive" immunometric assays for TSH. Clin Chim Acta 1991; 203:S5-16
403. Sadler WA, Murray LM, Turner JG. What does "functional sensitivity" mean? Clin Chem 1996; 42:2051
404. Giovanella L, Feldt-Rasmussen U, Verburg FA, Grebe SK, Plebani M, Clark PM. Thyroglobulin measurement by highly sensitive assays: focus on laboratory challenges. Clin Chem Lab Med 2015; 53:1301-1314
405. Reix N, Massart C, Gasser F, Heurtault B, Agin A. Should functional sensitivity of a new thyroid stimulating hormone immunoassay be monitored routinely? The ADVIA Centaur TSH3-UL assay experience. Clin Biochem 2012; 45:1260-1262
406. Rigo RB, Panyella MG, Bartolome LR, Ramos PA, Soria PR, Navarro MA. Variations observed for insulin concentrations in an interlaboratory quality control program may be due to interferences between reagents and the matrix of the control materials. Clin Biochem 2007; 40:1088-1091
407. Ross HA, Netea-Maier RT, Schakenraad E, Bravenboer B, Hermus AR, Sweep FC. Assay bias may invalidate decision limits and affect comparability of serum thyroglobulin assay methods: an approach to reduce interpretation differences. Clin Chim Acta 2008; 394:104-109
408. Algeciras-Schimnich A, Bruns DE, Boyd JC, Bryant SC, La Fortune KA, Grebe SK. Failure of current laboratory protocols to detect lot-to-lot reagent differences: findings and possible solutions. Clin Chem 2013; 59:1187-1194
409. Hayden JA, Schmeling M, Hoofnagle AN. Lot-to-lot variations in a qualitative lateral-flow immunoassay for chronic pain drug monitoring. Clin Chem 2014; 60:896-897
410. Thaler MA, Iakoubov R, Bietenbeck A, Luppa PB. Clinically relevant lot-to-lot reagent difference in a commercial immunoturbidimetric assay for glycated hemoglobin A1c. Clin Biochem 2015; 48:1167-1170
411. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 2008; 29 Suppl 1:S49-52

412. Spencer C, Fatemi S, Singer P, Nicoloff J, Lopresti J. Serum Basal thyroglobulin measured by a second-generation assay correlates with the recombinant human thyrotropin-stimulated thyroglobulin response in patients treated for differentiated thyroid cancer. Thyroid 2010; 20:587-595
413. Giovanella L, Clark PM, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, Leenhardt L, Luster M, Schalin-Jantti C, Schott M, Seregni E, Rimmele H, Smit J, Verburg FA. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. Eur J Endocrinol 2014; 171:R33-46
414. Rawlins ML, Roberts WL. Performance characteristics of six third-generation assays for thyroid-stimulating hormone. Clin Chem 2004; 50:2338-2344
415. Iervasi A, Iervasi G, Bottoni A, Boni G, Annicchiarico C, Di Cecco P, Zucchelli GC. Diagnostic performance of a new highly sensitive thyroglobulin immunoassay. J Endocrinol 2004; 182:287-294
416. Smallridge RC, Meek SE, Morgan MA, Gates GS, Fox TP, Grebe S, Fatourechi V. Monitoring thyroglobulin in a sensitive immunoassay has comparable sensitivity to recombinant human TSH-stimulated thyroglobulin in follow-up of thyroid cancer patients. J Clin Endocrinol Metab 2007; 92:82-87
417. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab 2002; 87:1068-1072
418. Boas M, Forman JL, Juul A, Feldt-Rasmussen U, Skakkebaek NE, Hilsted L, Chellakooty M, Larsen T, Larsen JF, Petersen JH, Main KM. Narrow intra-individual variation of maternal thyroid function in pregnancy based on a longitudinal study on 132 women. Eur J Endocrinol 2009; 161:903-910
419. Meikle AW, Stringham JD, Woodward MG, Nelson JC. Hereditary and environmental influences on the variation of thyroid hormones in normal male twins. J Clin Endocrinol Metab 1988; 66:588-592
420. Panicker V, Wilson SG, Spector TD, Brown SJ, Falchi M, Richards JB, Surdulescu GL, Lim EM, Fletcher SJ, Walsh JP. Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. Clin Endocrinol 2008; 68:652-659
421. Nilsson SE, Read S, Berg S, Johansson B. Heritabilities for fifteen routine biochemical values: findings in 215 Swedish twin pairs 82 years of age or older. Scand J Clin Lab Invest 2009; 69:562-569
422. Hansen PS, Brix TH, Sørensen TI, Kyvik KO, Hegedüs L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. J Clin Endocrinol Metab 2004; 89:1181-1187
423. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. Eur J Endocrinol 2006; 155:655-662
424. Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Sestu N, Maschio A, Busonero F, Albai G, Dei M, Lai S, Mulas A, Crisponi L, Tanaka T, Bandinelli S, Guralnik JM, Loi A, Balaci L, Sole G, Prinzis A, Mariotti S, Shuldiner AR, Cao A, Schlessinger D, Uda M, Abecasis GR, Nagaraja R, Sanna S, Naitza S. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. American journal of human genetics 2008; 82:1270-1280
425. Shields BM, Freathy RM, Knight BA, Hill A, Weedon MN, Frayling TM, Hattersley AT, Vaidya B. Phosphodiesterase 8B gene polymorphism is associated with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab 2009; 94:4608-4612
426. Bertalan R, Sallai A, Solyom J, Lotz G, Szabo I, Kovacs B, Szabo E, Patocs A, Racz K. Hyperthyroidism caused by a germline activating mutation of the thyrotropin receptor gene: difficulties in diagnosis and therapy. Thyroid 2010; 20:327-332

427. Biebermann H, Winkler F, Handke D, Gruters A, Krude H, Kleinau G. Molecular description of non-autoimmune hyperthyroidism at a neonate caused by a new thyrotropin receptor germline mutation. Thyroid research 2011; 4 Suppl 1:S8
428. Scaglia PA, Chiesa A, Bastida G, Pacin M, Domene HM, Gruneiro-Papendieck L. Severe congenital non-autoimmune hyperthyroidism associated to a mutation in the extracellular domain of thyrotropin receptor gene. Arq Bras Endocrinol Metabol 2012; 56:513-518
429. Agretti P, Segni M, De Marco G, Ferrarini E, Di Cosmo C, Corrias A, Weber G, Larizza D, Calcaterra V, Pelizzo MR, Cesaretti G, Vitti P, Tonacchera M. Prevalence of activating thyrotropin receptor and Gsalpha gene mutations in paediatric thyroid toxic adenomas: a multicentric Italian study. Clin Endocrinol (Oxf) 2013; 79:747-749
430. Grob F, Deladoey J, Legault L, Spigelblatt L, Fournier A, Vassart G, Van Vliet G. Autonomous adenomas caused by somatic mutations of the thyroid-stimulating hormone receptor in children. Horm Res Paediatr 2014; 81:73-79
431. Nakamura A, Morikawa S, Aoyagi H, Ishizu K, Tajima T. A Japanese family with nonautoimmune hyperthyroidism caused by a novel heterozygous thyrotropin receptor gene mutation. Pediatr Res 2014; 75:749-753
432. Kleinau G, Biebermann H. Constitutive activities in the thyrotropin receptor: regulation and significance. Advances in pharmacology (San Diego, Calif) 2014; 70:81-119
433. Larsen CC, Karaviti LP, Seghers V, Weiss RE, Refetoff S, Dumitrescu AM. A new family with an activating mutation (G431S) in the TSH receptor gene: a phenotype discussion and review of the literature. International journal of pediatric endocrinology 2014; 2014:23
434. Alberti L, Proverbio MC, Costagliola S, Romoli R, Boldrighini B, Vigone MC, Weber G, Chiumello G, Beck-Peccoz P, Persani L. Germline mutations of TSH receptor gene as cause of nonautoimmune subclinical hypothyroidism. J Clin Endocrinol Metab 2002; 87:2549-2555
435. Persani L, Calebiro D, Cordella D, Weber G, Gelmini G, Libri D, de Filippis T, Bonomi M. Genetics and phenomics of hypothyroidism due to TSH resistance. Mol Cell Endocrinol 2010; 322:72-82
436. Calebiro D, Gelmini G, Cordella D, Bonomi M, Winkler F, Biebermann H, de Marco A, Marelli F, Libri DV, Antonica F, Vigone MC, Cappa M, Mian C, Sartorio A, Beck-Peccoz P, Radetti G, Weber G, Persani L. Frequent TSH receptor genetic alterations with variable signaling impairment in a large series of children with nonautoimmune isolated hyperthyrotropinemia. J Clin Endocrinol Metab 2012; 97:E156-160
437. Rapa A, Monzani A, Moia S, Vivenza D, Bellone S, Petri A, Teofoli F, Cassio A, Cesaretti G, Corrias A, de Sanctis V, Di Maio S, Volta C, Wasniewska M, Tatò L, Bona G. Subclinical hypothyroidism in children and adolescents: a wide range of clinical, biochemical, and genetic factors involved. J Clin Endocrinol Metab 2009; 94:2414-2420
438. De Marco G, Agretti P, Camilot M, Teofoli F, Tatò L, Vitti P, Pinchera A, Tonacchera M. Functional studies of new TSH receptor (TSHr) mutations identified in patients affected by hypothyroidism or isolated hyperthyrotrophinaemia. Clin Endocrinol 2009; 70:335-338
439. Brouwer JP, Appelhof BC, Peeters RP, Hoogendijk WJ, Huyser J, Schene AH, Tijssen JG, Van Dyck R, Visser TJ, Wiersinga WM, Fliers E. Thyrotropin, but not a polymorphism in type II deiodinase, predicts response to paroxetine in major depression. Eur J Endocrinol 2006; 154:819-825
440. Takeda K, Mishiba M, Sugiura H, Nakajima A, Kohama M, Hiramatsu S. Evaluated reference intervals for serum free thyroxine and thyrotropin using the conventional outliner rejection test without regard to presence of thyroid antibodies and prevalence of thyroid dysfunction in Japanese subjects. Endoc J 2009; 56:1059-1066
441. Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for

interpretation of thyroid function tests. Thyroid 2003; 13:1069-1078
442. Jensen E, Petersen PH, Blaabjerg O, Hegedüs L. Biological variation of thyroid autoantibodies and thyroglobulin. Clin Chem Lab Med 2007; 45:1058-1064
443. Ankrah-Tetteh T, Wijeratne S, Swaminathan R. Intraindividual variation in serum thyroid hormones, parathyroid hormone and insulin-like growth factor-1. Ann Clin Biochem 2008; 45:167-169
444. van de Ven AC, Netea-Maier RT, Medici M, Sweep FC, Ross HA, Hofman A, de Graaf J, Kiemeney LA, Hermus AR, Peeters RP, Visser TJ, den Heijer M. Underestimation of effect of thyroid function parameters on morbidity and mortality due to intra-individual variation. J Clin Endocrinol Metab 2011; 96:E2014-2017
445. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev 2008; 29:76-131
446. Cooper DS, Biondi B. Subclinical thyroid disease. Lancet 2012; 379:1142-1154
447. Silvio R, Swapp KJ, La'ulu SL, Hansen-Suchy K, Roberts WL. Method specific second-trimester reference intervals for thyroid-stimulating hormone and free thyroxine. Clin Biochem 2009; 42:750-753
448. Coene KL, Demir AY, Broeren MA, Verschuure P, Lentjes EG, Boer AK. Subclinical hypothyroidism: a 'laboratory-induced' condition? Eur J Endocrinol 2015; 173:499-505
449. Strich D, Karavani G, Levin S, Edri S, Gillis D. Normal limits for serum thyrotropin vary greatly depending on method. Clin Endocrinol (Oxf) 2016; 85:110-115
450. Solberg HE. The IFCC recommendation on estimation of reference intervals. Clin Chem Lab Med 2004; 42:710-714
451. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. Clin Endocrinol (Oxf) 2012; 77:773-779
452. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002; 87:489-499
453. Hollowell JG, Staehling NW, Hannon WH, Flanders DW, Gunter EW, Maberly GF, Braverman LE, Pino S, Miller DT, Garbe PL, DeLozier DM, Jackson RJ. Iodine nutrition in the United States. Trends and public health implications: iodine excretion data from National Health and Nutrition Examination Surveys I and III (1971-1974 and 1988-1994). J Clin Endocrinol Metab 1998; 83:3401-3408
454. McNeil AR, Stanford PE. Reporting Thyroid Function Tests in Pregnancy. Clin Biochem Rev 2015; 36:109-126
455. Kristensen GB, Rustad P, Berg JP, Aakre KM. Analytical Bias Exceeding Desirable Quality Goal in 4 out of 5 Common Immunoassays: Results of a Native Single Serum Sample External Quality Assessment Program for Cobalamin, Folate, Ferritin, Thyroid-Stimulating Hormone, and Free T4 Analyses. Clin Chem 2016; 62:1255-1263
456. Spencer CA, Hollowell JG, Kazarosyan M, Braverman LE. National Health and Nutrition Examination Survey III thyroid-stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. J Clin Endocrinol Metab 2007; 92:4236-4240
457. Bjoro T, Holmen J, Kruger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 2000; 143:639-647
458. Pedersen OM, Aardal NP, Larssen TB, Varhaug JE, Myking O, Vik-Mo H. The value of ultrasonography in predicting autoimmune thyroid disease. Thyroid 2000; 10:251-259
459. Jensen E, Hyltoft Petersen P, Blaabjerg O, Hansen PS, Brix TH, Kyvik KO, Hegedus

L. Establishment of a serum thyroid stimulating hormone (TSH) reference interval in healthy adults. The importance of environmental factors, including thyroid antibodies. Clin Chem Lab Med 2004; 42:824-832
460. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab 2007; 92:4575-4582
461. Zelaya AS, Stotts A, Nader S, Moreno CA. Antithyroid peroxidase antibodies in patients with high normal range thyroid stimulating hormone. Fam Med 2010; 42:111-115
462. Sichieri R, Baima J, Marante T, de Vasconcellos MT, Moura AS, Vaisman M. Low prevalence of hypothyroidism among black and Mulatto people in a population-based study of Brazilian women. Clin Endocrinol 2007; 66:803-807
463. Boucai L SM. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. Clin Endocrinol 2009; 70:788-793
464. Vejbjerg P, Knudsen N, Perrild H, Carlé A, Laurberg P, Pedersen IB, Rasmussen LB, Ovesen L, Jørgensen T. The impact of smoking on thyroid volume and function in relation to a shift towards iodine sufficiency. Eur J Epidemiol 2008; 23:423-429
465. Buchinger W, Lorenz-Wawschinek O, Semlitsch G, Langsteger W, Binter G, Bonelli RM, Eber O. Thyrotropin and thyroglobulin as an index of optimal iodine intake: correlation with iodine excretion of 39,913 euthyroid patients. Thyroid 1997; 7:593-597
466. Nyrnes A, Jorde R, Sundsfjord J. Serum TSH is positively associated with BM. Int J Obes 2006; 30:100-105
467. Knudsen N, Laurberg P, Rasmussen LB, Bulow I, Perrild H, Ovesen L, Jorgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 2005; 90:4019-4024
468. Kok P, Roelfsema F, Langendonk JG, Frolich M, Burggraaf J, Meinders AE, Pijl H. High circulating thyrotropin levels in obese women are reduced after body weight loss induced by caloric restriction. J Clin Endocrinol Metab 2005; 90:4659-4663
469. Chikunguwo S, Brethauer S, Nirujogi V, Pitt T, Udomsawaengsup S, Chand B, Schauer P. Influence of obesity and surgical weight loss on thyroid hormone levels. Surg Obes Relat Dis 2007; 3:631-635
470. Fox CS, Pencina MJ, D'Agostino RB, Murabito JM, Seely EW, Pearce EN, Vasan RS. Relations of thyroid function to body weight: cross-sectional and longitudinal observations in a community-based sample. Arch Intern Med 2008; 168:587-592
471. Friedrich N, Rosskopf D, Brabant G, Völzke H, Nauck M, Wallaschofski H. Associations of anthropometric parameters with serum TSH, prolactin, IGF-I, and testosterone levels: results of the study of health in Pomerania (SHIP). Exp Clin Endocrinol Diabetes 2010; 118:266-273
472. Biondi B. Thyroid and obesity: an intriguing relationship. J Clin Endocrinol Metab 2010; 95:3614-3617
473. Solanki A, Bansal S, Jindal S, Saxena V, Shukla US. Relationship of serum thyroid stimulating hormone with body mass index in healthy adults. Indian journal of endocrinology and metabolism 2013; 17:S167-169
474. Taylor PN, Razvi S, Pearce SH, Dayan CM. Clinical review: A review of the clinical consequences of variation in thyroid function within the reference range. J Clin Endocrinol Metab 2013; 98:3562-3571
475. Duntas LH, Biondi B. The interconnections between obesity, thyroid function, and autoimmunity: the multifold role of leptin. Thyroid 2013; 23:646-653
476. Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L, Biondi B. Mechanisms in endocrinology: the crosstalk between thyroid gland and adipose tissue: signal integration in health and disease. Eur J Endocrinol 2014; 171:R137-152
477. Betry C, Challan-Belval MA, Bernard A, Charrie A, Drai J, Laville M, Thivolet C,

Disse E. Increased TSH in obesity: Evidence for a BMI-independent association with leptin. Diabetes & metabolism 2015; 41:248-251
478. Asvold BO, Vatten LJ, Nilsen TI, Bjoro T. The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. Eur J Endocrinol 2007; 156:181-186
479. Soldin OP, Goughenour BE, Gilbert SZ, Landy HJ, Soldin SJ. Thyroid Hormone Levels Associated with Active and Passive Cigarette Smoking. Thyroid 2010; 20:0-0
480. Surks MI, Boucai L. Age- and race-based serum Thyrotropin Reference Limits. J Clin Endocrinol Metab 2010; 95:496 -502
481. Atzmon G, Barzilai N, Hollowell JG, Surks MI, Gabriely I. Extreme longevity is associated with increased serum thyrotropin. J Clin Endocrinol Metab 2009; 94:1251-1254
482. Bremner AP, Feddema P, Leedman PJ, Brown SJ, Beilby JP, Lim EM, Wilson SG, O'Leary PC, Walsh JP. Age-related changes in thyroid function: a longitudinal study of a community-based cohort. J Cln Endocrinol Metab 2012; 97:1554-1562
483. Hoogendoorn EH, Hermus AR, de Vegt F, Ross HA, Verbeek AL, Kiemeney LA, Swinkels DW, Sweep FC, den Heijer M. Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. Clin Chem 2006; 52:104-111
484. Volzke H, Alte D, Kohlmann T, Ludemann J, Nauck M, John U, Meng W. Reference intervals of serum thyroid function tests in a previously iodine-deficient area. Thyroid 2005; 15:279-285
485. Kratzsch J, Fiedler GM, Leichtle A, Brugel M, Buchbinder S, Otto L, Sabri O, Matthes G, Thiery J. New reference intervals for thyrotropin and thyroid hormones based on national academy of clinical biochemistry criteria and regular ultrasonography of the thyroid. Clin Chem 2005; 51:1480-1486
486. Berghout A, Wiersinga WM, Smits NJ, touber JL. Interrelationships between age, thyroid volume, thyroid nodularity, and thyroid function in patients with sporadic nontoxic goiter. Amer J Med 1990; 89:602-608
487. Meisinger C, Ittermann T, Wallaschofski H, Heier M, Below H, Kramer A, Doring A, Nauck M, Volzke H. Geographic variations in the frequency of thyroid disorders and thyroid peroxidase antibodies in persons without former thyroid disease within Germany. Eur J Endocrinol 2012; 167:363-371
488. Ittermann T, Khattak RM, Nauck M, Cordova CM, Volzke H. Shift of the TSH reference range with improved iodine supply in Northeast Germany. Eur J Endocrinol 2015; 172:261-267
489. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frölich M, Westendorp RG. Thyroid status, disability and cognitive function, and survival in old age. JAMA 2004; 292:2591-2599
490. Simonsick EM, Newman AB, Ferrucci L, Satterfield S, Harris TB, Rodondi N, Bauer DC. Subclinical hypothyroidism and functional mobility in older adults. Arch Intern Med 2009; 169:2011-2017
491. Atzmon G, Barzilai N, Surks MI, Gabriely I. Genetic predisposition to elevated serum thyrotropin is associated with exceptional longevity. J Clin Endocrinol Metab 2009; 94:4768-4775
492. Rozing MP, Houwing-Duistermaat JJ, Slagboom PE, Beekman M, Frölich M, de Craen AJ, Westendorp RG, van Heemst D. Familial longevity is associated with decreased thyroid function. J Clin Endocrinol Metab 2010; 95:4979-4984
493. van den Beld AW, Visser TJ, Feelders RA, Grobbee DE, Lamberts SW. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. J Clin Endocrinol Metab 2005; 90:6403-6409
494. Akirov A, Gimbel H, Grossman A, Shochat T, Shimon I. Elevated TSH in adults

treated for hypothyroidism is associated with increased mortality. Eur J Endocrinol 2016;
495. Taylor PN, Panicker V, Sayers A, Shields B, Iqbal A, Bremner AP, Beilby JP, Leedman PJ, Hattersley AT, Vaidya B, Frayling T, Evans J, Tobias JH, Timpson NJ, Walsh JP, Dayan CM. A meta-analysis of the associations between common variation in the PDE8B gene and thyroid hormone parameters, including assessment of longitudinal stability of associations over time and effect of thyroid hormone replacement. Eur J Endocrinol 2011; 164:773-780
496. Somwaru LL, Rariy CM, Arnold AM, Cappola AR. The natural history of subclinical hypothyroidism in the elderly: the cardiovascular health study. J Clin Endocrinol Metab 2012; 97:1962-1969
497. Magner JA. Thyroid-Stimulating Hormone: Biosynthesis, Cell Biology, and Bioactivity. Endocrinol Rev 1990; 11:354-385
498. Estrada JM, Soldin D, Buckey TM, Burman KD, Soldin OP. Thyrotropin isoforms: implications for thyrotropin analysis and clinical practice. Thyroid 2014; 24:411-423
499. Persani L. Hypothalamic thyrotropin-releasing hormone and thyrotropin biological activity. Thyroid 1998; 8:941-946
500. Sergi I, Papandreou MJ, Medri G, Canonne C, Verrier B, Ronin C. Immunoreactive and bioactive isoforms of human thyrotropin. Endocrinology 1991; 128:3259-3268
501. 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; 43:519-530
502. Schaaf L, Trojan J, Helton TE, Usadel KH, Magner JA. Serum thyrotropin (TSH) heterogeneity in euthyroid subjects and patients with subclinical hypothyroidism: the core fucose content of TSH-releasing hormone-released TSH is altered, but not the net charge of TSH. J Endocrinol 1995; 144:561-571
503. 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
504. Oliveira JH, Barbosa ER, Kasamatsu T, Abucham J. Evidence for thyroid hormone as a positive regulator of serum thyrotropin bioactivity. J Clin Endocrinol Metab 2007; 92:3108-3113
505. Barreca T, Franceschini R, Messina V, Bottaro L, Rolandi E. 24-hour thyroid-stimulating hormone secretory pattern in elderly men. Gerentology 1985; 31:119-123
506. van Coevorden A, Laurent E, Decoster C, Kerkhofs M, Neve P, van Cauter E, Mockel J. Decreased basal and stimulated thyrotropin secretion in healthy elderly men. J Clin Endocrinol Metab 1989; 69:177-185
507. Romijn JA, Wiersinga WM. Decreased nocturnal surge of thyrotropin in nonthyroidal illness. J Clin Endocrinol Metab 1990; 70:35-42
508. Persani L, Asteria C, Tonacchera M, Vitti P, Krishna V, Chatterjee K, Beck-Peccoz P. Evidence for the secretion of thyrotropin with enhanced bioactivity in syndromes of thyroid hormone resistance. J Clin Endocrinol Metab 1994; 78:1034-1039
509. Persani L. Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. J Clin Endocrinol Metab 2012; 97:3068-3078
510. Chan MK, Seiden-Long I, Aytekin M, Quinn F, Ravalico T, Ambruster D, Adeli K. Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER): pediatric reference intervals for an integrated clinical chemistry and immunoassay analyzer, Abbott ARCHITECT ci8200. Clin Biochem 2009; 42:885-891
511. Zurakowski D, Di Canzio J, Majzoub JA. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin and free thyroxine. Clin Chem 1999; 45:1087-1091
512. Kapelari K, Kirchlechner C, Högler W, Schweitzer K, Virgolini I, Moncayo R. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study.

BMC endocrine disorders 2008; 8:15
513. Lazar L, Frumkin RB, Battat E, Lebenthal Y, Phillip M, Meyerovitch J. Natural history of thyroid function tests over 5 years in a large pediatric cohort. J Clin Endocrinol Metab 2009; 94:1678-1682
514. Strich D, Edri S, Gillis D. Current normal values for TSH and FT3 in children are too low: evidence from over 11,000 samples. J Pediatr Endocrinol Metab 2012; 25:245-248
515. d'Herbomez M, Jarrige V, Darte C. Reference intervals for serum thyrotropin (TSH) and free thyroxine (FT4) in adults using the Access Immunoassay System. Clin Chem Lab Med 2005; 43:102-105
516. Zarković M, Cirić J, Beleslin B, Cirić S, Bulat P, Topalov D, Trbojević B. Further studies on delineating thyroid-stimulating hormone (TSH) reference range. Horm Metab Res 2011; 43:970-976
517. Hamilton TE, Davis S, Onstad L, Kopecky KJ. Thyrotropin levels in a population with no clinical, autoantibody, or ultrasonographic evidence of thyroid disease: implications for the diagnosis of subclinical hypothyroidism. J Clin Endocrinol Metab 2008; 93:1224-1230
518. Goichot B, Sapin R, Schlienger JL. Subclinical hyperthyroidism: considerations in defining the lower limit of the thyrotropin reference interval. Clin Chem 2009; 55:420-424
519. Völzke H, Schmidt CO, John U, Wallaschofski H, Dörr M, Nauck M. Reference levels for serum thyroid function tests of diagnostic and prognostic significance. Horm Metab Res 2010; 42:809-814
520. O'Leary PC, Feddema PH, Michelangeli VP, Leedman PJ, Chew GT, Knuiman M, Kaye J, Walsh JP. Investigations of thyroid hormones and antibodies based on a community health survey: the Busselton thyroid study. Clin Endocrinol 2006; 64:97-104
521. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado Thyroid Disease Prevalence Study. Arch Intern Med 2000; 160:19-27
522. Sawin CT. Subclinical hyperthyroidism and atrial fibrillation. Thyroid 2002; 12:501-503
523. Choi AR, Manning P. Overshooting the mark: subclinical hyperthyroidism secondary to excess thyroid hormone treatment may be more prevalent than we realise. NZ Med J 2009; 122:93-94
524. Ochs N, Auer R, Bauer DC, Nanchen D, Gussekloo J, Cornuz J, Rodondi N. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. Ann Intern Med 2008; 148:832-845
525. Murphy E, Gluer CC, Reid DM, Felsenberg D, Roux C, Eastell R, Williams GR. Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 2010; 95:3173-3181
526. Blum MR, Bauer DC, Collet TH, Fink HA, Cappola AR, da Costa BR, Wirth CD, Peeters RP, Asvold BO, den Elzen WP, Luben RN, Imaizumi M, Bremner AP, Gogakos A, Eastell R, Kearney PM, Strotmeyer ES, Wallace ER, Hoff M, Ceresini G, Rivadeneira F, Uitterlinden AG, Stott DJ, Westendorp RG, Khaw KT, Langhammer A, Ferrucci L, Gussekloo J, Williams GR, Walsh JP, Juni P, Aujesky D, Rodondi N. Subclinical thyroid dysfunction and fracture risk: a meta-analysis. Jama 2015; 313:2055-2065
527. Collet TH, Gussekloo J, Bauer DC, den Elzen WP, Cappola AR, Balmer P, Iervasi G, Asvold BO, Sgarbi JA, Volzke H, Gencer B, Maciel RM, Molinaro S, Bremner A, Luben RN, Maisonneuve P, Cornuz J, Newman AB, Khaw KT, Westendorp RG, Franklyn JA, Vittinghoff E, Walsh JP, Rodondi N. Subclinical hyperthyroidism and the risk of coronary heart disease and mortality. Arch Intern Med 2012; 172:799-809
528. Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrinol Metab 2005; 90:5483-5488
529. Surks MI. Should the upper limit of the normal reference range for TSH be lowered?

Nat Clin Pract Endocrinol Metab 2008; 4:370-371
530. Laurberg P, Andersen S, Carlé A, Karmisholt J, Knudsen N, Pedersen IB. The TSH upper reference limit: where are we at? Nat Rev Endocrinol 2011; 7:232-239
531. McQuade C, Skugor M, Brennan DM, Hoar B, Stevenson C, Hoogwerf BJ. Hypothyroidism and moderate subclinical hypothyroidism are associated with increased all-cause mortality independent of coronary heart disease risk factors: a PreCIS database study. Thyroid 2011; 21:837-843
532. Vanderpump MPJ, Tunbridge WMG, French JM, Appleton D, Bates D, Rodgers H, Evans JG, Clark F, Tunbridge F, Young ET. The incidence of thyroid disorders in the community; a twenty year follow up of the Whickham survey. Clin Endocrinol 1995; 43:55-68
533. Helfand M. Screening for subclinical thyroid dysfunction in nonpregnant adults: a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2004; 140:128-141
534. Rugge B, Balshem H, Sehgal R, Relevo R, Gorman P, Helfand M. AHRQ Comparative Effectiveness Reviews. Screening and Treatment of Subclinical Hypothyroidism or Hyperthyroidism. Rockville (MD): Agency for Healthcare Research and Quality (US); 2011.
535. Asvold BO, Vatten LJ, Midthjell K, Bjoro T. Serum TSH within the reference range as a predictor of future hypothyroidism and hyperthyroidism: 11-year follow-up of the HUNT Study in Norway. J Clin Endocrinol Metab 2012; 97:93-99
536. Asvold BO, Bjoro T, Vatten LJ. Association of thyroid function with estimated glomerular filtration rate in a population-based study: the HUNT study. Eur J Endocrinol 2011; 164:101-105
537. Ittermann T, Thamm M, Wallaschofski H, Rettig R, Volzke H. Serum thyroid-stimulating hormone levels are associated with blood pressure in children and adolescents. J Clin Endocrinol Metab 2012; 97:828-834
538. Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. J Clin Endocrinol Metab 2003; 88:2438-2444
539. Iqbal A, Figenschau Y, Jorde R. Blood pressure in relation to serum thyrotropin: The Tromso study. J Hum Hypertens 2006; 20:932-936
540. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, Gussekloo J. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. Jama 2010; 304:1365-1374
541. Daswani R, Jayaprakash B, Shetty R, Rau NR. Association of Thyroid Function with Severity of Coronary Artery Disease in Euthyroid Patients. Journal of clinical and diagnostic research : JCDR 2015; 9:Oc10-13
542. Andersen MN, Olsen AS, Madsen JC, Kristensen SL, Faber J, Torp-Pedersen C, Gislason GH, Selmer C. Long-Term Outcome in Levothyroxine Treated Patients With Subclinical Hypothyroidism and Concomitant Heart Disease. J Clin Endocrinol Metab 2016; 101:4170-4177
543. Ittermann T, Lorbeer R, Dorr M, Schneider T, Quadrat A, Hesselbarth L, Wenzel M, Lehmphul I, Kohrle J, Mensel B, Volzke H. High levels of thyroid-stimulating hormone are associated with aortic wall thickness in the general population. European radiology 2016; 26:4490-4496
544. Iqbal A, Jorde R, Figenschau Y. Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Tromso Study. J Intern Med 2006; 260:53-61
545. Asvold BO, Bjoro T, Vatten LJ. Associations of TSH levels within the reference range with future blood pressure and lipid concentrations: 11-year follow-up of the HUNT study.

Eur J Endocrinol 2013; 169:73-82
546. Witte T, Ittermann T, Thamm M, Riblet NB, Volzke H. Association between serum thyroid-stimulating hormone levels and serum lipids in children and adolescents: a population-based study of german youth. J Clin Endocrinol Metab 2015; 100:2090-2097
547. Javed Z, Sathyapalan T. Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits. Therapeutic advances in endocrinology and metabolism 2016; 7:12-23
548. Ajmani SN, Aggarwal D, Bhatia P, Sharma M, Sarabhai V, Paul M. Prevalence of overt and subclinical thyroid dysfunction among pregnant women and its effect on maternal and fetal outcome. Journal of Obstetrics and Gynaecology of India 2014; 64:105-110
549. Krassas G, Karras SN, Pontikides N. Thyroid diseases during pregnancy: a number of important issues. Hormones (Athens) 2015; 14:59-69
550. Nazarpour S, Ramezani Tehrani F, Simbar M, Azizi F. Thyroid dysfunction and pregnancy outcomes. Iranian journal of reproductive medicine 2015; 13:387-396
551. Casey BM, Dashe JS, Wells CE, McIntire DD, Leveno KJ, Cunningham FG. Subclinical hyperthyroidism and pregnancy outcomes. Obstet Gynecol 2006; 107:337-341
552. Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Leveno KJ, Cunningham FG. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol 2005; 105:239-245
553. Shan ZY, Chen YY, Teng WP, Yu XH, Li CY, Zhou WW, Gao B, Zhou JR, Ding B, Ma Y, Wu Y, Liu Q, Xu H, Liu W, Li J, Wang WW, Li YB, Fan CL, Wang H, Guo R, Zhang HM. A study for maternal thyroid hormone deficiency during the first half of pregnancy in China. Eur J Clin Invest 2009; 39:37-42
554. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev 2010; 31:702-755
555. Lazarus JH. Thyroid function in pregnancy. British medical bulletin 2011; 97:137-148
556. Brabant G, Peeters RP, Chan SY, Bernal J, Bouchard P, Salvatore D, Boelaert K, Laurberg P. Management of subclinical hypothyroidism in pregnancy: are we too simplistic? Eur J Endocrinol 2015; 173:P1-p11
557. Negro R, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Stagnaro-Green A. Increased pregnancy loss rate in thyroid antibody negative women with TSH levels between 2.5 and 5.0 in the first trimester of pregnancy. J Clin Endocrinol Metab 2010; 95:E44-48
558. Liu H, Shan Z, Li C, Mao J, Xie X, Wang W, Fan C, Wang H, Zhang H, Han C, Wang X, Liu X, Fan Y, Bao S, Teng W. Maternal subclinical hypothyroidism, thyroid autoimmunity, and the risk of miscarriage: a prospective cohort study. Thyroid 2014; 24:1642-1649
559. Maraka S, Ospina NM, O'Keeffe DT, Espinosa De Ycaza AE, Gionfriddo MR, Erwin PJ, Coddington CC, 3rd, Stan MN, Murad MH, Montori VM. Subclinical Hypothyroidism in Pregnancy: A Systematic Review and Meta-Analysis. Thyroid 2016; 26:580-590
560. Kaprara A, Krassas GE. Thyroid autoimmunity and miscarriage. Hormones (Athens) 2008; 7:294-302
561. Wang S, Teng WP, Li JX, Wang WW, Shan ZY. Effects of maternal subclinical hypothyroidism on obstetrical outcomes during early pregnancy. J Endocrinol Invest 2012; 35:322-325
562. Negro R, Schwartz A, Stagnaro-Green A. Impact of Levothyroxine in Miscarriage and Preterm Delivery Rates in First Trimester Thyroid Antibody-Positive Women with TSH<2.5mIU/L. J Clin Endocrinol Metab 2016:jc20161803
563. Wilson KL, Casey BM, McIntire DD, Halvorson LM, Cunningham FG. Subclinical thyroid disease and the incidence of hypertension in pregnancy. Obstet Gynecol 2012; 119:315-320
564. Negro R, Formoso G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications. J Clin Endocrinol Metab 2006; 91:2587-2591

565. Chen X, Jin B, Xia J, Tao X, Huang X, Sun L, Yuan Q. Effects of Thyroid Peroxidase Antibody on Maternal and Neonatal Outcomes in Pregnant Women in an Iodine-Sufficient Area in China. International journal of endocrinology 2016; 2016:6461380
566. Mannisto T, Vaarasmaki M, Pouta A, Hartikainen AL, Ruokonen A, Surcel HM, Bloigu A, Jarvelin MR, Suvanto-Luukkonen E. Perinatal outcome of children born to mothers with thyroid dysfunction or antibodies: a prospective population-based cohort study. J Clin Endocrinol Metab 2009; 94:772-779
567. Saki F, Dabbaghmanesh MH, Ghaemi SZ, Forouhari S, Ranjbar Omrani G, Bakhshayeshkaram M. Thyroid function in pregnancy and its influences on maternal and fetal outcomes. International journal of endocrinology and metabolism 2014; 12:e19378
568. Chen LM, Zhang Q, Si GX, Chen QS, Ye EL, Yu LC, Peng MM, Yang H, Du WJ, Zhang C, Lu XM. Associations between thyroid autoantibody status and abnormal pregnancy outcomes in euthyroid women. Endocrine 2015; 48:924-928
569. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999; 341:549-555
570. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, Teng X, Guo R, Wang H, Li J, Chen Y, Wang W, Chawinga M, Zhang L, Yang L, Zhao Y, Hua T. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. Clin Endocrinol (Oxf) 2010; 72:825-829
571. Nazarpour S, Ramezani Tehrani F, Simbar M, Tohidi M, Alavi Majd H, Azizi F. Effects of levothyroxine treatment on pregnancy outcomes in pregnant women with autoimmune thyroid disease. Eur J Endocrinol 2016;
572. Smallridge RC, Ladenson PW. Hypothyroidism in pregnancy: consequences to neonatal health. J Clin Endocrinol Metab 2001; 86:2349-2353
573. Korevaar TI, Steegers EA, de Rijke YB, Visser WE, Jaddoe VW, Visser TJ, Medici M, Peeters RP. Placental Angiogenic Factors Are Associated With Maternal Thyroid Function and Modify hCG-Mediated FT4 Stimulation. J Clin Endocrinol Metab 2015; 100:E1328-1334
574. Korevaar TI, Steegers EA, Pop VJ, Broeren MA, Chaker L, de Rijke YB, Jaddoe VW, Medici M, Visser TJ, Tiemeier H, Peeters RP. Thyroid autoimmunity impairs the thyroidal response to hCG: two population-based prospective cohort studies. J Clin Endocrinol Metab 2016:jc20162942
575. Pekonen F, Alfthan H, Stenman UH, Ylikorkala O. Human chorionic gonadotropin (hCG) and thyroid function in early human pregnancy: circadian variation and evidence for intrinsic thyrotropic activity of hCG. J Clin Endocrinol Metab 1988; 66:853-856
576. Goodwin TM, Montoro M, Mestman JH, Pekary AE, Hershman JM. The role of chorionic gonadotropin in transient hyperthyroidism of hyperemesis gravidarum. J Clin Endocrinol Metab 1992; 75:1333-1337
577. Grun JP, Meuris S, De Nayer P, Glinoer D. The thyrotrophic role of human chorionic gonadotrophin (hCG) in the early stages of twin (versus single) pregnancies. Clin Endocrinol (Oxf) 1997; 46:719-725
578. Li C, Shan Z, Mao J, Wang W, Xie X, Zhou W, Li C, Xu B, Bi L, Meng T, Du J, Zhang S, Gao Z, Zhang X, Yang L, Fan C, Teng W. Assessment of thyroid function during first-trimester pregnancy: what is the rational upper limit of serum TSH during the first trimester in Chinese pregnant women? J Clin Endocrinol Metab 2014; 99:73-79
579. Medici M, Korevaar TI, Schalekamp-Timmermans S, Gaillard R, de Rijke YB, Visser WE, Visser W, de Muinck Keizer-Schrama SM, Hofman A, Hooijkaas H, Bongers-Schokking JJ, Tiemeier H, Jaddoe VW, Visser TJ, Peeters RP, Steegers EA. Maternal early-pregnancy thyroid function is associated with subsequent hypertensive disorders of pregnancy: the

generation R study. J Clin Endocrinol Metab 2014; 99:E2591-2598
580. Rajput R, Singh B, Goel V, Verma A, Seth S, Nanda S. Trimester-specific reference interval for thyroid hormones during pregnancy at a Tertiary Care Hospital in Haryana, India. Indian journal of endocrinology and metabolism 2016; 20:810-815
581. Lambert-Messerlian G, McClain M, Haddow JE, Palomaki GE, Canick JA, Cleary-Goldman J, Malone FD, Porter TF, Nyberg DA, Bernstein P, D'Alton ME. First- and second-trimester thyroid hormone reference data in pregnant women: a FaSTER (First- and Second-Trimester Evaluation of Risk for aneuploidy) Research Consortium study. Am J Obstet Gyneol 2008; 199:62: e61-66
582. Tozzoli R, D'Aurizio F, Ferrari A, Castello R, Metus P, Caruso B, Perosa AR, Sirianni F, Stenner E, Steffan A, Villalta D. The upper reference limit for thyroid peroxidase autoantibodies is method-dependent: A collaborative study with biomedical industries. Clin Chim Acta 2016; 452:61-65
583. Weeke J, Gundersen HJ. Circadian and 30 minute variations in serum TSH and thyroid hormones in normal subjects. Acta Endocrinol 1978; 89:659-672
584. Evans PJ, Weeks I, Jones MK, Woodhead JS, Scanlon MF. The circadian variation of thyrotrophin in patients with primary thyroidal disease. CLIN Endocrinol (Oxf) 1986; 24:343-348
585. Brabant G, Prank K, Hoang-Vu C, von zur Muhlen A. Hypothalamic regulation of pulsatile thyrotropin secretion. J Clin Endocrinol Metab 1991; 72:145-150
586. Roelfsema F, Pereira AM, Veldhuis JD, Adriaanse R, Endert E, Fliers E, Romijn JA. Thyrotropin secretion profiles are not different in men and women. J Clin Endocrinol Metab 2009; 94:3964-3967
587. Ladenson PW, Singer PA, Ain KB, Bagchi N, Bigos ST, Levy EG, Smith SA, Daniels GH, Cohen HD. American Thyroid Association guidelines for detection of thyroid dysfunction. Arch Intern Med 2000; 160:1573-1575
588. Singer PA, Cooper DS, Levy EG, Ladenson PW, Braverman LE, Daniels G, Greenspan FS, McDougall IR, Nikolai TF. Treatment guidelines for patients with hyperthyroidism and hypothyroidism. Standards of Care Committee, American Thyroid Association. JAMA 1995; 273:808-812
589. Carr D, McLeod DT, Parry G, Thornes HM. Fine adjustment of thyroxine replacement dosage: comparison of the thyrotrophin releasing hormone test using a sensitive thyrotrophin assay with measurement of free thyroid hormones and clinical assessment. Clin Endocrinol 1988; 28:325-333
590. Walsh JP, Ward LC, Burke V, Bhagat CI, Shiels L, Henley D, Gillett MJ, Gilbert R, Tanner M, Stuckey BG. Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being, or quality of life: results of a double-blind, randomized clinical trial. J Clin Endocrinol Metab 2006; 91:2624-2630
591. Jonklaas J, Sarlis NJ, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis M, Maxon HR, Sherman SI. Outcomes of patients with differentiated thyroid carcinoma following initial therapy. Thyroid 2006;
592. Pujol P, Daures JP, Nsakala N, Baldet L, Bringer J, Jaffiol C. Degree of thyrotropin suppression as a prognostic determinant in differentiated thyroid cancer. J Clin Endocrinol Metab 1996; 81:4318-4323
593. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009; 19:1167-1124
594. Wartofsky L, Burman KD. Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome". Endocrinol Rev 1982; 3:164-217

595. Mebis L, van den Berghe G. The hypothalamus-pituitary-thyroid axis in critical illness. Neth J Med 2009; 67:332-340
596. Lamb EJ, Martin J. Thyroid function tests: often justified in the acutely ill. Ann Clin Biochem 2000; 37:158-164
597. Mebis L, Paletta D, Debaveye Y, Ellger B, Langouche L, D'Hoore A, Darras VM, Visser TJ, Van den Berghe G. Expression of thyroid hormone transporters during critical illness. Eur J Endocrinol 2009; 161:243-250
598. Bunevicius R, Steibliene V, Prange AJ, Jr. Thyroid axis function after in-patient treatment of acute psychosis with antipsychotics: a naturalistic study. BMC psychiatry 2014; 14:279
599. Verhoye E, Van den Bruel A, Delanghe JR, Debruyne E, Langlois MR. Spuriously high thyrotropin values due to anti-thyrotropin antibodies in adult patients. Clin Chem Lab Med 2009; 47:604-606
600. Uy HL, Reasner CA, Samuels MH. Pattern of recovery of the hypothalamic-pituitary-thyroid axis following radioactive iodine therapy in patients with Graves' disease. Am J Med 1995; 99:173-179
601. Kempers MJ, van der Sluijs Veer L, Nijhuis-van der Sanden RW, Lanting CI, Kooistra L, Wiedijk BM, Last BF, de Vijlder JJ, Grootenhuis MA, Vulsma T. Neonatal screening for congenital hypothyroidism in the Netherlands: cognitive and motor outcome at 10 years of age. J Clin Endocrinol Metab 2007; 92:919-924
602. Schaaf L, Theodoropoulou M, Gregori A, Leiprecht A, Trojan J, Klostermeier J, Stalla GK. Thyrotropin-releasing hormone time-dependently influences thyrotropin microheterogeneity--an in vivo study in euthyroidism. J Endocrinol 2000; 166:137-143
603. Tjornstrand A, Gunnarsson K, Evert M, Holmberg E, Ragnarsson O, Rosen T, Filipsson Nystrom H. The incidence rate of pituitary adenomas in western Sweden for the period 2001-2011. Eur J Endocrinol 2014; 171:519-526
604. Beck-Peccoz P, Lania A, Beckers A, Chatterjee K, Wemeau J-L. 2013 European Thyroid Association Guidelines for the Diagnosis and treatment of TSH-secreting pituitary tumors. in press 2013;
605. Refetoff S, Bassett JH, Beck-Peccoz P, Bernal J, Brent G, Chatterjee K, De Groot LJ, Dumitrescu AM, Jameson JL, Kopp PA, Murata Y, Persani L, Samarut J, Weiss RE, Williams GR, Yen PM. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. Thyroid 2014; 24:407-409
606. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev 1993; 14:348-399
607. Refetoff S, DeGroot LJ, Benard B, DeWind LT. Studies of a sibship with apparent hereditary resistance to the intracellular action of thyroid hormone. Metabolism 1972; 21:723-756
608. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta 2013; 1830:3987-4003
609. Laurberg P. Persistent problems with the specificity of immunometric TSH assays. Thyroid 1993; 4:279-283
610. Sapin R, d'Herbomez M, Schlienger JL, Wemeau JL. Anti-thyrotropin antibody interference in thyrotropin assays. Clin Chem 1998; 44:2557-2559
611. Hattori N, Ishihara T, Shimatsu A. Variability in the detection of macro TSH in different immunoassay systems. Eur J Endocrinol 2016; 174:9-15
612. Drees JC1, Stone JA, Reamer CR, Arboleda VE, Huang K, Hrynkow J, Greene DN, Petrie MS, Hoke C, Lorey TS, Dlott RS. Falsely Undetectable TSH in a Cohort of South Asian Euthyroid Patients. J Clin Endocrinol Metab 2014; 99:1171-1179
613. Pappa T, Johannesen J, Scherberg N, Torrent M, Dumitrescu A, Refetoff S. A TSHbeta Variant with Impaired Immunoreactivity but Intact Biological Activity and Its Clinical

Implications. Thyroid 2015; 25:869-876
614. Medeiros-Neto G, Herodotou DT, Rajan S, Kommareddi S, de Lacerda L, Sandrini R, Boguszewski MC, Hollenberg AN, Radovick S, Wondisford FE. A circulating, biologically inactive thyrotropin caused by a mutation in the beta subunit gene. J Clin Invest 1996; 97:1250-1256
615. Sertedaki A, Papadimitriou A, Voutetakis A, Dracopoulou M, Maniati-Christidi M, Dacou-Voutetakis C. Low TSH congenital hypothyroidism: identification of a novel mutation of the TSH beta-subunit gene in one sporadic case (C85R) and of mutation Q49stop in two siblings with congenital hypothyroidism. Pediatr Res 2002; 52:935-941
616. Grunert SC, Schmidts M, Pohlenz J, Kopp MV, Uhl M, Schwab KO. Congenital Central Hypothyroidism due to a Homozygous Mutation in the TSHbeta Subunit Gene. Case reports in pediatrics 2011; 2011:369871
617. Saravanan P, Dayan CM. Thyroid autoantibodies. Endocrinol Metab Clin N Am 2001; 30:315-337
618. Schardt CW, McLaughlan SM, Matheson J, Smith BR. An enzyme-linked immunoassay for thyroid microsomal antibodies. J Immunol Methods 1982; 55:155-168
619. Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metab 1990; 71:661-669
620. Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N. TSH receptor autoantibody immunoassay in patients with Graves' disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. Autoimmun Rev 2012; 12:107-113
621. Kahaly GJ. Bioassays for TSH Receptor Antibodies: Quo Vadis? Eur Thyroid J 2015; 4:3-5
622. Massart C, Sapin R, Gibassier J, Agin A, d'Herbomez M. Intermethod variability in TSH-receptor antibody measurement: implication for the diagnosis of Graves disease and for the follow-up of Graves ophthalmopathy. Clin Chem 2009; 55:183-186
623. Tozzoli R, Villalta D, Bizzaro N. Challenges in the Standardization of Autoantibody Testing: a Comprehensive Review. Clinical reviews in allergy & immunology 2016;
624. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endoc Rev 1998; 19:673-616
625. Davies T, Marians R, Latif R. The TSH receptor reveals itself. J Clin Invest 2002; 110:161-164
626. Smith BR, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. Endocrinol Rev 1988; 9:106-121
627. Adams DD. Long-acting thyroid stimulator: how receptor autoimmunity was discovered. Autoimmunity 1988; 1:3-9
628. McKenzie MJ, Zakarija M. Antibodies in autoimmune thyroid disease. 6th. Edition ed. Philadelphia: J B Lippincott.
629. Ando T, Latif R, Davies TF. Thyrotropin receptor antibodies: new insights into their actions and clinical relevance. Best Pract Res Clin Endocrinol Metab 2005; 19:33-52
630. Noh JY, Hamada N, Inoue Y, Abe Y, Ito K, Ito K. Thyroid-Stimulating Antibody is Related to Graves' Ophthalmopathy, But Thyrotropin-Binding Inhibitor Immunoglobulin is Related to Hyperthyroidism in Patients with Graves' Disease. Thyroid 2000; 10:809-813
631. Mizutori Y, Chen CR, Latrofa F, McLachlan SM, Rapoport B. Evidence that shed thyrotropin receptor A subunits drive affinity maturation of autoantibodies causing Graves' disease. J Clin Endocrinol Metab 2009; 94:927-935
632. Woo YJ, Jang SY, Lim TH, Yoon JS. Clinical Association of Thyroid Stimulating Hormone Receptor Antibody Levels with Disease Severity in the Chronic Inactive Stage of Graves' Orbitopathy. Korean journal of ophthalmology : KJO 2015; 29:213-219
633. Gupta MK. Thyrotropin-receptor antibodies in thyroid diseases: advances in

detection techniques and clinical applications. Clin Chim Acta 2000; 293:1-29
634. Kahaly GJ, Diana T, Glang J, Kanitz M, Pitz S, Konig J. Thyroid Stimulating Antibodies Are Highly Prevalent in Hashimoto's Thyroiditis and Associated Orbitopathy. J Clin Endocrinol Metab 2016; 101:1998-2004
635. Morgenthaler NG, Ho SC, Minich WB. Stimulating and blocking thyroid-stimulating hormone (TSH) receptor autoantibodies from patients with Graves' disease and autoimmune hypothyroidism have very similar concentration, TSH receptor affinity, and binding sites. J Clin Endocrinol Metab 2007; 92:1058-1065
636. Yoshida K, Aizawa Y, Kaise N, Fukazawa H, Kiso Y, Sayama N, Mori K, Hori H, Abe K. Relationship between thyroid-stimulating antibodies and thyrotropin-binding inhibitory immunoglobulins years after administration of radioiodine for Graves' disease: retrospective clinical survey. J Endocrinol Invest 1996; 19:682-686
637. Quadbeck B, Hoermann R, Hahn S, Roggenbuck U, Mann K, Janssen OE. Binding, stimulating and blocking TSH receptor antibodies to the thyrotropin receptor as predictors of relapse of Graves' disease after withdrawal of antithyroid treatment. Horm Metab Res 2005; 37:745-750
638. McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. Thyroid 2013; 23:14-24
639. Seetharamaiah GS, Kurosky A, Desai RK, Dallas JS, Prabhakar VS. A recombinant extracellular domain of the thyrotropin (TSH) receptor bindsTSH in the absence of membranes. Endocrinol 1994; 134:549-554
640. Sugawa H, Ueda Y, M. U, al e. Immunization with the "immunogenic peptide" of TSH receptor induces oligoclonal antibodies with various biological activities. Peptides 1998; 19:1301-1307
641. Davies TF, Ando T, Lin RY, Tomer Y, Latif R. Thyrotropin receptor-associated diseases: from adenomata to Graves disease. J Clin Invest 2005; 115:1972-1983
642. Takasu N, oshiro C, Akamine H, Komiya I, Nagata A, Sato Y, Yoshimura H, Ito K. Thyroid-stimulating antibody and TSH-binding inhibitor immunoglobulin in 277 Graves' patients and in 686 normal subjects. J Endocrinol Invest 1997; 20:452-461
643. Kung AW, Jones BM. A change from stimulatory to blocking antibody activity in Graves' disease during pregnancy. J Clin Endocrinol Metab 1998; 83:514-518
644. Michalek K, Morshed SA, Latif R, Davies TF. TSH receptor autoantibodies. Autoimmun Rev 2009; 9:113-116
645. Kamijo K. TSH-receptor antibodies determined by the first, second and third generation assays and thyroid-stimulating antibody in pregnant patients with Graves' disease. Endocr J 2007; 54:619-624
646. Tozzoli R, D'Aurizio F, Villalta D, Giovanella L. Evaluation of the first fully automated immunoassay method for the measurement of stimulating TSH receptor autoantibodies in Graves' disease. Clin Chem Lab Med 2017; 55:58-64
647. Kasagi K, Konishi J, Iida Y, Ikekubo K, Mori T, Kuma K, Torizuka K. A new in vitro assay for human thyroid stimulator using cultured thyroid cells: effect of sodium chloride on adenosine 3',5'-monophosphate increase. 54 1982:108-114
648. Shewring G, Rees-Smith B. An improved radioreceptor assay for TSH receptor antibodies. Clin Endocrinol 1982; 17:409-417
649. Tokuda Y, Kasagi K, LidaY, Hatabu H, Hidaka A, Misaki T, Konishi J. Sensitive, practical bioassay of thyrotropin, with use of FRTL-5 thyroid cells and magnetizable solid-phase-bound antibodies. Clin Chem 1988; 34:2360-2364
650. McKenzie JM, Zakarija M. LATS in Graves' disease. Recent Prog Horm Res 1976; 33:29-57
651. Kasagi K, Konishi J, Iida Y, Arai K, Endo K, Torizuka K. A sensitive and practical

assay for thyroid stimulating antibodies using FRTL-5 thyroid cells. Acta Endocrinol 1987; 115:30-36
652. Morris JC, Hay ID, Nelson RE, Jiang N-S. Clinical utility of thyrotropin-receptor antibody assays: comparison of radioreceptor and bioassay methods. Mayo Clin Proc 1988; 63:707-717
653. Michelangeli VP, Munro DS, Poon CW, Frauman AG, Colman PG. Measurement of thyroid stimulating immunoglobulins in a new cell line transfected with a functional human TSH receptor (JPO9 cells), compared with an assay using FRTL-5 cells. Clin Endocrinol 1994; 40:645-652
654. Minich WB, Behr M, Loos U. Expression of a functional tagged human thyrotropin receptor in HeLa cells using recombinant vaccinia virus. Exp Clin Endocrinol Diab 1997; 105:282-290
655. Morgenthaler NG, Pampel I, Aust G, SeisslerJ, Scherbaum WA. Application of a bioassay with CHO cells for the routine detection of stimulating and blocking autoantibodies to the TSH-receptor. Horm Metab Res 1998; 30:162-168
656. Kamijo K, Murayama H, Uzu T, Togashi K, Olivo PD, Kahaly GJ. Similar clinical performance of a novel chimeric thyroid-stimulating hormone receptor bioassay and an automated thyroid-stimulating hormone receptor binding assay in Graves' disease. Thyroid 2011; 21:1295-1299
657. Atger M, Misrahi M, Young J, Jolivet A, Orgiazzi J, Schaison G, Milgrom E. Autoantibodies interacting with purified native thyrotropin receptor. Eur J Biochem 1999; 265:1022-1031
658. Iida Y, Konishi J, Kasagi K, Kuma K, Torizuka K. Detection of TSH-binding inhibitor immunoglobulins by using the triton-solubilized receptor from human thyroid membranes. Endocrinol Jpn 1982; 29:227-231
659. Costagliola S, Morganthaler NG, Hoermann R, Badenhoop K, Struck J, Freitag D, Poertl s, Weglohner W, Hollidt JM, Quadbeck B, Dumont JE, Schumm-Draeger PM, Bergmann A, Mann K, Vassart G, Usadel KH. Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. J Clin Endocrinol Metab 1999; 84:90-97
660. Schott M, Feldkamp J, Bathan C, Fritzen R, Scherbaum WA, Seissler J. Detecting TSH-Receptor antibodies with the recombinant TBII assay: technical and clinical evaluation. Horm Metab Research 2000; 32:429-435
661. Giovanella L, Ceriani L, Garancini S. Clinical applications of the 2nd. generation assay for anti-TSH receptor antibodies in Graves' disease. Evaluation in patients with negative 1st. generation test. Clin Chem Lab med 2001; 39:25-28
662. Smith BR, Bolton J, Young S, Collyer A, Weeden A, Bradbury J, Weightman D, Perros P, Sanders J, Furmaniak J. A new assay for thyrotropin receptor autoantibodies. Thyroid 2004; 14:830-835
663. Massart C, Gibassier J, d'Herbomez M. Clinical value of M22-based assays for TSH-receptor antibody (TRAb) in the follow-up of antithyroid drug treated Graves' disease: comparison with the second generation human TRAb assay. Clin Chim Acta 2009; 407:62-66
664. Zöphel K, Roggenbuck D, Wunderlich G, Schott M. Continuously increasing sensitivity over three generations of TSH receptor autoantibody assays. Horm Metab Res 2010; 42:900-902
665. Costagliola S, Swillens S, Niccoli P, Dumont JE, Vassart G, Ludgate M. Binding assay for thyrotropin receptor autoantibodies using the recombinant receptor protein. J Clin Endocrinol Metab 1992; 75:1540-1544
666. Morgenthaler NG, Hodak K, Seissler J, Steinbrenner H, Pampel I, Gupta M, McGregor AM, Scherbaum WA, Banga JP. Direct binding of thyrotropin receptor

autoantibody to in vitro translated thyrotropin receptor: a comparison to radioreceptor assay and thyroid stimulating bioassay. Thyroid 1999; 9:466-475
667. Feldt-Rasmussen. Meta-analysis evaluation of the impact of thyrotropin receptor antibodies on long-term remission after medical therapy of Graves' disease. J Clin Endocrinol Metab 1994; 78:98-102
668. Cho BY, Shong MH, Yi KH, Lee HK, Koh CS, Min HK. Evaluation of serum basal thyrotropin levels and thyrotropin receptor antibody levels and thyrotropin receptor antibody activities as prognostic markers for discontinuation of antithyroid drug treatment in patients with Graves' disease. Clin Endocrinol 1992; 36:585-590
669. Shibayama K, Ohyama Y, Yokota Y, Ohtsu S, Takubo N, Matsuura N. Assays for thyroid-stimulating antibodies and thyrotropin-binding inhibitory immunoglobulins in children with Graves' disease. Endoc J 2005; 52:505-510
670. Pedersen IB, Knudsen N, Perrild H, Ovesen L, Laurberg P. TSH-receptor antibody measurement for differentiation of hyperthyroidism into Graves' disease and multinodular toxic goitre: a comparison of two competitive binding assays. Clin Endocrinol 2001; 55:381-390
671. Tan K, Loh TP, Sethi S. Lack of standardized description of TRAb assays. Endocrine 2012;
672. Barbesino G, Tomer Y. Clinical review: Clinical utility of TSH receptor antibodies. J Clin Endocrinol Metab 2013; 98:2247-2255
673. Evans M, Sanders J, Tagami T, Sanders P, Young S, Roberts E, Wilmot J, Hu X, Kabelis K, Clark J, Holl S, Richards T, Collyer A, Furmaniak J, Smith BR. Monoclonal autoantibodies to the TSH receptor, one with stimulating activity and one with blocking activity, obtained from the same blood sample. CLIN Endocrinol (Oxf) 2010; 73:404-412
674. Bartalena L, Marcocci C, Bogazzi F, al e. Relation between therapy for hyperthyroidism and the course of Graves' disease. N Engl J Med 1998; 338:73-78
675. Eckstein AK, Plicht M, Lax H, Neuhäuser M, Mann K, Lederbogen S, Heckmann C, Esser J, Morgenthaler NG. Thyrotropin receptor autoantibodies are independent risk factors for Graves' ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab 2006; 91:3464-3470
676. Bech K. Immunological aspects of Graves' disease and importance of thyroid stimulating immunoglobulins. Acta Endocrinol (Copenh) Suppl 1983; 103:5-38
677. Feldt-Rasmussen U. Serum thyroglobulin and thyroglobulin autoantibodies in thyroid disease. Allergy 1983; 38:369-387
678. Nygaard B, Metcalfe RA, Phipps J, Weetman AP, Hegedus L. Graves' disease and thyroid-associated opthalmopathy triggered by 131I treatment of non-toxic goitre. J Endocrinol Invest 1999; 22:481-485
679. Gerding MN, van der Meer Jolanda WC, Broenink M, Bakker O, WM W, Prummel MF. Association of thyrotropin receptor antibodies with the clinical features of Graves' opthalmopathy. Clin Endocrinol 2000; 52:267-271
680. Takamura Y, Nakano K, Uruno T, Ito Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, Kuma K, Miyauchi A. Changes in serum TSH receptor antibody (TRAb) values in patients with Graves' disease after total or subtotal thyroidectomy. Endoc J 2003; 50:595-601
681. Zimmermann-Belsing T, Nygaard B, Rasmussen AK, Feldt-Rasmussen U. Use of the 2nd generation TRAK human assay did not improve prediction of relapse after antithyroid medical therapy of Graves' disease. Eur J Endocrinol 2002; 146:173-177
682. Schott M, Morgenthaler NG, Fritzen R, Feldkamp J, Willenberg HS, Scherbaum WA, Seissler J. Levels of autoantibodies against human TSH receptor predict relapse of hyperthyroidism in Graves' disease. Horm Metab Res 2004; 36:92-96
683. Carella C, Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Nersita R, Iorio S,

Amato G, Braverman LE, Roti E. Serum thyrotropin receptor antibodies concentrations in patients with Graves' disease before, at the end of methimazole treatment, and after drug withdrawal: evidence that the activity of thyrotropin receptor antibody and/or thyroid response modify during the observation period. Thyroid 2006; 16:295-302
684. Morris JC. Clinical use of immunological assays of TSH Receptor autoantibodies. Thyroid Today 1998; 21:1-7
685. Michelangeli V, Poon C, taft J, Newnham H, Topliss D, Colman P. The prognostic value of thyrotropin receptor antibody measurement in the early stages of treatment of Graves' disease with antithyroid drugs. Thyroid 1998; 8:119-124
686. McKenzie JM, Zakarija M. Fetal and neonatal hyperthyroidism and hypothyroidism due to maternal TSH receptor antibodies [Review]. Thyroid 1992; 2:155-159
687. Chan GW, Mandel SJ. Therapy insight: management of Graves' disease during pregnancy. Nat Clin Pract Endocrinol Metab 2007; 3:470-478
688. Nor Azlin MI, Bakin YD, Mustafa N, Wahab NA, Johari MJ, Kamarudin NA, Jamil MA. Thyroid autoantibodies and associated complications during pregnancy. j Obstet Gynaecol 2010; 30:675-678
689. Hamada N, Momotani N, Ishikawa N, Yoshimura Noh J, Okamoto Y, Konishi T, Ito K, Ito K. Persistent high TRAb values during pregnancy predict increased risk of neonatal hyperthyroidism following radioiodine therapy for refractory hyperthyroidism. Endoc J 2011; 58:55-58
690. Heithorn R, Hauffa BP, Reinwein D. Thyroid antibodies in children of mothers with autoimmune thyroid disorders. Eur J Pediatr 1999; 158:24-28
691. Abeillon-du Payrat J, Chikh K, Bossard N, Bretones P, Gaucherand P, Claris O, Charrie A, Raverot V, Orgiazzi J, Borson-Chazot F, Bournaud C. Predictive value of maternal second-generation thyroid-binding inhibitory immunoglobulin assay for neonatal autoimmune hyperthyroidism. Eur J Endocrinol 2014; 171:451-460
692. McLachlan SM, Rapoport B. Thyroid peroxidase autoantibody epitopes revisited. Clin Endocrinol 2008; 69:526-527
693. Jaume JC, Costante G, Nishikawa T, Phillips DI, Rapoport B, McLachlan SM. Thyroid peroxidase autoantibody fingerprints in hypothyroid and euthyroid individuals. I. Cross-sectional study in elderly women. J Clin Endocrinol Metab 1995; 80:994-999
694. Jaume JC, Burek CL, Hoffman WH, Rose NR, McLachlan SM, Rapoport B. Thyroid peroxidase autoantibody epitopic 'fingerprints' in juvenile Hashimoto's thyroiditis: evidence for conservation over time and in families. J Clin Endocrinol Metab 1996; 104:115-123
695. Czarnocka B, Szabolcs I, Pastuszko D, Feldkamp J, Dohán O, Podoba J, Wenzel B. In old age the majority of thyroid peroxidase autoantibodies are directed to a single TPO domain irrespective of thyroid function and iodine intake. Clin Endocrinol 1998; 48:803-808
696. Nielsen CH, Brix TH, Gardas A, Banga JP, Hegedüs L. Epitope recognition patterns of thyroid peroxidase autoantibodies in healthy individuals and patients with Hashimoto's thyroiditis. Clin Endocrinol 2008; 69:664-668
697. Ehlers M, Thiel A, Bernecker C, Porwol D, Papewalis C, Willenberg HS, Schinner S, Hautzel H, Scherbaum WA, Schott M. Evidence of a combined cytotoxic thyroglobulin and thyroperoxidase epitope-specific cellular immunity in Hashimoto's thyroiditis. J Clin Endocrinol Metab 2012; 97:1347-1354
698. Trotter WR, Belyavin G, Waddams A. Precipitating and complement fixing antibodies in Hashimoto's disease. Proc R Soc Med 1957; 50:961-?
699. Cayzer I, Chalmers SR, Doniach d, Swana G. An evaluation of two new haemagglutination tests for the rapid diagnosis of autoimmune thyroid disease. J Clin Pathol 1978; 31:1147-1151
700. Mariotti S, Russova A, Pisani S, Pinchera A. A new solid phase immunoradiometric assay for antithyroid microsomal antibody. J Clin Endocrinol Metab 1983; 56:467-473

701. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS Lett 1985; 190:147-152
702. Mariotti S, Anelli S, Ruf J, Bechi R, Czarnocka B, Lombardi A, Carayon P, Pinchera A. Comparison of serum thyroid microsomal and thyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metab 1987; 65:987-993
703. Hoier-Madsen M, Feldt-Rasmussen U, Hegedus L, Perrild H, Hansen HS. Enzyme-linked immunosorbent assay for determination of thyroglobulin autoantibodies. Acta Pathol Microbiol Scand 1984; 92:377-382
704. Ruf J, Czarnocka B, Ferrand M, Doullais F, Carayon P. Novel routine assay of thyroperoxidase autoantibodies. Clin Chem 1988; 34:2231-2234
705. Yokoyama N, Taurog A, Klee GG. Thyroid peroxidase and thyroid microsomal autoantibodies. J Clin Endocrinol Metab 1989; 68:766-773
706. Beever K, Bradbury J, Phillips D, McLachlan SM, Pegg C, Goral A, Overbeck W, Feifel G, BR< S. Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem 1989; 35:1949-1954
707. Groves CJ, Howells RD, Williams S, Darke C, Parkes AB. Primary standardization for the ELISA of serum thyroperoxidase and thyroglobulin antibodies and their prevalence in a normal Welsh population. J Clin Lab Immunol 1990; 32:147-151
708. Laurberg P, Pedersen KM, Vittinghus E, Ekelund S. Sensitive enzyme-linked immunosorbent assay for measurement of autoantibodies to human thyroid peroxidase. Scand J Clin Lab Invest 1992; 52:663-669
709. Finke R, Bogner u, Kotulla P, Schleusener H. Anti-TPO antibody determinations using different methods. Exp Clin Endocrinol 1994; 102:145-150
710. La'ulu SL, Slev PR, Roberts WL. Performance characteristics of 5 automated thyroglobulin autoantibody and thyroid peroxidase autoantibody assays. Clin Chim Acta 2007; 376:88-95
711. Feldt-Rasmussen U, Hoin-Madsen M, Beck K, al e. Anti-thyroid peroxidase antibodies in thyroid disorders and non thyroid autoimmune diseases. Autoimmunity 1991; 9:245-251
712. Kasagi K, Takahashi N, Inoue G, Honda T, Kawachi Y, Izumi Y. Thyroid function in Japanese adults as assessed by a general health checkup system in relation with thyroid-related antibodies and other clinical parameters. Thyroid 2009; 19:937-944
713. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT. Serum Thyroglobulin Autoantibodies: Prevalence, influence on serum thyroglobulin measurement and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 1998; 83:1121-1127
714. Guo J, Jaume JC, Rapoport B, McLachlan SM. Recombinant thyroid peroxidase-specific Fab converted to immunoglobulin G (IgG)molecules: evidence for thyroid cell damage by IgG1, but not IgG4, autoantibodies. J Clin Endocrinol Metab 1997; 82:925-931
715. Rebuffat SA, Nguyen B, Robert B, Castex F, Peraldi-Roux S. Antithyroperoxidase antibody-dependent cytotoxicity in autoimmune thyroid disease. J Clin Endocrinol Metab 2008; 93:929-934
716. Chiovato L, Bassi P, Santini F, Mammoli C, Lapi P, Carayon P, Pinchera A. Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. J Clin Endocrinol Metab 1993; 77:1700-1705
717. Karanikas G, Schuetz M, Wahl K, Paul M, Kontur S, Pietschmann P, Kletter K, Dudczak R, Willheim M. Relation of anti-TPO autoantibody titre and T-lymphocyte cytokine production patterns in Hashimoto's thyroiditis. Clin Endocrinol 2005; 63:191-196
718. Carmel R, Spencer CA. Clinical and subclinical thyroid disorders associated with pernicious anemia. Observations on abnormal thyroid-stimulating hormone levels and on a

possible association of blood group O with hyperthyroidism. Arch Intern Med 1982; 142:1465-1469
719. Vestgaard M, Nielsen LR, Rasmussen AK, Damm P, Mathiesen ER. Thyroid peroxidase antibodies in pregnant women with type 1 diabetes: impact on thyroid function, metabolic control and pregnancy outcome. Acta Obstet Gynecol Scand 2008; 87:1336-1342
720. Nakamura H, Usa T, Motomura M, Ichikawa T, Nakao K, Kawasaki E, Tanaka M, Ishikawa K, Eguchi K. Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders. J Endocrinol Invest 2008; 31:861-865
721. **Huber G**, Staub JJ, Meier C, Mitrache C, Guglielmetti M, **Huber P**, Braverman LE. Prospective Study of the Spontaneous Course of Subclinical Hypothyroidism: Prognostic Value of Thyrotropin, Thyroid Reserve, and Thyroid Antibodies. J Clin Endocrinol Metab 2002; 87:3221-3226
722. Mariotti S, Barbesino G, Caturegli P, Atzeni F, Manetti L, Marinò M, Grasso L, Velluzzi F, Loviselli A, Pinchera A. False negative results observed in anti-thyroid peroxidase autoantibody determination by competitive radioimmunoassays using monoclonal antibodies. Eur J Endocrinol 1994; 130:552-558
723. Schmidt M, Voell M, Rahlff I, Dietlein M, Kobe C, Faust M, Schicha H. Long-term follow-up of antithyroid peroxidase antibodies in patients with chronic autoimmune thyroiditis (Hashimoto's thyroiditis) treated with levothyroxine. Thyroid 2008; 18:755-760
724. Johnston AM, Eagles JM. Lithium-associated clinical hypothyroidism. Prevalence and risk factors. Br J Psychiatry 1999; 175:336-339
725. Bell TM, Bansal AS, Shorthouse C, et al. Low titre autoantibodies predict autoimmune disease during interferon alpha treatment of chronic hepatitis C. J Gastroenterol Hepatol 1999; 14:419-422
726. Daniels GH. Amiodarone-induced thyrotoxicosis. J Clin Endocrinol Metab 2001; 86:3-8
727. Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of Amiodarone on the Thyroid. Endoc Rev 2001; 22:240-254
728. Eskes SA, Wiersinga WM. Amiodarone and thyroid. Best Pract Res Clin Endocrinol Metab 2009; 23:735-751
729. Bocchetta A, Mossa P, Velluzzi F, Mariotti S, Zompo MD, Loviselli A. Ten-year follow-up of thyroid function in lithium patients. J Clin Psychopharmacol 2001; 21:594-598
730. Tsang W, Houlden RL. Amiodarone-induced thyrotoxicosis: a review. Can J Cardiol 2009; 25:421-424
731. Mecacci F, Parretti E, Cioni R, Lucchetti R, Magrini A, La Torre P, Mignosa M, Acanfora L, Mello G. Thyroid autoimmunity and its association with non-organ-specific antibodies and subclinical alterations of thyroid function in women with a history of pregnancy loss or preeclampsia. J Reprod Immunol 2000; 46:39-50
732. Stagnaro-Green A, Roman SH, Cobin RH, el-Harazy E, Alvarez-Marfany M, Davies TF. Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. JAMA 1990; 264:1422-1425
733. Glinoer D, Riahi M, Grun JP, Kinthaert J. Risk of subclinical hypothyroidism in pregnant women with asymptomatic autoimmune thyroid disorders. J Clin Endocrinol Metab 1994; 79:1970
734. Premawardhana LD, Parkes AB, AMMARI F, John R, Darke C, Adams H, Lazarus JH. Postpartum thyroiditis and long-term thyroid status: prognostic influence of Thyroid Peroxidase Antibodies and ultrasound echogenicity. J Clin Endocrinol Metab 2000; 85:71-75
735. Bussen S, Steck T, Dietl J. Increased prevalence of thyroid antibodies in euthyroid women with a history of recurrent in-vitro fertilization failure. Hum Reprod 2000; 15:545-548
736. Muller AF, Drexhage HA, Berghout A. Postpartum thyroiditis and autoimmune thyroiditis in women of childbearing age: recent insights and consequences for antenatal

and postnatal care. . Endocrinol Rev 2001; 22:605-630
737. Vaquero E, Lazzarin N, De Carolis C, Valensise H, Moretti C, Romanini C. Mild thyroid abnormalities and recurrent spontaneous abortion: diagnostic and therapeutical approach. Am J Reprod Immunol 2000; 43:204-208
738. Kim CH, Chae HD, Kang BM, Chang YS. Influence of antithyroid antibodies in euthyroid women on in vitro fertilization-embryo transfer outcome. Am J Reprod Immunol 1998; 40:2-8
739. Poppe K, Glinoer D, tournaye H, Devroey P, Van Steirteghem a, Kaufman L, Velkeniers B. Assisted reproduction and thyroid autoimmunity: an unfortunate combination? J Clin Endocrinol Metab 2003; 88:4149-4152
740. Negro R, Formoso G, Coppola L, Presicce G, Mangieri T, Pezzarossa A, Dazzi D. Euthyroid women with autoimmune disease undergoing assisted reproduction technologies: the role of autoimmunity and thyroid function. J Endocrinol Invest 2007; 30:3-8
741. Karakosta P, Alegakis D, Georgiou V, Roumeliotaki T, Fthenou E, Vassilaki M, Boumpas D, Castanas E, Kogevinas M, Chatzi L. Thyroid Dysfunction and Autoantibodies in Early Pregnancy Are Associated with Increased Risk of Gestational Diabetes and Adverse Birth Outcomes. J Clin Endocrinol Metab 2012; 97:4464-4472
742. He X, Wang P, Wang Z, He X, Xu D, Wang B. Thyroid antibodies and risk of preterm delivery: a meta-analysis of prospective cohort studies. Eur J Endocrinol 2012; 167:455-464
743. McLachlan SM, Rapoport B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies. Thyroid 2004; 14:510-520
744. Ericsson UB, Christensen SB, Thorell JI. A high prevalence of thyroglobulin autoantibodies in adults with and without thyroid disease as measured with a sensitive solid-phase immunosorbent radioassay. Clin Immunol Immunopathol 1985; 37:154-162
745. Krahn J, Dembinski T. Thyroglobulin and anti-thyroglobulin assays in thyroid cancer monitoring. Clin Biochem 2009; 42:416-419
746. Latrofa F, Ricci D, Montanelli L, Rocchi R, Piaggi P, Sisti E, Grasso L, Basolo F, Ugolini C, Pinchera A, Vitti P. Thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: comparison of different assays and evaluation of causes of discrepancies. J Clin Endocrinol Metab 2012; 97:3974-3982
747. Pickett AJ, Jones M, Evans C. Causes of discordance between thyroglobulin antibody assays. Ann Clin Biochem 2012; 49:463-467
748. Pinchera A, Mariotti S, Vitti P, Tosi M, Grasso L, Facini F, Buti R, Baschieri L. Interference of serum thyroglobulin in the radioassay for serum antithyroglobulin antibodies. J Clin Endocrinol Metab 1977; 45:1077-1088
749. Mariotti S, Pinchera A, Vitti P, Chiovato L, Marcocci C, Urbano C, Tosi M, Baschieri L. Comparison of radioassay and haemagglutination methods for anti-thyroid microsomal antibodies. Clin Exp Immunol 1978; 34:118-125
750. Gao Y, Yuan Z, Yu Y, Lu H. Mutual interference between serum thyroglobulin and antithyroglobulin antibody in an automated chemiluminescent immunoassay. Clin Biochem 2007; 40:735-738
751. Ruf J, Henry M, DeMicco C, Carayon P. Characterization of monoclonal and autoimmune antibodies to thyroglobulin : application to clinical investigation. In: Hufner M, Reiners C, eds Thyroglobulin and thyrolobulin antibodies in the follow-up of thyroid cancer and endemic goiter Stuttgart: G Thieme Verlag 1987:21-30
752. Okosieme OE, Evans C, Moss L, Parkes AB, Premawardhana LD, Lazarus JH. Thyroglobulin antibodies in serum of patients with differentiated thyroid cancer: relationship between epitope specificities and thyroglobulin recovery. Clin Chem 2005; 51:729-734
753. Taylor KP, Parkington D, Bradbury S, Simpson HL, Jefferies SJ, Halsall DJ. Concordance between thyroglobulin antibody assays. Ann Clin Biochem 2011; 48:367-369
754. Jensen EA, Petersen PH, Blaabjerg O, Hansen PS, Brix TH, Hegedüs L.

Establishment of reference distributions and decision values for thyroid antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). Clin Chem Lab Med 2006; 44:991-998
755. Ruf J, Carayon P, Lissitzky S. Various expressions of a unique anti-human thyroglobulin antibody repertoire in normal state and autoimmune disease. Eur J Immunol 1985; 15:268-272
756. Doullay F, Ruf J, Codaccioni JL, et al. Prevalence of autoantibodies to thyroperoxidase in patients with various thyroid and autoimmune diseases. Autoimmunity 1991; 9:237-244
757. Aras G, Gültekin SS, Küçük NO. The additive clinical value of combined thyroglobulin and antithyroglobulin antibody measurements to define persistent and recurrent disease in patients with differentiated thyroid cancer. Nucl Med Commun 2008; 29:880-884
758. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. The American Thyroid Association Guidelines Taskforce. Thyroid 2006; 16:109-142
759. Kumar A, Shah DH, Shrihari U, Dandekar SR, Vijayan U, Sharma SM. Significance of antithyroglobulin autoantibodies in differentiated thyroid carcinoma. Thyroid 1994; 4:199-202
760. Gorges R, Maniecki M, Jentzen W, Sheu SN, Mann K, Bockisch A, Janssen OE. Development and clinical impact of thyroglobulin antibodies in patients with differentiated thyroid carcinoma during the first 3 years after thyroidectomy. Eur J Endocrinol 2005; 153:49-55
761. Spencer CA. Clinical review: Clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol Metab 2011; 96:3615-3627
762. Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, Ryu JS, Gong G, Hong SJ, Shong YK. Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 2008; 93:4683-4689
763. Pacini F, Mariotti S, Formica N, Elisei R. Thyroid autoantibodies in thyroid cancer: Incidence and relationship with tumor outcome. Acta Endocrinol (Copenh) 1988; 119:373-380
764. Rubello D, Casara D, Girelli ME, Piccolo M, Busnardo B. Clinical meaning of circulating antithyroglobulin antibodies in differentiated thyroid cancer: a prospective study. J Nucl Med 1992; 33:1478-1480
765. Spencer CA. New Insights for Using Serum Thyroglobulin (Tg) Measurement for Managing Patients with Differentiated Thyroid Carcinomas. Thyroid International 2003; 4:1-14
766. Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, Grasso L, Pinchera A. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. Ann Intern Med 2003; 139:346-351
767. Tomer Y, Greenberg D. The thyroglobulin gene as the first thyroid-specific susceptibility gene for autoimmune thyroid disease. Trends Mol Med 2004; 10:306-308
768. Van den Briel T, West CE, Hautvast JG, Vulsma T, de Vijlder JJ, Ategbo EA. Serum thyroglobulin and urinary iodine concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply in schoolchildren in Benin. J Nutr 2001; 131:2701-2706
769. Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Carlé A, Pedersen IB, Rasmussen LB, Ovesen L, Jørgensen T. Thyroglobulin as a marker of iodine nutrition status in the general

population. Eur J Endocrinol 2009; 161:475-481
770. Wang Z, Zhang H, Zhang X, Sun J, Han C, Li C, Li Y, Teng X, Fan C, Liu A, Shan Z, Liu C, Weng J, Teng W. Serum thyroglobulin reference intervals in regions with adequate and more than adequate iodine intake. Medicine (Baltimore) 2016; 95:e5273
771. Bath SC, Pop VJ, Furmidge-Owen VL, Broeren MA, Rayman MP. Thyroglobulin as a Functional Biomarker of Iodine Status in a Cohort Study of Pregnant Women in the United Kingdom. Thyroid 2016;
772. Vali M, Rose NR, Caturegli P. Thyroglobulin as autoantigen: structure-function relationships. Rev Endocr Metab Disord 2000; 1:69-77
773. Knobel M, Medeiros-Neto G. An outline of inherited disorders of the thyroid hormone generating system. Thyroid 2003; 13:771-801
774. Niu DM, Hsu JH, Chong KW, Huang CH, Lu YH, Kao CH, Yu HC, Lo MY, Jap TS. Six new mutations of the thyroglobulin gene discovered in taiwanese children presenting with thyroid dyshormonogenesis. J Clin Endocrinol Metab 2009; 94:5045-5052
775. Citterio CE, Machiavelli GA, Miras MB, Gruñeiro-Papendieck L, Lachlan K, Sobrero G, Chiesa A, Walker J, Muñoz L, Testa G, Belforte FS, González-Sarmiento R, Rivolta CM, Targovnik HM. New insights into thyroglobulin gene: Molecular analysis of seven novel mutations associated with goiter and hypothyroidism. Mol Cell Endocrinol 2013; 365:277-291
776. Fugazzola L, Persani L, Mannavola D, Reschini E, Vannucchi G, Weber G, Beck-Peccoz P. Recombinant human TSH testing is a valuable tool for differential diagnosis of congenital hypothyroidism during L-thyroxine replacement. Clin Endocrinol (Oxf) 2003; 59:230-236
777. Simsek E, Karabay M, Kocabay K. Neonatal screening for congenital hypothyroidism in West Black Sea area, Turkey. Int J Clin Pract 2005; 59:336-341
778. Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L, Pinchera A. Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med 1982; 307:410-412
779. Pacini F, Pinchera A. Serum and tissue thyroglobulin measurement: clinical applications in thyroid disease. Biochimie 1999; 81:463-467
780. Chow E, Siddique F, Gama R. Thyrotoxicosis factitia: role of thyroglobulin. Ann Clin Biochem 2008; 45:447-448
781. Torrens JI, Burch HB. Serum thyroglobulin measurement. Utility in clinical practice. Endocrinol Metab Clin N Amer 2001; 30:429-467
782. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 2006; 154:787-803
783. Iervasi A, Iervasi G, Carpi A, Zucchelli GC. Serum thyroglobulin measurement: clinical background and main methodological aspects with clinical impact. Biomed Pharmacother 2006; 60:414-424
784. Spencer CA, Petrovic I. Thyroglobuin measurement. In: Thyroid Function Testing Editor Gregory Brent MD Springer Science, MA, USA 2010
785. Tuttle RM, Leboeuf R. Follow up Approaches in Thyroid Cancer: A Risk Adapted Paradigm. Endocrinol Metab Clin North Am 2008; 37:419-435
786. Lin JD. Thyroglobulin and human thyroid cancer. Clin Chim Acta 2008; 388:15-21
787. Pitoia F, Ward L, Wohllk N, Friguglietti C, Tomimori E, Gauna A, Camargo R, Vaisman M, Harach R, Munizaga F, Corigliano S, Pretell E, Niepomniszcze H. Recommendations of the Latin American Thyroid Society on diagnosis and management of differentiated thyroid cancer. Arq Bras Endocrinol Metabol 2009; 53:884-887
788. Brassard M, Borget I, Edet-Sanson A, Giraudet AL, Mundler O, Toubeau M, Bonichon F, Borson-Chazot F, Leenhardt L, Schvartz C, Dejax C, Brenot-Rossi I, Toubert

ME, Torlontano M, Benhamou E, Schlumberger M. Long-term follow-up of patients with papillary and follicular thyroid cancer: a prospective study on 715 patients. J Clin Endocrinol Metab 2011; 96:1352-1359
789. Rosario PW, Borges MA, Fagundes TA, Franco AC, Purisch S. Is stimulation of thyroglobulin (Tg) useful in low-risk patients with thyroid carcinoma and undetectable Tg on thyroxin and negative neck ultrasound? Clin Endocrinol (Oxf) 2005; 62
790. Grebe SKG. Diagnosis and management of thyroid carcinoma: a focus on serum thyroglobulin. Expert Rev Endocrinol Metab 2009; 4:25-43
791. Malandrino P, Latina A, Marescalco S, Spadaro A, Regalbuto C, Fulco RA, Scollo C, Vigneri R, Pellegriti G. Risk-adapted management of differentiated thyroid cancer assessed by a sensitive measurement of basal serum thyroglobulin. J Clin Endocrinol Metab 2011; 96:1703-1709
792. Chindris AM, Diehl NN, Crook JE, Fatourechi V, Smallridge RC. Undetectable sensitive serum thyroglobulin (<0.1 ng/ml) in 163 patients with follicular cell-derived thyroid cancer: results of rhTSH stimulation and neck ultrasonography and long-term biochemical and clinical follow-up. J Clin Endocrinol Metab 2012; 97:2714-2723
793. Trimboli P, La Torre D, Ceriani L, Condorelli E, Laurenti O, Romanelli F, Ventura C, Signore A, Valabrega S, Giovanella L. High sensitive thyroglobulin assay on thyroxine therapy: can it avoid stimulation test in low and high risk differentiated thyroid carcinoma patients? Horm Metab Res 2013; 45:664-668
794. Mariotti S, Barbesino G, Caturegli P, Marino M, Manetti L, Pacini F, Centoni R, Pinchera A. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: an unobtainable goal? J Clin Endocrinol Metab 1995; 80:468-472
795. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyroglobulin assays. Clin Chem 1996; 42:164-173
796. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 1998; 83:1121-1127
797. Clark P, Franklyn J. Can we interpret serum thyroglobulin results? Ann Clin Biochem 2012; 49:313-322
798. Latrofa F, Ricci D, Sisti E, Piaggi P, Nencetti C, Marino M, Vitti P. Significance of Low Levels of Thyroglobulin Autoantibodies Associated with Undetectable Thyroglobulin After Thyroidectomy for Differentiated Thyroid Carcinoma. Thyroid 2016; 26:798-806
799. Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, Spencer CA, Turcu AF, Algeciras-Schimnich A. Thyroglobulin (Tg) Testing Revisited: Tg Assays, TgAb Assays, and Correlation of Results With Clinical Outcomes. J Clin Endocrinol Metab 2015; 100:E1074-1083
800. Jindal A, Khan U. Is Thyroglobulin Level by Liquid Chromatography Tandem-Mass Spectrometry Always Reliable for Follow-Up of DTC After Thyroidectomy: A Report on Two Patients. Thyroid 2016; 26:1334-1335
801. Azmat U, Porter K, Senter L, Ringel MD, Nabhan F. Thyroglobulin Liquid Chromatography-Tandem Mass Spectrometry Has a Low Sensitivity for Detecting Structural Disease in Patients with Antithyroglobulin Antibodies. Thyroid 2016;
802. Rosario PW, Mourao GF, Calsolari MR. Low postoperative nonstimulated thyroglobulin as a criterion for the indication of low radioiodine activity in patients with papillary thyroid cancer of intermediate risk 'with higher risk features'. Clin Endocrinol (Oxf) 2016; 85:453-458
803. Spencer CA, Wang CC. Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls. Endocrinol Metab Clin North Am 1995; 24:841-863
804. Spencer CA, Wang CC. Thyroglobulin measurement:- Techniques, clinical benefits

and pitfalls. Endocrinol Metab Clin N Amer 1995; 24:841-863
805. Zucchelli G, Iervasi A, Ferdeghini M, Iervasi G. Serum thyroglobulin measurement in the follow-up of patients treated for differentiated thyroid cancer. Q J Nucl Med Mol Imaging 2009; 53:482-489
806. Zophel K, Wunderlich G, Smith BR. Serum thyroglobulin measurements with a high sensitivity enzyme-linked immunosorbent assay: is there a clinical benefit in patients with differentiated thyroid carcinoma? Thyroid 2003; 13:861-865
807. Iervasi A, Iervasi G, Ferdeghini M, Solimeo C, Bottoni A, Rossi L, Colato C, Zucchelli GC. Clinical relevance of highly sensitive Tg assay in monitoring patients treated for differentiated thyroid cancer. Clin Endocrinol (Oxf) 2007; 67:434-441
808. Giovanella L. Highly sensitive thyroglobulin measurements in differentiated thyroid carcinoma management. Clin Chem Lab Med 2008; 46:1067-1073
809. Tomoda C, Miyauchi A. Undetectable serum thyroglobulin levels in patients with medullary thyroid carcinoma after total thyroidectomy without radioiodine ablation. Thyroid 2012; 22:680-682
810. Angell TE, Spencer CA, Rubino BD, Nicoloff JT, LoPresti JS. In Search of an Unstimulated Thyroglobulin Baseline Value in Low-Risk Papillary Thyroid Carcinoma Patients Not Receiving Radioactive Iodine Ablation. Thyroid 2014; 24:1127-1133
811. Haugen BR, Ladenson PW, Cooper DS, Pacini F, Reiners C, Luster M, Schlumberger M, Sherman SI, Samuels M, Graham K, Braverman LE, Skarulis MC, Davies TF, DeGroot L, Mazzaferri EL, Daniels GH, Ross DC, Becker DV, Mazon HR, Cavalieri RR, Spencer CA, McEllin K, Weintraub BD, EC. R. A comparison of Recombinant Human Thyrotropin and Thyroid Hormone Withdrawal for the Detection of Thyroid Remnant or Cancer. J Clin Endocrinol Metab 1999; 84:3877-3885
812. Pacini F, Castagna MG. Diagnostic and therapeutic use of recombinant human TSH (rhTSH) in differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab 2008; 22:1009-1021
813. Kloos RT, Mazzaferri EL. A single recombinant human thyrotropin-stimulated serum thyroglobulin measurement predicts differentiated thyroid carcinoma metastases three to five years later. J Clin Endocrinol Metab 2005; 90:5047-5057
814. Mazzaferri EL. Will highly sensitive thyroglobulin assays change the management of thyroid cancer? Clin Endocrinol (Oxf) 2007; 67:321-323
815. Persoon AC, Jager PL, Sluiter WJ, Plukker JT, Wolffenbuttel BH, Links TP. A sensitive Tg assay or rhTSH stimulated Tg: what's the best in the long-term follow-up of patients with differentiated thyroid carcinoma. PLoS ONE 2007; 2:e816
816. Spencer CA, Lopresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab 2008; 4:223-233
817. Spencer CA, Lopresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab 2008; 4:223-233
818. Bachelot A, Cailleux AF, Klain M, Baudin E, Ricard M, Bellon N, Caillou B, Travagli JP, Schlumberger M. Relationship between tumor burden and serum thyroglobulin level in patients with papillary and follicular thyroid carcinoma. Thyroid 2002; 12:707-711
819. Pacini F, Molinaro E, Castagna MG, Agate L, Elisei R, Ceccarelli C, Lippi F, Taddei D, Grasso L, Pinchera A. Recombinant human thyrotropin-stimulated serum thyroglobulin combined with neck ultrasonography has the highest sensitivity in monitoring differentiated thyroid carcinoma. J Clin Endocrinol Metab 2003; 88:3668-3673
820. Heilo A, Sigstad E, Fagerlid KH, Håskjold OI, Grøholt KK, Berner A, Bjøro T, Jørgensen LH. Efficacy of ultrasound-guided percutaneous ethanol injection treatment in patients with a limited number of metastatic cervical lymph nodes from papillary thyroid carcinoma. J Clin Endocrinol Metab 2011; 96:2750-2755
821. Hay ID, Lee RA, Davidge-Pitts C, Reading CC, Charboneau JW. Long-term outcome

of ultrasound-guided percutaneous ethanol ablation of selected "recurrent" neck nodal metastases in 25 patients with TNM stages III or IVA papillary thyroid carcinoma previously treated by surgery and 131I therapy. Surgery 2013; 154:1448-1454; discussion 1454-1445
822. Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, Carayon P, Ericsson UB, Koutras DA, Lamas de Leon L, DeNayer P, Pacini F, Palumbo G, Santos A, Schlumberger M, Seidel C, Van Herle AJ, DeVijlder JJM. Human thyroglobulin reference material (CRM 457) 1st part: Assessment of homogeneity, stability and immunoreactivity. Ann Biol Clin 1996; 54:337-342
823. Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, Carayon P, Ericsson UB, Koutras DA, Lamas de Leon L, DeNayer P, Pacini F, Palumbo G, Santos A, Schlumberger M, Seidel C, Van Herle AJ, DeVijlder JJM. Human thyroglobulin reference material (CRM 457) 2nd part: Physicochemical characterization and certification. Ann Biol Clin 1996; 54:343-348
824. Kim M, Jeon MJ, Kim WG, Lee JJ, Ryu JS, Cho EJ, Ko DH, Lee W, Chun S, Min WK, Kim TY, Shong YK, Kim WB. Comparison of Thyroglobulin Measurements Using Three Different Immunoassay Kits: A BRAMHS Tg-Plus RIA Kit, a BRAMHS hTg Sensitive Kryptor Kit, and a Beckman Coulter ACCESS Immunoassay Kit. Endocrinology and metabolism (Seoul, Korea) 2016;
825. Feldt-Rasmussen U, Petersen PH, Blaabjerg O, Horder M. Long-term variability in serum thyroglobulin and thyroid related hormones in healthy subjects. Acta Endocrinol (Copenh) 1980; 95:328-334
826. Heilig B, Hufner M, Dorken B, Schmidt-Gayk H. Increased heterogeneity of serum thyroglobulin in thyroid cancer patients as determined by monoclonal antibodies. Klin Wochenschr 1986; 64:776-780
827. Schulz R, Bethauser H, Stempka L, Heilig B, Moll A, Hufner M. Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. Eur J Clin Invest 1989; 19:459-463
828. de Micco C, Ruf J, Carayon P, Chrestian MA, Henry JF, Toga M. Immunohistochemical study of thyroglobulin in thyroid carcinomas with monoclonal antibodies. Cancer 1987; 59:471-476
829. Kim PS, Dunn AD, Dunn JT. Altered immunoreactivity of thyroglobulin in thyroid disease. J Clin Endocrinol Metab 1988; 67:161-168
830. Cubero JM, Rodríguez-Espinosa J, Gelpi C, Estorch M, Corcoy R. Thyroglobulin autoantibody levels below the cut-off for positivity can interfere with thyroglobulin measurement. Thyroid 2003; 13:659-661
831. Cole TG, Johnson D, Eveland BJ, Nahm MH. Cost-effective method for detection of "hook effect" in tumor marker immunometric assays. Clin Chem 1993; 39:695-696
832. Basuyau JP, Leroy M, Brunelle P. Determination of Tumor Markers in Serum. Pitfalls and Good Practice. Clin Chem Lab Med 2001; 39:1227-1233
833. Leboeuf R, Langlois MF, Martin M, Ahnadi CE, Fink GD. "Hook effect" in calcitonin immunoradiometric assay in patients with metastatic medullary thyroid carcinoma: case report and review of the literature. J Clin Endocrinol Metab 2006; 91:361-364
834. Jassam N, Jones CM, Briscoe T, Horner JH. The hook effect: a need for constant vigilance. Ann Clin Biochem 2006; 43:314-317
835. Selby C. Interference in immunoassay. Ann Clin Biochem 1999; 36:704-721
836. Morgenthaler NG, Froehlich J, Rendl J, Willnich M, Alonso C, Bergmann A, Reiners C. Technical evaluation of a new immunoradiometric and a new immunoluminometric assay for thyroglobulin. Clin Chem 2002; 48:1077-1083
837. Netzel BC, Grebe SK, Algeciras-Schimnich A. Usefulness of a thyroglobulin liquid chromatography-tandem mass spectrometry assay for evaluation of suspected heterophile interference. Clin Chem 2014; 60:1016-1018

838. Schaadt B, Feldt-Rasmussen U, Rasmussen B, Torring H, Foder B, Jorgensen K, Sand Hansen H. Assessment of the influence of thyroglobulin (Tg) autoantibodies and other interfering factors on the use of serum Tg as tumor marker in differentiated thyroid carcinoma. Thyroid 1995; 5:165-170
839. Spencer CA. Recoveries cannot be used to authenticate thyroglobulin (Tg) measurements when sera contain Tg autoantibodies. Clin Chem 1996; 42:661-663
840. Weigle WO, High GJ. The behaviour of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. J Immunol 1967; 98:1105-1114
841. Sellitti DF, Suzuki K. Intrinsic regulation of thyroid function by thyroglobulin. Thyroid 2014; 24:625-638
842. Igawa T, Haraya K, Hattori K. Sweeping antibody as a novel therapeutic antibody modality capable of eliminating soluble antigens from circulation. Immunological reviews 2016; 270:132-151
843. Tozzoli R, Bizzaro N, Tonutti E, Pradella M, Manoni F, Vilalta D, Bassetti D, Piazza A, Rizzotti P. Immunoassay of anti-thyroid autoantibodies: high analytical variability in second generation methods. Clin Chem Lab Med 2002; 40:568-573
844. Benvenga S, Burek CL, Talor M, Rose NR, Trimarchi F. Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in hashimoto's thyroiditis and non-autoimmune thyroid diseases. J Endocrinol Invest 2002; 25:977-982
845. Rosário PW, Maia FF, Fagundes TA, Vasconcelos FP, Cardoso LD, Purisch S. Antithyroglobulin antibodies in patients with differentiated thyroid carcinoma: methods of detection, interference with serum thyroglobulin measurement and clinical significance. Arq Bras Endocrinol Metabol 2004; 48:487-492
846. McLachlan SM, B. R. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies. Thyroid 2004; 14:510-520
847. Schneider AB, Pervos R. Radioimmunoassay of human thyroglobulin: effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 1978; 47:126-137
848. Feldt-Rasmussen U, Rasmussen AK. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vivo and in vitro. J Endocrinol Invest 1985; 8:571-576
849. Crane MS, Strachan MW, Toft AD, Beckett GJ. Discordance in thyroglobulin measurements by radioimmunoassay and immunometric assay: a useful means of identifying thyroglobulin assay interference. Ann Clin Biochem 2013; 50:421-432
850. Spencer C, Fatemi S. Thyroglobulin antibody (TgAb) methods - Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab 2013; 27:701-712
851. Feldt-Rasmussen U, Petersen PH, Date J, Madsen CM. Sequential changes in serum thyroglobulin (Tg) and its autoantibodies (TgAb) following subtotal thyroidectomy of patients with preoperatively detectable TgAb. Clin Endocrinol (Oxf) 1980; 12:29-38
852. Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. Blood 1988; 72:512-519
853. van der Laken CJ, Voskuyl AE, Roos JC, Stigter van Walsum M, de Groot ER, Wolbink G, Dijkmans BA, Aarden LA. Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. Ann Rheum Dis 2007; 66:253-256
854. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, Fontana M, Moon JC, Pinzani M, Gillmore JD, Hawkins PN, Pepys MB. Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component. N Engl J Med 2015; 373:1106-1114
855. Van Herle AJ, Uller RP. Elevated serum thyroglobulin: a marker of metastases in

differentiated thyroid carcinomas. J Clin Invest 1975; 56:272-277
856. Spencer CA, Platler BW, Nicoloff JT. The effect of 125-I thyroglobulin tracer heterogeneity on serum Tg RIA measurement. Clin Chim Acta 1985; 153:105-115
857. Spencer CA, Platler B, Guttler RB, Nicoloff JT. Heterogeneity of 125-I labelled thyroglobulin preparations. Clin Chim Acta 1985; 151:121-132
858. Black EG, Hoffenberg R. Should one measure serum thyroglobulin in the presence of anti-thyroglobulin antibodies? Clin Endocrinol (Oxf) 1983; 19:597-601
859. Weightman DR, Mallick UK, Fenwick JD, Perros P. Discordant serum thyroglobulin results generated by two classes of assay in patients with thyroid carcinoma: correlation with clinical outcome after 3 years of follow-up. Cancer 2003; 98:41-47
860. Jahagirdar VR, Strouhal P, Holder G, Gama R, Singh BM. Thyrotoxicosis factitia masquerading as recurrent Graves' disease: endogenous antibody immunoassay interference, a pitfall for the unwary. Ann Clin Biochem 2008; 45:325-327
861. Bayer MF, Kriss JP. Immunoradiometric assay for serum thyroglobulin: semiquantitative measurement of thyroglobulin in antithyroglobulin-positive sera. J Clin Endocrinol Metab 1979; 49:557-564
862. Latrofa F, Ricci D, Grasso L, Vitti P, Masserini L, Basolo F, Ugolini C, Mascia G, Lucacchini A, Pinchera A. Characterization of thyroglobulin epitopes in patients with autoimmune and non-autoimmune thyroid diseases using recombinant human monoclonal thyroglobulin autoantibodies. J Clin Endocrinol Metab 2008; 93:591-596
863. Rahmoun MN, Bendahmane I. Anti-thyroglobulin antibodies in differentiated thyroid carcinoma patients: Study of the clinical and biological parameters. Ann Endocrinol (Paris) 2014; 75:15-18
864. Marquet PY, Daver A, Sapin R, Bridgi B, Muratet JP, Hartmann DJ, Paolucci F, Pau B. Highly sensitive immunoradiometric assay for serum thyroglobulin with minimal interference from autoantibodies. Clin Chem 1996; 42:258-262
865. Haapala AM, Soppi E, Morsky P, al. e. Thyroid antibodies in association with thyroid malignancy II: qualitative properties of thyroglobulin antibodies. Scand J Clin Lab Invest 1995; 55:317-322
866. Netzel BC, Grant RP, Hoofnagle AN, Rockwood AL, Shuford CM, Grebe SK. First Steps toward Harmonization of LC-MS/MS Thyroglobulin Assays. Clin Chem 2016; 62:297-299
867. Petrovic I, S. Fatemi, J. LoPresti, S.K. Grebe, A. Algeciras-Schimnich, B.C. Netzel, C. Spencer. SerumTg is frequently undetectable by mass spectrometry (Tg-MS) IN TgAb-positive differentiated thyroid cancer (DTC) patients with structural disease. . Thyroid 2015; 25:A251
868. Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, Lee DS, Lee MC, Cho BY. Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol (Oxf) 2002; 57:215-221
869. Küçük ON, Aras G, Kulak HA, Ibiş E. Clinical importance of anti-thyroglobulin auto-antibodies in patients with differentiated thyroid carcinoma: comparison with 99mTc-MIBI scans. Nucl Med Commun 2006; 27:873-876
870. Thomas D, Liakos V, Vassiliou E, Hatzimarkou F, Tsatsoulis A, Kaldrimides P. Possible reasons for different pattern disappearance of thyroglobulin and thyroid peroxidase autoantibodies in patients with differentiated thyroid carcinoma following total thyroidectomy and iodine-131 ablation. J Endocrinol Invest 2007; 30:173-180
871. Feldt-Rasmussen U, Rasmussen AK. Autoimmunity in differentiated thyroid cancer: significance and related clinical problems. Hormones (Athens) 2010; 9:109-117
872. Hsieh CJ, Wang PW. Sequential changes of serum antithyroglobulin antibody levels are a good predictor of disease activity in thyroglobulin-negative patients with papillary thyroid carcinoma. Thyroid 2014; 24:488-493

873. Feldt-Rasmussen U, Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, Rimmele H, Seregni E, Smit JW, Theimer C, Giovanella L. Thyroglobulin autoantibodies as surrogate biomarkers in the management of patients with differentiated thyroid carcinoma. Current medicinal chemistry 2014; 21:3687-3692
874. Rosario PW, Carvalho M, Mourao GF, Calsolari MR. Comparison of Antithyroglobulin Antibody Concentrations Before and After Ablation with 131I as a Predictor of Structural Disease in Differentiated Thyroid Carcinoma Patients with Undetectable Basal Thyroglobulin and Negative Neck Ultrasonography. Thyroid 2016; 26:525-531
875. Nascimento C, Borget I, Troalen F, Al Ghuzlan A, Deandreis D, Hartl D, Lumbroso J, Chougnet CN, Baudin E, Schlumberger M, Leboulleux S. Ultrasensitive serum thyroglobulin measurement is useful for the follow-up of patients treated with total thyroidectomy without radioactive iodine ablation. Eur J Endocrinol 2013; 169:689-693
876. Spencer C, Fatemi S. Thyroglobulin antibody (TgAb) methods - Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab 2013; 27:701-712
877. Uller RP, Van Herle AJ. Effect of therapy on serum thyroglobulin levels in patients with Graves' disease. J Clin Endocrinol Metab 1978; 46:747-755
878. Feldt-Rasmussen U, Blichert-Toft M, Christiansen C, Date J. Serum thyroglobulin and its autoantibody following subtotal thyroid resection of Graves' disease. Eur J Clin Invest 1982; 12:203-208
879. Benvenga S, Bartolone L, Squadrito S, Trimarchi F. Thyroid hormone autoantibodies elicited by diagnostic fine needle biopsy. J Clin Endocrinol Metab 1997; 82:4217-4223
880. Polyzos SA, Anastasilakis AD. Alterations in serum thyroid-related constituents after thyroid fine-needle biopsy: a systematic review. Thyroid 2010; 20:265-271
881. Izumi M, Larsen PR. Correlation of sequential changes in serum thyroglobulin, triiodothyronine, and thyroxine in patients with Graves' disease and subacute thyroiditis. Metabolism 1978; 27:449-460
882. Gardner DF, Rothman J, Utiger RD. Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to Graves' disease: effects of T3, iodide, 131I and antithyroid drugs. Clin Endocrinol (Oxf) 1979; 11:585-594
883. Feldt-Rasmussen U, Bech K, Date J, Hyltoft Pedersen P, Johansen K, Nistrup Madsen S. Thyroid stimulating antibodies, thyroglobulin antibodies and serum proteins during treatment of Graves' disease with radioiodine or propylthiouracil. Allergy 1982; 37:161-167
884. Feldt-Rasmussen U, Bech K, Date J, Petersen PH, Johansen K. A prospective study of the differential changes in serum thyroglobulin and its autoantibodies during propylthiouracil or radioiodine therapy of patients with Graves' disease. Acta Endocrinol (Copenh) 1982; 99:379-385
885. Yamada O, Miyauchi A, Ito Y, Nakayama A, Yabuta T, Masuoka H, Fukushima M, Higashiyama T, Kihara M, Kobayashi K, Miya A. Changes in serum thyroglobulin antibody levels as a dynamic prognostic factor for early-phase recurrence of thyroglobulin antibody-positive papillary thyroid carcinoma after total thyroidectomy. Endocr J 2014; 61:961-965
886. Tsushima Y, Miyauchi A, Ito Y, Kudo T, Masuoka H, Yabuta T, Fukushima M, Kihara M, Higashiyama T, Takamura Y, Kobayashi K, Miya A, Kikumori T, Imai T, Kiuchi T. Prognostic significance of changes in serum thyroglobulin antibody levels of pre- and post-total thyroidectomy in thyroglobulin antibody-positive papillary thyroid carcinoma patients. Endocr J 2013; 60:871-876
887. Tumino S, Belfiore A. Appearance of antithyroglobulin antibodies as the sole sign of metastatic lymph nodes in a patient operated on for papillary thyroid cancer: a case report. Thyroid 2000; 10:431-433
888. Donegan D, McIver B, Algeciras-Schimnich A. Clinical Consequences of a Change in

Anti-Thyroglobulin Antibody Assays During the Follow-Up of Patients with Differentiated Thyroid Cancer. Endocr Pract 2014:1-19
889. Lupoli GA, Okosieme OE, Evans C, Clark PM, Pickett AJ, Premawardhana LD, Lupoli G, Lazarus JH. Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. J Clin Endocrinol Metab 2015; 100:100-108
890. Sapin R, d'Herbomez M, Gasser F, Meyer L, Schlienger JL. Increased sensitivity of a new assay for anti-thyroglobulin antibody detection in patients with autoimmune thyroid disease. Clin Biochem 2003; 36:611-616
891. Gianoukakis AG. Thyroglobulin antibody status and differentiated thyroid cancer: what does it mean for prognosis and surveillance? Current opinion in oncology 2015; 27:26-32
892. Kim ES, Lim DJ, Baek KH, Lee JM, Kim MK, Kwon HS, Song KH, Kang MI, Cha BY, Lee KW, Son HY. Thyroglobulin Antibody Is Associated with Increased Cancer Risk in Thyroid Nodules. Thyroid 2010; 20:885-891
893. Vasileiadis I, Boutzios G, Charitoudis G, Koukoulioti E, Karatzas T. Thyroglobulin antibodies could be a potential predictive marker for papillary thyroid carcinoma. Ann Surg Oncol 2014; 21:2725-2732
894. Grani G, Calvanese A, Carbotta G, D'Alessandri M, Nesca A, Bianchini M, Del Sordo M, Vitale M, Fumarola A. Thyroid autoimmunity and risk of malignancy in thyroid nodules submitted to fine-needle aspiration cytology. Head Neck 2015; 37:260-264
895. Karatzas T, Vasileiadis I, Zapanti E, Charitoudis G, Karakostas E, Boutzios G. Thyroglobulin antibodies as a potential predictive marker of papillary thyroid carcinoma in patients with indeterminate cytology. Am J Surg 2016;
896. Petrovic I, S. Fatemi, J. LoPresti, C. Spencer. Follow-Up Time Needed for TgAb+ Differentiated Thyroid Cancer (DTC) Patients To Convert to TgAb-
Negativity Following Successful Surgery Relates to Initial TgAb Concentration and Not Radioiodine (RAI) Remnant
Ablation. Thyroid 2015; 25:A130
897. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity 1998; 8:363-372
898. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. Journal of cancer epidemiology 2013; 2013:965212
899. Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer "epidemic"--screening and overdiagnosis. N Engl J Med 2014; 371:1765-1767
900. Oda H, Miyauchi A, Ito Y, Yoshioka K, Nakayama A, Sasai H, Masuoka H, Yabuta T, Fukushima M, Higashiyama T, Kihara M, Kobayashi K, Miya A. Incidences of Unfavorable Events in the Management of Low-Risk Papillary Microcarcinoma of the Thyroid by Active Surveillance Versus Immediate Surgery. Thyroid 2016; 26:150-155
901. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006; 295:2164-2167
902. Cairong Zhu, Tongzhang Zheng, Briseis A. Kilfoy, Xuesong Han, Shuangge Ma, Yue Ba, Yana Bai, Rong Wang, Yong Zhu, Zhang Y. A Birth Cohort Analysis of the Incidence of Papillary Thyroid Cancer in the United States, 1973–2004. Thyroid 2009; 19:1061-1066
903. Udelsman R, Zhang Y. The epidemic of thyroid cancer in the United States: the role of endocrinologists and ultrasounds. Thyroid 2014; 24:472-479
904. Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. N Engl J Med 2016; 375:614-617
905. Hundahl SA, Cady B, Cunningham MP, Mazzaferri E, McKee RF, Rosai J, Shah JP, Fremgen AM, Stewart AK, Holzer S. Initial results from a prospective cohort study of 5583

cases of thyroid carcinoma treated in the united states during 1996. U.S. and German Thyroid Cancer Study Group. An American College of Surgeons Commission on Cancer Patient Care Evaluation study. Cancer 2000; 89:202-217
906. Mazzaferri EL, Kloos RT. Current Approaches to Primary Therapy for Papillary and Follicular Thyroid Cancer. J Clin Endocrinol Metab 2001; 86:1447-1463
907. Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, McIver B, Mullan BP, Oberg AL, Powell CC, van Heerden JA, Goellner JR. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. World J Surg 2002; 26:879-885
908. Pitoia F, Bueno F, Urciuoli C, Abelleira E, Cross G, Tuttle RM. Outcomes of patients with differentiated thyroid cancer risk-stratified according to the american thyroid association and latin american thyroid society risk of recurrence classification systems. Thyroid 2013; 23:1401-1407
909. Ashcraft MW, Van Herle AJ. The comparative value of serum thyroglobulin measurements and iodine 131 total body scans in the follow-up of patients with treated differentiated thyroid cancer. Am J Med 1981; 71:806-814
910. Pineda JD, Lee T, Ain K, Reynolds JC, Robbins J. Iodine-131 therapy for thyroid cancer patients with elevated thyroglobulin and negative diagnostic sca. J Clin Endocrinol Metab 1995; 80:1488-1492
911. Baudin E, Do Cao C, Cailleux AF, Leboulleux S, Travagli JP, Schlumberger M. Positive predictive value of serum thyroglobulin levels, measured during the first year of follow-up after thyroid hormone withdrawal, in thyroid cancer patients. J Clin Endocrinol Metab 2003; 88:1107-1111
912. Smallridge RC, Diehl N, Bernet V. Practice trends in patients with persistent detectable thyroglobulin and negative diagnostic radioiodine whole body scans: a survey of American Thyroid Association members. Thyroid 2014; 24:1501-1507
913. Lamartina L, Durante C, Filetti S, Cooper DS. Low-risk differentiated thyroid cancer and radioiodine remnant ablation: a systematic review of the literature. J Clin Endocrinol Metab 2015; 100:1748-1761
914. Giovanella L, Imperiali M, Ferrari A, Palumbo A, Furlani L, Graziani MS, Castello R. Serum thyroglobulin reference values according to NACB criteria in healthy subjects with normal thyroid ultrasound. Clin Chem Lab Med 2012; 50:891-893
915. Djemli A, Van Vliet G, Belgoudi J, Lambert M, Delvin EE. Reference intervals for free thyroxine, total triiodothyronine, thyrotropin and thyroglobulin for Quebec newborns, children and teenagers. Clin Biochem 2004; 37:328-330
916. Sobrero G, Munoz L, Bazzara L, Martin S, Silvano L, Iorkansky S, Bergoglio L, Spencer C, Miras M. Thyroglobulin reference values in a pediatric infant population. Thyroid 2007; 17:1049-1054
917. Unger J, De Maertelaer V, Golstein J, Decoster C, Jonckheer MH. Relationship between serum thyroglobulin and intrathyroidal stable iodine in human simple goiter. Clin Endocrinol 1985; 23:1-6
918. Grebe SK. Soluble thyroid tumor markers – old and new challenges and potential solutions. NZ J Med Lab Science 2013:76-87
919. Shih ML, Lee JA, Hsieh CB, Yu JC, Liu HD, Kebebew E, Clark OH, Duh QY. Thyroidectomy for Hashimoto's thyroiditis: complications and associated cance. Thyroid 2008; 18:729-734
920. Hrafnkelsson J, Tulinius H, Kjeld M, Sigvaldason H, Jonasson JG. Serum thyroglobulin as a risk factor for thyroid carcinoma. Acta Oncol 2000; 39:973-977
921. Sands NB, Karls S, Rivera J, Tamilia M, Hier MP, Black MJ, Gologan O, Payne RJ. Preoperative serum thyroglobulin as an adjunct to fine-needle aspiration in predicting well-

differentiated thyroid cancer. J Otolaryngol Head Neck Surg 2010; 39:669-673
922. Petric R, Perhavec A, Gazic B, Besic N. Preoperative serum thyroglobulin concentration is an independent predictive factor of malignancy in follicular neoplasms of the thyroid gland. J Surg Oncol 2012; 105:351-356
923. Lee EK, Chung KW, Min HS, Kim TS, Kim TH, Ryu JS, Jung YS, Kim SK, Lee YJ. Preoperative serum thyroglobulin as a useful predictive marker to differentiate follicular thyroid cancer from benign nodules in indeterminate nodules. J Korean Med Sci 2012; 27:1014-1018
924. Rinaldi S, Plummer M, Biessy C, Tsilidis KK, Ostergaard JN, Overvad K, Tjonneland A, Halkjaer J, Boutron-Ruault MC, Clavel-Chapelon F, Dossus L, Kaaks R, Lukanova A, Boeing H, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Agnoli C, Tumino R, Vineis P, Panico S, Bueno-de-Mesquita HB, Peeters PH, Weiderpass E, Lund E, Quiros JR, Agudo A, Molina E, Larranaga N, Navarro C, Ardanaz E, Manjer J, Almquist M, Sandstrom M, Hennings J, Khaw KT, Schmidt J, Travis RC, Byrnes G, Scalbert A, Romieu I, Gunter M, Riboli E, Franceschi S. Thyroid-stimulating hormone, thyroglobulin, and thyroid hormones and risk of differentiated thyroid carcinoma: the EPIC study. Journal of the National Cancer Institute 2014; 106:dju097
925. Scheffler P, Forest VI, Leboeuf R, Florea AV, Tamilia M, Sands NB, Hier MP, Mlynarek AM, Payne RJ. Serum Thyroglobulin Improves the Sensitivity of the McGill Thyroid Nodule Score for Well-Differentiated Thyroid Cancer. Thyroid 2014; 24:852-857
926. Trimboli P, Treglia G, Giovanella L. Preoperative measurement of serum thyroglobulin to predict malignancy in thyroid nodules: a systematic review. Horm Metab Res 2015; 47:247-252
927. Ericsson UB, Tegler L, Lennquist S, Christensen SB, Ståhl E, Thorell JI. Serum thyroglobulin in differentiated thyroid carcinoma. Acta Chir Scand 1984; 150:367-375
928. Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, Barbi F, Avenia N, Scipioni A, Verrienti A, Tosi E, Cavaliere A, Gulino A, Filetti S, Russo D. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab 2007; 92:2840-2843
929. Gibelli B, Tredici P, De Cicco C, Bodei L, Sandri MT, Renne G, Bruschini R, Tradati N. Preoperative determination of serum thyroglobulin to identify patients with differentiated thyroid cancer who may present recurrence without increased thyroglobulin. Acta Otorhinolaryngol Ital 2005; 25:94-99
930. Giovanella L, Ceriani L, Ghelfo A, Maffioli M, Keller F. Preoperative undetectable serum thyroglobulin in differentiated thyroid carcinoma: incidence, causes and management strategy. Clin Endocrinol 2007; 67:547-551
931. Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S, Schlumberger M. Expression of the Na+/I- symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab 1999; 84:3228-3234
932. Ronga G, Filesi M, Ventroni G, Vestri AR, Signore A. Value of the first serum thyroglobulin level after total thyroidectomy for the diagnosis of metastases from differentiated thyroid carcinoma. Eur J Nucl Med 1999; 26:1448-1452
933. Lima N, Cavaliere E, Tomimori E, Knobel M, Medeieros-Neto G. Prognostic value of serial serum thyroglobulin determinations after total thyroidectomy for differentiated thyroid cancer. J Endocrinol Invest 2002; 25:110-115
934. Lin JD, Huang MJ, Hsu BR, Chao TC, Hsueh C, Liu FH, Liou MJ, Weng HF. Significance of postoperative serum thyroglobulin levels in patients with papillary and follicular thyroid carcinomas. J Surg Oncol 2002; 80:45-51
935. Hall FT, Beasley NJ, Eski SJ, Witterick IJ, Walfish PG, Freeman JL. Predictive value of serum thyroglobulin after surgery for thyroid carcinoma. Laryngoscope 2003; 113:77-81
936. Toubeau M, Touzery C, Arveux P, Chaplain G, Vaillant G, Berriolo A, Riedinger JM,

Boichot C, Cochet A, Brunotte F. Predictive value for disease progression of serum thyroglobulin levels measured in the postoperative period and after (131)I ablation therapy in patients with differentiated thyroid cancer. J Nucl Med 2004; 45:988-994
937. Kim TY, Kim WB, Kim ES, Ryu JS, Yeo JS, Kim SC, Hong SJ, Shong YK. Serum thyroglobulin levels at the time of 131I remnant ablation just after thyroidectomy are useful for early prediction of clinical recurrence in low-risk patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 2005; 90:1440-1445
938. Makarewicz J, Adamczewski Z, Knapska-Kucharska M, Lewiński A. Evaluation of the diagnostic value of the first thyroglobulin determination in detecting metastases after differentiated thyroid carcinoma surgery. Exp Clin Endocrinol Diabetes 2006; 114:485-489
939. Heemstra KA, Liu YY, Stokkel M, Kievit J, Corssmit E, Pereira AM, Romijn JA, Smit JW. Serum thyroglobulin concentrations predict disease-free remission and death in differentiated thyroid carcinoma. Clin Endocrinol (Oxf) 2007; 66:58-64
940. Giovanella L, Ceriani L, Suriano S, Ghelfo A, Maffioli M. Thyroglobulin measurement before rhTSH-aided (131)I ablation in detecting metastases from differentiated thyroid carcinoma. Clin Endocrinol (Oxf) 2008; 68:659-663
941. Feldt-Rasmussen U, Petersen PH, Nielsen H, Date J. Thyroglobulin of varying molecular sizes with different disappearence rates in plasma following subtotal thyroidectomy. Clin Endocrinol (Oxf) 1978; 9:205-214
942. Padovani RP, Robenshtok E, Brokhin M, Tuttle RM. Even without additional therapy, serum thyroglobulin concentrations often decline for years after total thyroidectomy and radioactive remnant ablation in patients with differentiated thyroid cancer. Thyroid 2012; 22:778-783
943. Durante C, Montesano T, Attard M, Torlontano M, Monzani F, Costante G, Meringolo D, Ferdeghini M, Tumino S, Lamartina L, Paciaroni A, Massa M, Giacomelli L, Ronga G, Filetti S. Long-Term Surveillance of Papillary Thyroid Cancer Patients Who Do Not Undergo Postoperative Radioiodine Remnant Ablation: Is There a Role for Serum Thyroglobulin Measurement? J Clin Endocrinol Metab 2012; 97:2748-2753
944. Pacini F, Agate L, Elisei R, Capezzone M, Ceccarelli C, Lippi F, Molinaro E, Pinchera A. Outcome of differentiated thyroid cancer with detectable serum Tg and negative diagnostic (131)I whole body scan: comparison of patients treated with high (131)I activities versus untreated patients. J Clin Endocrinol Metab 2001; 86:4092-4097
945. Schaap J, Eustatia-Rutten CF, Stokkel M, Links TP, Diamant M, van der Velde EA, Romijn JA, Smit JW. Does radioiodine therapy have disadvantageous effects in non-iodine accumulating differentiated thyroid carcinoma. Clin Endocrinol (Oxf) 2002; 57:117-124
946. Valadão MM, Rosário PW, Borges MA, Costa GB, Rezende LL, Padrão EL, Barroso AL, Purisch S. Positive predictive value of detectable stimulated tg during the first year after therapy of thyroid cancer and the value of comparison with Tg-ablation and Tg measured after 24 months. Thyroid 2006; 16:1145-1149
947. Rosario P, Borges M, Reis J, Alves MF. Effect of suppressive therapy with levothyroxine on the reduction of serum thyroglobulin after total thyroidectomy. Thyroid 2006; 16:199-200
948. Huang SH, Wang PW, Huang YE, Chou FF, Liu RT, Tung SC, Chen JF, Kuo MC, Hsieh JR, Hsieh HH. Sequential follow-up of serum thyroglobulin and whole body scan in thyroid cancer patients without initial metastasis. Thyroid 2006; 16:1273-1278
949. Miyauchi A, Kudo T, Miya A, Kobayashi K, Ito Y, Takamura Y, Higashiyama T, Fukushima M, Kihara M, Inoue H, Tomoda C, Yabuta T, Masuoka H. Prognostic impact of serum thyroglobulin doubling-time under thyrotropin suppression in patients with papillary thyroid carcinoma who underwent total thyroidectomy. Thyroid 2011; 21:707-716
950. Pacini F, Sabra MM, Tuttle RM. Clinical relevance of thyroglobulin doubling time in the management of patients with differentiated thyroid cancer. Thyroid 2011; 21:691-692

951. Giovanella L, Trimboli P, Verburg FA, Treglia G, Piccardo A, Foppiani L, Ceriani L. Thyroglobulin levels and thyroglobulin doubling time independently predict a positive 18F-FDG PET/CT scan in patients with biochemical recurrence of differentiated thyroid carcinoma. Eur J Nucl Med Mol Imaging 2013; 40:874-880
952. Miyauchi A, Kudo T, Kihara M, Higashiyama T, Ito Y, Kobayashi K, Miya A. Relationship of biochemically persistent disease and thyroglobulin-doubling time to age at surgery in patients with papillary thyroid carcinoma. Endocr J 2013; 60:415-421
953. Yim JH, Kim EY, Bae Kim W, Kim WG, Kim TY, Ryu JS, Gong G, Hong SJ, Yoon JH, Shong YK. Long-term consequence of elevated thyroglobulin in differentiated thyroid cancer. Thyroid 2013; 23:58-63
954. Elisei R, Agate L, Viola D, Matrone A, Biagini A, Molinaro E. How to manage patients with differentiated thyroid cancer and a rising serum thyroglobulin level. Endocrinol Metab Clin North Am 2014; 43:331-344
955. Kelders A, Kennes LN, Krohn T, Behrendt FF, Mottaghy FM, Verburg FA. Relationship between positive thyroglobulin doubling time and 18F-FDG PET/CT-positive, 131I-negative lesions. Nucl Med Commun 2014; 34:176-181
956. Rossing RM, Jentzen W, Nagarajah J, Bockisch A, Gorges R. Serum Thyroglobulin Doubling Time in Progressive Thyroid Cancer. Thyroid 2016; 26:1712-1718
957. Schlumberger M CP, Fragu P, Lumbroso J, Parmentier C and Tubiana M,. Circulating thyrotropin and thyroid hormones in patients with metastases of differentiated thyroid carcinoma: relationship to serum thyrotropin levels. J Clin Endocrinol Metab 1980; 51:513-519
958. Robbins RJ, Srivastava S, Shaha A, Ghossein R, Larson SM, Fleisher M, Tuttle RM. Factors influencing the basal and recombinant human thyrotropin-stimulated serum thyroglobulin in patients with metastatic thyroid carcinoma. J Clin Endocrinol Metab 2004; 89:6010-6016
959. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT. Detection of residual and recurrent differentiated thyroid carcinoma by serum Thyroglobulin measurement. Thyroid 1999; 9:435-441
960. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, Haugen BR, Sherman SI, Cooper DS, Braunstein GD, Lee S, Davies TF, Arafah BM, Ladenson PW, Pinchera A. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003; 88:1433-1441
961. Mazzaferri EL, Kloos RT. Is diagnostic iodine-131 scanning with recombinant human TSH useful in the follow-up of differentiated thyroid cancer after thyroid ablation? J Clin Endocrinol Metab 2002; 87:1486-1489
962. Vitale G, Lupoli GA, Ciccarelli A, Lucariello A, Fittipaldi MR, Fonderico F, Panico A, Lupoli G. Influence of body surface area on serum peak thyrotropin (TSH) levels after recombinant human TSH administration. J Clin Endocrinol Metab 2003; 88:1319-1322
963. Montesano T, Durante C, Attard M, Crocetti U, Meringolo D, Bruno R, Tumino S, Rubello D, Al-Nahhas A, Colandrea M, Maranghi M, Travascio L, Ronga G, Torlontano M. Age influences TSH serum levels after withdrawal of l-thyroxine or rhTSH stimulation in patients affected by differentiated thyroid cancer. Biomed Pharmacother 2007; 61:468-471
964. Braverman L, Kloos RT, Law B Jr, Kipnes M, Dionne M, Magner J. Evaluation of various doses of recombinant human thyrotropin in patients with multinodular goiters. Endoc Pract 2008; 14:832-839
965. Over R, Nsouli-Maktabi H, Burman KD, Jonklaas J. Age modifies the response to recombinant human thyrotropin. Thyroid 2010; 20:1377-1384
966. Schlumberger M, Charbord P, Fragu P, Lumbroso J, C P, Tubiana M. Circulating thyrotropin and thyroid hormones in patients with metastases of differentiated thyroid

carcinoma: relationship to serum thyrotropin levels. J Clin Endocrinol Metab 1980; 51:513-519
967. Nakabashi CC, Kasamatsu TS, Crispim F, Yamazaki CA, Camacho CP, Andreoni DM, Padovani RP, Ikejiri ES, Mamone MC, Aldighieri FC, Wagner J, Hidal JT, Vieira JG, Biscolla RP, Maciel RM. Basal serum thyroglobulin measured by a second-generation assay is equivalent to stimulated thyroglobulin in identifying metastases in patients with differentiated thyroid cancer with low or intermediate risk of recurrence. Eur Thyroid J 2014; 3:43-50
968. Groen AH, Klein Hesselink MS, Plukker JT, Sluiter WJ, van der Horst-Schrivers AN, Brouwers AH, Lentjes EG, Muller Kobold AC, Links TP. Additional value of a high sensitive thyroglobulin assay in the follow-up of patients with differentiated thyroid carcinoma. Clin Endocrinol (Oxf) 2016;
969. Rotman-Pikielny P, Reynolds JC, Barker WC, Yen PM, Skarulis MC, Sarlis NJ. Recombinant human thyrotropin for the diagnosis and treatment of a highly functional metastatic struma ovarii. J Clin Endocrinol Metab 2000; 85:237-244
970. Russo M, Marturano I, Masucci R, Caruso M, Fornito MC, Tumino D, Tavarelli M, Squatrito S, Pellegriti G. Metastatic malignant struma ovarii with coexistence of Hashimoto's thyroiditis. Endocrinology, diabetes & metabolism case reports 2016:160030
971. Trimboli P, D'Aurizio F, Tozzoli R, Giovanella L. Measurement of thyroglobulin, calcitonin, and PTH in FNA washout fluids. Clin Chem Lab Med 2016;
972. Machens A, Holzhausen HJ, Dralle H. The prognostic value of primary tumor size in papillary and follicular thyroid carcinoma. Cancer 2005; 103:2269-2273
973. Passler C, Scheuba C, Prager G, Kaczirek K, Kaserer K, Zettinig G, Niederle B. Prognostic factors of papillary and follicular thyroid cancer: differences in an iodine-replete endemic goiter region. Endocr Relat Cancer 2004; 11:131-139
974. Randolph GW, Duh QY, Heller KS, LiVolsi VA, Mandel SJ, Steward DL, Tufano RP, Tuttle RM. The prognostic significance of nodal metastases from papillary thyroid carcinoma can be stratified based on the size and number of metastatic lymph nodes, as well as the presence of extranodal extension. Thyroid 2012; 22:1144-1152
975. Pezzi TA, Sandulache VC, Pezzi CM, Turkeltaub AE, Feng L, Cabanillas ME, Williams MD, Lai SY. Treatment and survival of patients with insular thyroid carcinoma: 508 cases from the National Cancer Data Base. Head Neck 2016; 38:906-912
976. Rosario PW, de Faria S, Bicalho L, Alves MF, Borges MA, Purisch S, Padrão EL, Rezende LL, Barroso AL. Ultrasonographic differentiation between metastatic and benign lymph nodes in patients with papillary thyroid carcinoma. J Ultrasound Med 2005; 24:1385-1389
977. Pacini F, Fugazzola L, Lippi F, Ceccarelli C, Centoni R, Miccoli P, Elisei R, Pinchera A. Detection of thyroglobulin in fine needle aspirates of nonthyroidal neck masses: a clue to the diagnosis of metastatic differentiated thyroid cancer. J Clin Endocrinol Metab 1992; 74:1401-1404
978. Uruno T, Miyauchi A, Shimizu K, Tomoda C, Takamura Y, Ito Y, Miya A, Kobayashi K, Matsuzuka F, Amino N, Kuma K. Usefulness of thyroglobulin measurement in fine-needle aspiration biopsy specimens for diagnosing cervical lymph node metastasis in patients with papillary thyroid cancer. World J Surg 2005; 29:483-485
979. Boi F, Baghino G, Atzeni F, Lai ML, Faa G, Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. J Clin Endocrinol Metab 2006; 91:1364-1369
980. Snozek CL, Chambers EP, Reading CC, Sebo TJ, Sistrunk JW, Singh RJ, Grebe SK. Serum thyroglobulin, high-resolution ultrasound, and lymph node thyroglobulin in diagnosis of differentiated thyroid carcinoma nodal metastases. J Clin Endocrinol Metab

2007; 92:4278-4281
981. Bruno R, Giannasio P, Chiarella R, Capula C, Russo D, Filetti S, Costante G. Identification of a neck lump as a lymph node metastasis from an occult contralateral papillary microcarcinoma of the thyroid: key role of thyroglobulin assay in the fine-needle aspirate. Thyroid 2009; 19:531-533
982. Jeon SJ, Kim E, Park JS, Son KR, Baek JH, Kim YS, Park do J, Cho BY, Na DG. Diagnostic benefit of thyroglobulin measurement in fine-needle aspiration for diagnosing metastatic cervical lymph nodes from papillary thyroid cancer: correlations with US features. Korean J Radiol 2009; 10:106-111
983. Cunha N, Rodrigues F, Curado F, Ilhéu O, Cruz C, Naidenov P, Rascão MJ, Ganho J, Gomes I, Pereira H, Real O, Figueiredo P, Campos B, Valido F. Thyroglobulin detection in fine-needle aspirates of cervical lymph nodes: a technique for the diagnosis of metastatic differentiated thyroid cancer. Eur J Endocrinol 2007; 157:101-107
984. Suh YJ, Son EJ, Moon HJ, Kim EK, Han KH, Kwak JY. Utility of Thyroglobulin Measurements in Fine-needle Aspirates of Space Occupying Lesions in the Thyroid Bed after Thyroid Cancer Operations. Thyroid 2012; 23:in press
985. Cappelli C, Pirola I, De Martino E, Gandossi E, Cimino E, Samoni F, Agosti B, Rosei EA, Casella C, Castellano M. Thyroglobulin measurement in fine-needle aspiration biopsy of metastatic lymph nodes after rhTSH stimulation. Head Neck 2013; 35:E21-23
986. Grani G, Fumarola A. Thyroglobulin in Lymph Node Fine-Needle Aspiration Washout: A Systematic Review and Meta-analysis of Diagnostic Accuracy. J Clin Endocrinol Metab 2014; 99:1970-1982
987. Torres MR, Nóbrega Neto SH, Rosas RJ, Martins AL, Ramos AL, da Cruz TR. Thyroglobulin in the washout fluid of lymph-node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? Thyroid 2014; 24:7-18
988. Chung J, Kim EK, Lim H, Son EJ, Yoon JH, Youk JH, Kim JA, Moon HJ, Kwak JY. Optimal indication of thyroglobulin measurement in fine-needle aspiration for detecting lateral metastatic lymph nodes in patients with papillary thyroid carcinoma. Head Neck 2014; 36:795-801
989. Tang S, Buck A, Jones C, Sara Jiang X. The utility of thyroglobulin washout studies in predicting cervical lymph node metastases: One academic medical center's experience. Diagn Cytopathol 2016;
990. Jeon MJ, Kim WG, Jang EK, Choi YM, Lee YM, Sung TY, Yoon JH, Chung KW, Hong SJ, Baek JH, Lee JH, Kim TY, Shong YK, Kim WB. Thyroglobulin level in fine-needle aspirates for preoperative diagnosis of cervical lymph node metastasis in patients with papillary thyroid carcinoma: two different cutoff values according to serum thyroglobulin level. Thyroid 2015; 25:410-416
991. Zanella AB, Meyer EL, Balzan L, Silva AC, Camargo J, Migliavacca A, Guimaraes JR, Maia AL. Thyroglobulin measurements in washout of fine needle aspirates in cervical lymph nodes for detection of papillary thyroid cancer metastases. Arq Bras Endocrinol Metabol 2010; 54:550-554
992. Baskin HJ. Detection of recurrent papillary thyroid carcinoma by thyroglobulin assessment in the needle washout after fine-needle aspiration of suspicious lymph nodes. Thyroid 2004; 14:959-963
993. Shin HJ, Lee HS, Kim EK, Moon HJ, Lee JH, Kwak JY. A Study on Serum Antithyroglobulin Antibodies Interference in Thyroglobulin Measurement in Fine-Needle Aspiration for Diagnosing Lymph Node Metastasis in Postoperative Patients. PLoS One 2015; 10:e0131096
994. Boi F, Maurelli I, Pinna G, Atzeni F, Piga M, Lai ML, Mariotti S. Calcitonin measurement in wash-out fluid from fine needle aspiration of neck masses in patients with primary and metastatic medullary thyroid carcinoma. J Clin Endocrinol Metab 2007;

92:2115-2118
995. Abraham D, Gault PM, Hunt J, Bentz J. Calcitonin estimation in neck lymph node fine-needle aspirate fluid prevents misinterpretation of cytology in patients with metastatic medullary thyroid cancer. Thyroid 2009; 19:1015-1016
996. Massaro F, Dolcino M, Degrandi R, Ferone D, Mussap M, Minuto F, Giusti M. Calcitonin assay in wash-out fluid after fine-needle aspiration biopsy in patients with a thyroid nodule and border-line value of the hormone. J Endocrinol Invest 2009; 32:308-312
997. Barzon L, Boscaro M, Pacenti M, Taccaliti A, Palù G. Evaluation of circulating thyroid-specific transcripts as markers of thyroid cancer relapse. Int J Cancer 2004; 110:914-920
998. Verburg FA, Lips CJ, Lentjes EG, Klerk Jd J. Detection of circulating Tg-mRNA in the follow-up of papillary and follicular thyroid cancer: how useful is it? Br J Cancer 2004; 91:1-5
999. Gupta M, Chia SY. Circulating thyroid cancer markers. Curr Opin Endocrinol Diabetes Obes 2007; 14:383-388
1000. Ringel MD, Ladenson PW, Levine MA. Molecular diagnosis of residual and recurrent thyroid cancer by amplification of thyroglobulin messenger ribonucleic acid in peripheral blood. J Clin Endocrinol Metab 1998; 83:4435-4442
1001. Ausavarat S, Sriprapaporn J, Satayaban B, Thongnoppakhun W, Laipiriyakun A, Amornkitticharoen B, Chanachai R, Pattanachak C. Circulating thyrotropin receptor messenger ribonucleic acid is not an effective marker in the follow-up of differentiated thyroid carcinoma. Thyroid research 2015; 8:11
1002. Biscolla RP, Cerutti JM, Maciel RM. Detection of recurrent thyroid cancer by sensitive nested reverse transcription-polymerase chain reaction of thyroglobulin and sodium/iodide symporter messenger ribonucleic acid transcripts in peripheral blood. J Clin Endocrinol Metab 2000; 85:3623-3627
1003. Chinnappa P, Taguba L, Arciaga R, Faiman C, Siperstein A, Mehta AE, Reddy SK, Nasr C, Gupta MK. Detection of thyrotropin-receptor messenger ribonucleic acid (mRNA) and thyroglobulin mRNA transcripts in peripheral blood of patients with thyroid disease: sensitive and specific markers for thyroid cancer. J Clin Endocrinol Metab 2004; 89:3705-3709
1004. Barbosa GF, Milas M. Peripheral thyrotropin receptor mRNA as a novel marker for differentiated thyroid cancer diagnosis and surveillance. Expert Rev Anticancer Ther 2008; 8:1415-1424
1005. Milas M, Shin J, Gupta M, Novosel T, Nasr C, Brainard J, Mitchell J, Berber E, Siperstein A. Circulating thyrotropin receptor mRNA as a novel marker of thyroid cancer: clinical applications learned from 1758 samples. Ann Surg 2010; 252:643-651
1006. Ringel MD. Editorial: molecular detection of thyroid cancer: differentiating "signal" and "noise" in clinical assays. J Clin Endocrinol Metab 2004; 89:29-32
1007. Chia SY, Milas M, Reddy SK, Siperstein A, Skugor M, Brainard J, Gupta MK. Thyroid-stimulating hormone receptor messenger ribonucleic acid measurement in blood as a marker for circulating thyroid cancer cells and its role in the preoperative diagnosis of thyroid cancer. J Clin Endocrinol Metab 2007; 92:468-475
1008. Kaufmann S, Schmutzler C, Schomburg L, Körber C, Luster M, Rendl J, Reiners C, Köhrle J. Real time RT-PCR analysis of thyroglobulin mRNA in peripheral blood in patients with congenital athyreosis and with differentiated thyroid carcinoma after stimulation with recombinant human thyrotropin. Endocr Regul 2004; 38:41-49
1009. Chelly J, Concordet JP, Kaplan JC, Kahn A. Illegitimate transcription: transcription of any gene in any cell type. Proc Natl Acad Sci USA 1989; 86:2617-2621
1010. Ghossein RA, Bhattacharya S. Molecular detection and characterisation of circulating tumour cells and micrometastases in solid tumours. Eur J Cancer 2000; 36:1681-1694

1011. Savagner F, Rodien P, Reynier P, Rohmer V, Bigorgne JC, Malthiery Y. Analysis of Tg transcripts by real-time RT-PCR in the blood of thyroid cancer patients. J Clin Endocrinol Metab 2002; 87:635-639
1012. Elisei R, Vivaldi A, Agate L, Molinaro E, Nencetti C, Grasso L, Pinchera A, Pacini F. Low specificity of blood thyroglobulin messenger ribonucleic acid assay prevents its use in the follow-up of differentiated thyroid cancer patients. J Clin Endocrinol Metab 2004; 89:33-39
1013. Denizot A, Delfino C, Dutour-Meyer A, Fina F, Ouafik L. Evaluation of quantitative measurement of thyroglobulin mRNA in the follow-up of differentiated thyroid cancer. Thyroid 2003; 13:867-872
1014. Amakawa M, Kato R, Kameko F, Maruyama M, Tajiri J. Thyroglobulin mRNA expression in peripheral blood lymphocytes of healthy subjects and patients with thyroid disease. Clin Chim Acta 2008; 390:97-103
1015. Sellitti DF, Akamizu T, Doi SQ, Kim GH, Kariyil JT, Kopchik JJ, Koshiyama H. Renal expression of two 'thyroid-specific' genes: thyrotropin receptor and thyroglobulin. Exp Nephrol 2000; 8:235-243
1016. Bojunga J, Roddiger S, Stanisch M, Kusterer K, Kurek R, Renneberg H, Adams S, Lindhorst E, Usadel KH, Schumm-Draeger PM. Molecular detection of thyroglobulin mRNA transcripts in peripheral blood of patients with thyroid disease by RT-PCR. Br J Cancer 2000; 82:1650-1655
1017. Endo T, Kobayashi T. Thyroid-stimulating hormone receptor in brown adipose tissue is involved in the regulation of thermogenesis. Am J Physiol Endocrinol Metab 2008; 295:E514-518
1018. Zhang W, Tian LM, Han Y, Ma HY, Wang LC, Guo J, Gao L, Zhao JJ. Presence of thyrotropin receptor in hepatocytes: not a case of illegitimate transcription. J Cell Mol Med 2009; 13:4636-4642
1019. Cianfarani F, Baldini E, Cavalli A, Marchioni E, Lembo L, Teson M, Persechino S, Zambruno G, Ulisse S, Odorisio T, D'Armiento M. TSH receptor and thyroid-specific gene expression in human skin. J Invest Dermatol 2010; 130:93-101