Published in final edited form as:

Clin Biochem. 2014 September ; 47(0): 1272–1278. doi:10.1016/j.clinbiochem.2014.06.007.

Total and free thyroxine and triiodothyronine: Measurement discrepancies, particularly in inpatients

Jacqueline Jonklaas^{a,*}, Anpalakan Sathasivam^{a,b}, Hong Wang^c, Jianghong Gu^d, Kenneth D. Burman^b, and Steven J. Soldin^d

^aDivision of Endocrinology, Georgetown University, Washington, DC, USA

^bSection of Endocrinology Medstar Washington Hospital Center, Washington, DC, USA

^cMedstar Health Research Institute, Hyattsville, MD, USA

^dDepartment of Laboratory Medicine, National Institutes of Health, Bethesda, MD, USA

Abstract

Objective—We compared the performance of tandem mass spectrometry versus immunoassay for measuring thyroid hormones in a diverse group of inpatients and outpatients.

Methods—Thyroxine (T4), triiodothyronine (T3), free thyroxine (FT4), and free triiodothyronine (FT3) were measured by liquid chromatography tandem mass spectrometry and immunoassay in 100 patients and the two assays were compared.

Results—T4 and T3 values measured by the two different assays correlated well with each other ($r = 0.91–0.95$). However, the correlation was less good at the extremes ($r = 0.51–0.75$). FT4 and FT3 concentrations measured by the two assays correlated less well with each other ($r = 0.75$ and 0.50 respectively). The studied analytes had poor inverse correlation with the log-transformed TSH values ($r = -0.22–0.51$) in the population as a whole. The strongest correlations were seen in the groups of outpatients ($r = -0.25–0.61$). The weakest degree of correlation was noted in the inpatient group, with many correlations actually being positive.

Conclusion—The worst between-assay correlation was demonstrated at low and high hormone concentrations, in the very concentration ranges where accurate assay performance is typically most clinically important. Based on the lesser susceptibility of mass spectrometry to interferences from conditions such as binding protein abnormalities, we speculate that mass spectrometry better reflects the clinical situation. In this mixed population of inpatients and outpatients, we also note failure of assays to conform to the anticipated inverse linear relationship between thyroid hormones and log-transformed TSH.

Keywords

Free thyroxine; Thyroxine; Free triiodothyronine; Triiodothyronine; Immunoassays; Tandem mass spectrometry

Introduction

Thyroid hormone assays should ideally be accurate and truly reflect the concentration of analyte in the sample. Barriers to accurate measurement using immunoassays (IA) can include changes in binding proteins, presence of heterophilic antibodies, and concentration of non-esterified free fatty acids [1,2]. Such factors may account for the method-dependent variation documented in thyroid hormone measurements made during such physiologic and medical conditions as pregnancy, renal failure, non-thyroidal illness, and genetic abnormalities in binding proteins [3–7]. Discrepant values between thyroid hormone assays can be illustrated by examining the correlation between the results obtained when using different assays to assess the same sample [8]. Another means of judging the validity of thyroid hormone measurements is to examine the relationship between thyroid hormone concentration and the logarithmically transformed thyroid stimulating hormone (TSH) value, which is shown to follow a complex, but generally inverse linear relationship [8–13].

The performance of thyroid hormone assays is of importance across all range of values. However, assay performance may be particularly important at the low and high values for thyroid hormones, as it is at these two extremes that presence of thyroid disorders is more likely. Erroneous values for thyroid hormones may prevent the correct and timely diagnosis of hypothyroidism or hyperthyroidism, particularly in difficult or challenging clinical cases in which the diagnosis may be confounded by incongruent laboratory values. Measurement by tandem mass spectrometry (MS) is accurate, precise, and more specific than immunoassays [14]. When coupled with physical separation methods it permits the reliable measurement of free thyroid hormone in any of the conditions that may result in changes in binding protein concentrations [7,14,15]. These situations include, for example, pregnancy, non-thyroidal illness, and renal disease.

We were therefore interested in the performance of these two assay methodologies in samples from a diverse group of patients. The goal of this analysis was to examine in the same sample analyte values generated by IA compared with the values generated by MS with particular attention to thyroid hormone concentrations in the lower and higher concentration ranges.

Methods

Patient eligibility and recruitment

One hundred patients were recruited for this study. Patients were recruited from the outpatient clinics and inpatient services at Georgetown University and Medstar Washington Hospital Center. This protocol had been approved by the Institutional Review Board at both institutions. Individuals of any age with any medical diagnoses were included in order to capture conditions that could potentially affect assay performance. All patients signed a

written informed consent form and donated a single blood sample. The status of each patient as an inpatient or outpatient was recorded.

Assays used in study

Blood was collected in red top tubes without serum separator, as the latter has been shown to inhibit signal by 80–90% in methods employing ESI ionization [16]. All sera were stored at -80°C , at which temperature they had excellent stability, and analyzed in one batch for each assay at the completion of the study. The thyroid hormone and related analytes measured by MS were thyroxine (T4) and triiodothyronine (T3) in one mass spectrometry method, and free thyroxine (FT4), and free triiodothyronine (FT3) in another mass spectrometry method. These analytes were also measured by IA.

T4 and T3 by MS

This method, instrumentation, conditions, and working parameters have been previously described [17,18]. An API-5000 tandem mass spectrometer equipped with TurbolonSpray source and Shimadzu HPLC system was employed. 100 μL of patient serum was deproteinized by adding 150 microliters (μL) of acetonitrile containing labeled internal standards. The supernatant was diluted with 500 μL deionized water and a 300 μL aliquot was injected onto an Agilent ZORBAX SB-C18 column ($2.1 \times 30 \text{ mm } 1.8 \mu\text{m}$). After washing the column for 4.5 min with mobile phase A (0.01% formic acid in 98% water, 2% methanol) at a flow rate of 0.25 mL/min, the switching valve was activated and the analytes of interest were eluted into the mass spectrometer with a gradient of 35% mobile phase B (methanol with 0.01% formic acid) to 64% B in 2.5 min before equilibration for the next injection. Quantification by multiple reaction-monitoring analysis was performed in the positive mode. The ESI source was operated with ionspray voltage at 5500 V and heated temperature at 650°C . Gas settings were as follows: curtain gas 35, collision gas 4, nebulizer and heated gas 50. Retention time and ion pair for each analyte, their internal standards and compound-dependent parameters are listed. T4 (9.4, m/z 777.9/634.3), T4 $^{13}\text{C}_6$ (9.4, m/z 783.8/640.2) DP = 120, CE = 37, CXP = 21. T3 (8.83, 9.2, m/z 651.9/606.1), T3- $^{13}\text{C}_6$ (8.83, 9.2, m/z 657.9/612.1) DP = 120, CE = 29, CXP = 13.

The within-day coefficients of variation (CVs) were $<8.9\%$ and between day CVs were between 1.6% and 7.6%. Recovery ranged from 92.8% to 95.4%. The lower limits of detection were 1.93 nmol/L for T4 and 0.015 nmol/L for T3. Normal reference intervals for females and males (Gaussian method on 130 females and 130 males) for each of the analytes are: T4 54.00–140.00 and 60.40–131.00 nmol/L; T3 1.15–2.60 and 1.29–2.65 nmol/L respectively [19].

FT4 and FT3 by MS

Method—This method was performed in the Department of Laboratory Medicine at the National Institutes of Health, Bethesda, MD. The updated method was similar to our previous method [20,21], with minor modifications. Human serum/plasma samples were thawed at room temperature. 400 μL of human serum/plasma was placed in a 30 kDa ultrafiltration device (Centrifree YM-30, Millipore) and centrifuged in an Eppendorf temperature controlled centrifuge with a fixed angle rotor at 2700 rpm and a temperature of

37 °C for 30 min. To a 1.5 mL conical plastic Eppendorf centrifuge tube, 150 µL of ultrafiltrate was added and mixed with 450 µL methanol containing internal standards, deuterium-labeled T4 (T4- d_5) and carbon-labeled T3 (T3- $^{13}C_6$), for deproteinization. The tube was capped, vortex mixed vigorously for 30 s, and centrifuged for 10 min at 13,000 rpm. After centrifugation, 500 µL of supernatant was diluted with 600 µL of distilled de-ionized water and 400 µL aliquot was injected onto a Phenomenex Kinetex 2.6 µm C18 column (75 × 2.1 mm). The procedure involved an online extraction step followed by activation of a built-in valco switching valve and subsequent sample introduction into the mass spectrometer. After washing, the switching valve was activated and the analyte compounds were eluted from the column with a water/methanol gradient into the MS/MS system (see Table 1a).

Instrumentation—An AB SCIEX QTRAP 5500 tandem mass spectrometer (AB Sciex LLC) equipped with TurboIonSpray source was operated in negative ion multiple reaction monitoring (MRM) mode to perform the analysis. HPLC system consisted of two Shimadzu LC-20AD pumps, a Shimadzu SIL-20ACHT autosampler, and a Shimadzu DGU-20A₅ degasser (Shimadzu Scientific Instruments). Transitions to be monitored and compound-dependent parameters are summarized in Table 1b. Nitrogen worked as curtain and collision gas. The main working parameters of the mass spectrometer are shown in Table 1c. Data were acquired and processed by Analyst 1.5.1 software package (AB Sciex).

Assay performance—The within-day and between-day CVs were below 9% for FT3 and below 7% for FT4 at all concentrations tested. Accuracy, determined by isotope dilution, ranged between 95% and 105%. Good linearity was also obtained within the concentration range of 6.5–65.0 pmol/L for FT4 and 1.5–38.0 pmol/L for FT3 ($r = 0.995$). The 2.5th–97.5th percentiles for FT4 and FT3 were 17.50–31.0 pmol/L and 2.30–9.30 pmol/L, respectively. These reference intervals were established using healthy adult males and females of 20–60 years and $n = 150$ for each sex (unpublished data). These are identical to our previously published pediatric reference intervals [22]. There is an approximately 1.5-fold difference (increase) in the reference interval for FT4 and FT3 depending on whether the ultrafiltration step is carried out at 37 °C versus 25 °C [22].

Analytes measured by IA

The thyroid hormones measured by IA were T4, T3, FT4, and FT3. Methodology was as follows. We measured T4 (reference interval 60.49–171.17 nmol/L), FT4 (reference interval 9.78–18.79 pmol/L), and FT3 (reference interval 2.76–6.45 pmol/L) by direct (analog) immunoassay method on a Siemens Vista analyzer (Diagnostic Products; Siemens Healthcare Diagnostics). TSH (reference interval 0.40–4.00 mIU/L) was measured on the same analyzer. We measured T3 (reference interval 1.08–2.93 nmol/L) by a direct immunoassay method on a Siemens Immulite 2500 analyzer (Diagnostic Products; Siemens Healthcare Diagnostics). CVs for these assays were between 3 and 8%.

Statistical analysis

The correlation between the thyroid hormone analytes was assessed using the Spearman correlation coefficient. TSH values were logarithmically transformed prior to analysis. In

order to evaluate the performance of thyroid hormone assays at the lower and higher range of values, the analytes were also divided into three groups based on the associated TSH values. The three groups were those associated with TSH values <0.35 mIU/L, $0.35\text{--}3.5$ mIU/L, and >3.5 mIU/L. P values <0.05 were considered statistically significant. Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Sixty-three patients were outpatients and the remaining 37 were inpatients. The average ages of the outpatients and inpatients were 49 and 57 years respectively. Females comprised 67% of the outpatients and 49% of the inpatients. Three percent of the inpatients were surgically athyreotic or had Hashimoto's hypothyroidism and were taking levothyroxine. Thirty-six percent of the outpatients also had hypothyroidism and were taking levothyroxine. The characteristics of the study population are described in greater detail in a recent publication [23].

1. Within-analyte, between-assay correlations

Each analyte measured by MS had reasonable correlation with its own measurement by IA (see Table 2). However, a major observation was that the disagreement between the two assays was greatest at the lower and higher extremes of the measured concentrations. Fig. 1a and b show these data for T4 and T3 respectively. The greatest discrepancy was seen for low concentrations of T3 (Fig. 1b). Fig. 2a and b show these data for FT4 and FT3 respectively. In this case the greatest discrepancy was seen for high concentrations of FT4. FT3 values were discrepant throughout the range.

2. Out of range analytes

Table 3 shows the number (percentage) of analytes that were outside of their reference interval. As can be seen, there are markedly dissimilar proportions of each analyte that fall outside of their reference interval when they are measured by the two difference assay methods.

Three samples were below the reference interval for all analytes. Six samples were low for all analytes except T4 (IA) and/or FT4 (IA). Nine samples were low for three analytes (T3 (MS), FT4 (MS) and FT3 (MS)) but were not low for T4 (MS), T4 (IA) and FT4 (IA). Eleven samples were low for only T3 (MS) and FT4 (MS). Seventeen samples were low for T3 (MS) only. At the high end of the range 4 samples were high in all of the following 5 analytes (T4 (MS), T4 (IA), FT4 (MS), FT4 (IA), and FT3 (IA)). Seven samples were high for just T4 (MS) and FT4 (IA), with a subset being high for T4 (IA) also.

3. Analytes divided by TSH groupings.

When T4, T3, FT4, and FT3 were divided into 3 groups according to their respective TSH values of <0.35 mIU/L, $0.35\text{--}3.5$ mIU/L, and >3.5 mIU/L, there was generally a decline in the analyte value as the TSH values increased (see Figs. 3 and 4). This was perhaps best seen for the FT3 values (see Fig. 4b). The tendency

of T4 and T3 values measured by IA to change little over the range of TSH concentrations can be seen in the left hand section of Fig. 3a and b. In each case, lower T4 and T3 values were more frequently reported for TSH levels above 3.5 mIU/L when measured by MS as compared to IA (see right hand box plot in each figure with the box plot extending down to lower values for the MS assay shown in the furthest right-sided bar of the figure). The data for FT4 and FT3 are shown in Fig. 4a and b.

4. Analyte correlations with TSH

When all patients were analyzed as one group, the analyte with the best inverse correlation with log TSH was FT4 (IA). The correlation coefficient, however, was low at -0.51 ($P < 0.01$), and similar to that for FT4 (MS) -0.47 ($P < 0.01$). The degree of correlation, although significant for all other analytes, ranged only between -0.22 and -0.46 (see Table 4). When patients were divided into outpatients and inpatients, the correlations were better for outpatients for all analytes. In fact, in most cases there was a positive correlation between the various thyroid hormones and log-TSH in inpatients.

5. Between-analyte correlations

As shown in Table 5, there was fair correlation between total and free thyroid hormone levels regardless of the assay used. For example, T3 (IA) correlated with both FT3 (IA) and FT3 (MS). There was also some correlation between the different analytes. For example, T3 (MS) correlated with T4 (MS) and T4 (IA), while FT3 (IA) correlated with T4 (MS).

Discussion

The first major finding from this analysis is that total thyroid hormone values measured by different assays show greater concordance than do free thyroid hormone assays. This finding is expected, as assay performance is affected by hormone concentrations and total hormone concentrations are obviously higher than free hormone concentrations.

A second finding is that different assays have discrepant values at the low and high end of the reference intervals. This is particularly true of T3 and FT4. This direction of the discrepancy was such that low values for T3 were more often demonstrated by MS, and high values for FT4 were more often demonstrated by IA.

As a third observation, when analyte concentrations were grouped according to TSH values, thyroid hormone values below the reference interval were more often seen in association with high TSH concentrations in the case of MS. However, at the other end of the spectrum, thyroid hormone values above the reference interval were seen in association with low TSH values with both MS and IA measurements.

These results are disconcerting, since, theoretically, an ideal assay would function equally well at all ranges of analyte values. It is extreme values that typically serve as the basis for clinical decision-making regarding perturbed thyroid hormone secretion.

The patient population we studied consists of both inpatients and outpatients. Because of this diversity and the presence of the inpatient setting, factors typically affecting IA performance would be expected to come into play. Indeed, analyzing a variety of clinical situations was an achieved goal of the present study. Inpatients, especially, are known to be exposed to a variety of medical conditions and medications that predispose them to a typical pattern of thyroid function abnormalities. This constellation of findings is referred to as “the euthyroid sick syndrome” and by traditional immunoassays these patients frequently have decreased T3 and FT3 levels, and may also have normal or low T4 and FT4. Presently, it is unknown if these measurements truly reflect endogenous biologic conditions, or whether they are, at least in part, artifactual related to measurement issues. MS assays are less susceptible to these interferences [14]. Also IAs used for measurement of FT4/FT3 do not separate the analytes from their binding proteins, while the MS methods do. Based on both these IA features, it is postulated that the values generated by our MS assays are a better reflection of the true clinical situation, as is suggested in our data regarding TSH groupings. We thus postulate that the low T3 values generally seen by MS in this study, even within patients with normal TSH values, may be a true reflection of the medical status of the mixed patient population, although we cannot completely exclude that MS assays are not performing appropriately in this situation.

Thyroid hormone values are generally expected to have an inverse log-linear relationship with TSH. Although, this relationship has been shown to be complex [24] and affected by age [25] and, recently in a large group of patients, also by gender [13]. In our group of patients, the degree of $\log_{10}$ -thyroid hormone correlation was not high in any instance, although it was significant for all analytes in the entire group and in outpatients. Part of the reason for this low degree of correlation could be that the TSH and thyroid hormone values may have been affected by illness in the inpatient subgroup, as suggested by the lesser TSH-thyroid hormone correlations in inpatients. For example, inpatients are on several drug regimens that may affect TSH values or may impact deiodinase activity and change the concentrations of thyroid hormones. The generally poor correlation in our present study differs from our prior studies, which have shown a good correlation between FT4 measured by MS and log TSH [8,9]. However, it is a limitation of our study that the numbers of patients within the various subgroups are small, making it difficult to examine correlations between hormones within sub-groups. In addition, we did not measure heterophilic antibodies or other interfering substances as part of the study and only compared our MS methodology with one particular IA for each analyte.

In conclusion, assays with consistent performance at concentrations at either extreme of their reference interval would be more helpful to clinicians than the mixed performance demonstrated here. Our preliminary data with TSH groupings suggest that MS may perform better than IA, although further research in this area is warranted. Our results indicate the need for better means of validating these assays. Efforts to develop accurate clinical markers of thyroid hormone action at the cellular level that would lead to an enhanced assessment of which analyte assays are more reflective of the clinical status would be of considerable benefit.

Acknowledgments

This project has been funded in part with Federal funds (UL1TR000101 previously UL1RR031975) from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health, through the Clinical and Translational Science Awards Program (CTSA), a trademark of DHHS, part of the Roadmap Initiative, “Re-Engineering the Clinical Research Enterprise”.

KDB has or has recently had research grants from Pfizer, Eisai, Amgen, Astra Zeneca, and Innovative Technologies. He is the Deputy Editor of the Endocrine Society Journal, the Journal of Clinical Endocrinology and Metabolism. He serves on the Food and Drug Administration Endocrine Advisory Committee as an ad hoc member.

References

1. d'Herbomez M, Forzy G, Gasser F, Massart C, Beaudonnet A, Sapin R. Clinical evaluation of nine free thyroxine assays: persistent problems in particular populations. *Clin Chem Lab Med*. 2003; 41(7):942–947. [PubMed: 12940522]
2. Steele BW, Wang E, Klee GG, Thienpont LM, Soldin SJ, Sokoll LJ, et al. 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(3):310–317. [PubMed: 15737023]
3. Nelson JC, Wang R, Asher DT, Wilcox RB. Underestimates and overestimates of total thyroxine concentrations caused by unwanted thyroxine-binding protein effects. *Thyroid*. 2005; 15(1):12–15. [PubMed: 15687815]
4. 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(1):31–39. [PubMed: 10691311]
5. 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(6): 753–767. [PubMed: 19942151]
6. Lee RH, Spencer CA, Mestman JH, Miller EA, Petrovic I, Braverman LE, et al. Free T4 immunoassays are flawed during pregnancy. *Am J Obstet Gynecol*. 2009; 200(3):260 e1–260 e6. [PubMed: 19114271]
7. Kahric-Janovic 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(4): 303–311. [PubMed: 17465859]
8. Jonklaas J, Kahric-Janovic 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(7):1380–1388. [PubMed: 19460839]
9. 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(1):122–127. [PubMed: 21097676]
10. Serdar MA, Ozgurtas T, Ispir E, Kenar L, Senes M, Yucel D, et al. Comparison of relationships between FT4 and log TSH in Access DXI 800 Unicel, Modular E170 and ADVIA Centaur XP Analyzer. *Clin Chem Lab Med*. 2012; 50(10):1849–1852. [PubMed: 23089718]
11. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory medicine practice guidelines Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid*. 2003; 13(1):3–126. [PubMed: 12625976]
12. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, et al. Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. *J Clin Endocrinol Metab*. 1990; 70(2):453–460. [PubMed: 2105333]
13. Hadlow NC, Rothacker KM, Wardrop R, Brown SJ, Lim EM, Walsh JP. The relationship between TSH and free T4 in a large population is complex and nonlinear and differs by age and sex. *J Clin Endocrinol Metab*. 2013; 98(7):2936–2943. [PubMed: 23671314]
14. van Deventer HE, Soldin SJ. The expanding role of tandem mass spectrometry in optimizing diagnosis and treatment of thyroid disease. *Adv Clin Chem*. 2013; 61:127–152. [PubMed: 24015601]

15. 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(4):642–651. [PubMed: 18258669]
16. Gounden V, Soldin SJ. Clinical use of reference intervals derived from some CALIPER studies questioned. *Clin Chem*. 2014; 60(2):416–417. [PubMed: 24363369]
17. Soukhova N, Soldin OP, Soldin SJ. Isotope dilution tandem mass spectrometric method for T4/T3. *Clin Chim Acta*. 2004; 343(1–2):185–190. [PubMed: 15115693]
18. Nguyen LT, Gu J, Soldin OP, Soldin SJ. Development and validation of an isotope dilution tandem mass spectrometry method for the simultaneous quantification of 3-iodothyronamine, thyroxine, triiodothyronine, reverse T3 and 3,3'-diiodo-L-thyronine in human serum. *Clin Chem*. 2011; 57(B-99):A82.
19. Soldin, SJ.; Nguyen, L.; Sun, K.; Greenan, G.; Hanak, M.; Soldin, OP. Adult reference intervals for 3-iodothyronamine, thyroxine, triiodothyronine, reverse T3 and 3,3'-diiodo-L-thyronine measured by isotope dilution HPLC tandem mass spectrometry in human serum. American Association of Clinical Chemists annual meeting; Atlanta, GA. 2011.
20. 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(1–2):113–118. [PubMed: 16018881]
21. Gu J, Soldin OP, Soldin SJ. Simultaneous quantification of free triiodothyronine and free thyroxine by isotope dilution tandem mass spectrometry. *Clin Biochem*. 2007; 40(18):1386–1391. [PubMed: 17936741]
22. Soldin OP, Jang M, Guo T, Soldin SJ. Pediatric reference intervals for free thyroxine and free triiodothyronine. *Thyroid*. 2009; 19(7):699–702. [PubMed: 19583487]
23. Jonklaas J, Sathasivam A, Wang H, Finigan D, Soldin OP, Burman KD, et al. 3,3'-diiodothyronine concentrations in hospitalized or thyroidectomized patients: results from a pilot study@. *Endocr Pract*. 2014:1–22.
24. 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(6):1123–1129. [PubMed: 20299491]
25. Over R, Mannan S, Nsouli-Maktabi H, Burman KD, Jonklaas J. Age and the thyrotropin response to hypothyroxinemia. *J Clin Endocrinol Metab*. 2010; 95(8):3675–3683. [PubMed: 20484476]

Fig. 1.

a. Correlation between T4 measured by immunoassay and mass spectrometry (dotted lines indicate the reference interval for each assay, with the area of overlap indicated in the central boxed area). b. Correlation between T3 measured by immunoassay and mass spectrometry (dotted lines indicate the reference interval for each assay, with the area of overlap indicated in the central boxed area).

Fig. 2.

a. Correlation between FT4 measured by immunoassay and mass spectrometry (dotted lines indicate the reference interval for each assay, with the area of overlap indicated in the central boxed area). b. Correlation between FT3 measured by immunoassay and mass spectrometry (dotted lines indicate the reference interval for each assay, with the area of overlap indicated in the central boxed area).

Fig. 3.

a. T4 measured by immunoassay and mass spectrometry divided into 3 groups according to increasing serum TSH values (dashed lines indicate reference interval). b. T3 measured by immunoassay and mass spectrometry divided into 3 groups according to increasing serum TSH values (dashed lines indicate reference interval).

Fig. 4.

a. FT4 measured by immunoassay and mass spectrometry divided into 3 groups according to increasing serum TSH values (dashed lines indicate reference interval). b. FT3 measured by immunoassay and mass spectrometry divided into 3 groups according to increasing serum TSH values (dashed lines indicate reference interval).

Table 1

Tandem mass spectrometry method for FT4 and FT3.

1a. Mass spectrometer gradient parameters			
	Time, min	Solvent A, %	Solvent B, %
Cleaning	0.00	70	30
	1.00	70	30
	2.00	54	46
	3.50	30	70
	4.00	30	70
	4.01	10	90
	5.00	10	90
Elution	5.01	70	30
	7.00	70	30

1b. MRM conditions for FT3, FT4 and internal standards in negative ion mode					
Compound	MRM transition	DP	EP	CE	CXP
FT3	649.7/126.8	-109	-10	-94	-15
T3-6C13	655.5/126.9	-110	-10	-99	-7
FT4	775.5/126.8	-90	-10	-110	-17
T4-d5	780.7/126.8	-99	-10	-90	-17

1c. Tandem mass spectrometer working parameters	
Parameter	Value
Collision gas (CAD)	High
Curtain gas (CUR)	30
Gas 1 (GS1)	35
Gas 2 (GS2)	65
Ionspray voltage, V	-4500
Probe temperature, °C	650
Dwell time, ms	250

Solvent A: 0.05% formic acid in 2% (v/v) methanol/water.

Solvent B: 50% (v/v) methanol/acetonitrile.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Within-analyte, between-assay correlations.

	Analyte 1	Analyte 2	Spearman correlation coefficient (r)	P	Slope and Y intercept
Across entire range	T3 IA	T3 MS	0.95	<0.01	$y = 1.1249x + 34.13$
	T4 IA	T4 MS	0.91	<0.01	$y = 1.0806x + 1.23$
	FT4 IA	FT4 MS	0.75	<0.01	$y = 0.4891x + 0.46$
Below reference interval	FT3 IA	FT3 MS	0.50	<0.01	$y = 0.3180x + 194.59$
	T3 IA	T3 MS	0.72	<0.01	$y = 0.8612x + 45.08$
	T4 IA	T4 MS	0.75	<0.01	$y = 2.9194x - 3.27$
	FT4 IA	FT4 MS	0.37	<0.01	$y = 0.3701x + 0.56$
	FT3 IA	FT3 MS	0.01	0.85	$y = 0.0339x + 190.67$
Above reference interval	T3 IA	T3 MS	–	–	–
	T4 IA	T4 MS	0.51	<0.01	$y = 1.0424x + 1.91$
	FT4 IA	FT4 MS	0.38	<0.01	$y = 0.1552x + 1.41$
	FT3 IA	FT3 MS	–	–	–

Table 3

Analytes falling outside their reference interval (below the 2.5th percentile or above the 97.5th percentile).

Analyte	Percentage below range	Percentage above range
T4 MS	9	23
T4 IA	3	18
T3 MS	46	0
T3 IA	13	4
FT4 MS	29	7
FT4 IA	4	25
FT3 MS	18	2
FT3 IA	14	6

Table 4

Correlation (spearman correlation coefficient) of thyroid hormone analytes with log₁₀ transformed TSH (all correlations are negative, except for those in inpatients).

	Analyte	r	P value
Outpatients and inpatients combined (n = 100)	T4 MS	-0.25	0.01
	T4 IA	-0.25	0.01
	T3 MS	-0.22	0.03
	T3 IA	-0.22	0.02
	FT4 MS	-0.47	<0.01
	FT4 IA	-0.51	<0.01
	FT3 MS	-0.25	0.01
	FT3 IA	-0.46	<0.01
Outpatients (n = 63)	T4 MS	-0.32	<0.01
	T4 IA	-0.35	<0.01
	T3 MS	-0.29	0.01
	T3 IA	-0.33	<0.01
	FT4 MS	-0.61	<0.01
	FT4 IA	-0.58	<0.01
	FT3 MS	-0.25	0.01
	FT3 IA	-0.74	<0.01
Inpatients (n = 37)	T4 MS	Positive correlation	–
	T4 IA	Positive correlation	–
	T3 MS	Positive correlation	–
	T3 IA	Positive correlation	–
	FT4 MS	-0.24	0.01
	FT4 IA	-0.30	<0.01
	FT3 MS	Positive correlation	–
	FT3 IA	Positive correlation	–

Table 5

Between analyte correlations with spearman correlation coefficients above 0.4.

Analyte 1	Analyte 2	r	P
T3 IA	FT3 IA	0.79	<0.01
T3 MS	FT3 IA	0.79	<0.01
T4 MS	T3 MS	0.71	<0.01
T4 IA	T3 MS	0.65	<0.01
T3 IA	T4 MS	0.65	<0.01
FT4 IA	T4 IA	0.64	<0.01
FT4 IA	T4 MS	0.64	<0.01
T4 IA	T3 IA	0.62	<0.01
FT3 IA	T4 MS	0.55	<0.01
T3 MS	FT3 MS	0.47	<0.01
T4 MS	FT4 MS	0.44	<0.01
T4 IA	FT4 MS	0.43	<0.01
T3 IA	FT3 MS	0.42	<0.01