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Development of a pregnancy-specific reference material for thyroid biomarkers, vitamin D, and nutritional trace elements in serum

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Abstract

Objectives: Matrix differences among serum samples from non-pregnant and pregnant patients could bias measurements. Standard Reference Material 1949, Frozen Human Prenatal Serum, was developed to provide a quality assurance material for the measurement of hormones and nutritional elements throughout pregnancy.

Methods: Serum from non-pregnant women and women in each trimester were bottled into four levels based on pregnancy status and trimester. Liquid chromatography tandem mass spectrometry (LC-MS/MS) methods were developed and applied to the measurement of thyroid hormones, vitamin D metabolites, and vitamin D-binding protein (VDBP). Copper, selenium, and zinc measurements were conducted by inductively coupled plasma dynamic reaction cell MS. Thyroid stimulating hormone (TSH), thyroglobulin (Tg), and thyroglobulin antibody concentrations were analyzed using immunoassays and LC-MS/MS (Tg only).

Results: Certified values for thyroxine and triiodothyronine, reference values for vitamin D metabolites, VDBP, selenium, copper, and zinc, and information values for reverse triiodothyronine, TSH, Tg, and Tg antibodies were assigned. Significant differences in serum concentrations were evident for all analytes across the four levels ($p \leq 0.003$).

TSH measurements were significantly different ($p < 0.0001$) among research-only immunoassays. Tg concentrations were elevated in research-only immunoassays vs. Federal Drug Administration-approved automated immunoassay and LC-MS/MS. Presence of Tg antibodies increased differences between automated immunoassay and LC-MS/MS.

Conclusions: The analyte concentrations' changes consistent with the literature and the demonstration of matrix interferences in immunoassay Tg measurements indicate the functionality of this material by providing a relevant matrix-matched reference material for the different stages of pregnancy.

Keywords: mass spectrometry; pregnancy; standard reference material; thyroid; trace elements; vitamin D.

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Introduction

Dietary iodine, vitamin D, copper, selenium, and zinc are important nutrients for a healthy pregnancy. Iodine is a nutritional element essential to thyroid hormone production. Thyroid hormones (thyroxine [T_4], triiodothyronine [T_3], and reverse triiodothyronine [rT_3]), thyroid

stimulating hormone (thyrotropin [TSH]), and thyroglobulin (thyroid hormone precursor [Tg]) support fetal development [1]. Maternal thyroid disorders can lead to adverse pregnancy and birth outcomes, such as pre-eclampsia, pre-term delivery, low birth weight neonates, and impaired neurological development [2–4]. Vitamin D promotes bone health in women and the newborn [5, 6]. Nutritional deficiencies of trace elements including copper, zinc, and selenium are linked to poor fertility, pregnancy loss, retention of the placenta, and teratogenicity [7]. Deficiency of these nutrients leads to deleterious outcomes for women and children.

In order to better address public health and research pregnancy outcomes related to thyroid and vitamin D biomarkers and trace elements, the National Institute of Standards and Technology (NIST), the Centers for Disease Control and Prevention (CDC), the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and the Mayo Clinic collaborated to develop a new Standard Reference Material (SRM) 1949, Frozen Human Prenatal Serum. This trimester-matched serum material was uniquely developed to represent the concentrations of hormones, trace elements, and proteins observed throughout pregnancy [8]. This is the first pregnancy-specific serum material certified for important nutritional biomarkers, which could provide better diagnostic quality assurance for maternal/fetal health.

This manuscript details the production of SRM 1949 as follows: (1) improvement of the thyroid hormone reference measurement procedures (RMPs) using liquid chromatography tandem mass spectrometry (LC-MS/MS), (2) application of candidate reference measurement procedures using LC-MS/MS methods for vitamin D metabolites and vitamin D-binding protein (VDBP) to pregnancy serum, (3) application of a CDC-developed method to measure copper, zinc, and selenium in pregnancy serum, and (4) investigation of multiple commercially available research only grade enzyme-linked immunosorbent assays (ELISAs), Food and Drug Administration (FDA) approved automated immunoassays (IAs), and a LC-MS/MS method to explore TSH and Tg measurements.

Materials and methods

Material collection and production

Serum was acquired from Equitech-Bio Inc (Kerrville, TX) in four units of 500 mL per level using FDA guidelines and Good Laboratory Practices. Donor types were non-pregnant (NP) reproductive age women (n=12), first trimester (FT) women 6–10 weeks pregnant (n=40), second trimester (ST) women 18–21 weeks pregnant (n=69), and third trimester (TT) women 32–35 weeks pregnant (n=60). The mean donor age for each pool was 29.

Pools were prepared in a Class 100 cleanroom at the CDC. Materials expected to contact serum were screened for trace metals prior to use [9]. Serum was thawed and homogenized on nutating mixers at 4 °C. Cold serum was filtered through sterile gauze to remove particulates. After equilibration to room temperature, serum was dispensed (1.8 mL) into cryovials using a Microlab 600 automated dispenser system (Hamilton Company, Reno, NV), with continuous mixing. Vials were capped immediately and stored at −70 °C. Shipment to NIST and collaborators was on dry ice and samples were maintained at −80 °C.

Thyroid hormones

Metrological traceability to the International System of Units (SI) for T_4 and T_3 was established through purity characterization of the neat chemical calibrants (Supplemental Table 1). Calibrant mass purities were assessed directly by ^{1}H-qNMR using internal standards (IS). Calibration of ^{1}H-qNMR measurements were performed using a dimethyl sulfone IS traceable to the SI measurement units for chemical mass fraction through the NIST PS1 Primary Standard for quantitative NMR (Benzoic Acid [10]). Data was acquired by a Bruker Avance II 600 MHz spectrometer equipped with a 5-mm broadband inverse detection probe and operating with Topspin (Version 3.2) software (Billerica, MA). LC-MS/MS multiple reaction monitoring (MRM) scans of individual calibrants indicated an impurity of T_3 in T_4 stocks. Therefore, independent calibration curves for each analyte were prepared to avoid underquantifying T_3.

Samples were extracted according to the RMPs for total T_4 and total T_3 by Tai et al. [11, 12]. Briefly, six aliquots per level (0.5 g) were gravimetrically spiked with a mixture of labeled IS containing approximately 5 ng of each T_4-$^{13}C_6$, T_3-$^{13}C_6$, and rT_3-$^{13}C_6$ (Supplemental Table 1). SRM 971, Hormones in Frozen Human Serum, certified for total T_4 and total T_3, served as quality control. Samples were alkalized with ammonium hydroxide before solid phase extraction using Waters Oasis MAX cartridges (3 cc, 60 mg bed weight, Milford, MA). The extract was evaporated to dryness then reconstituted in 100 µL of methanol, water, with formic acid mixture (50:50:0.025, volume fraction, heretofore v/v).

An Agilent 1100 Series LC with an Agilent Eclipse Plus C18 column (2.1×150 mm, 5 µm particle size; Santa Clara, CA) at 20 °C under isocratic conditions of methanol and water with formic acid (60:40:0.5 v/v) at 200 µL/min for 10 min was used to conduct a novel separation that successfully baseline delimited all three thyroid hormone metabolites. Instrument parameters for an AB Sciex API 4000 LC-MS/MS system (Toronto, Canada) were CAD=6 psig, CUR=20 psig, GS1=40 psig, GS2=70 psig, IS=4500 V, TEM=600 °C, injection=10 µL, positive mode, electrospray ionization. Two transitions per analyte were monitored using MRM (Supplemental Table 1).

Vitamin D

Vitamin D metabolites were measured using a new high throughput isotope dilution LC-MS/MS method that complements the current RMP [13]. Serum (0.75 g) was spiked with an IS solution (25-(OH)D_3-$^{13}C_5$=162 ng, 25-(OH)D_2-$^{13}C_3$=12.4 ng, and 3-epi-25(OH)D_3-d_3=1.72 ng; Supplemental Table 1). After equilibration at room temperature for 1 h,

sample pH was adjusted to pH 9.8±0.2 with carbonate buffer. Sample liquid-liquid extraction was conducted twice with a mixture of hexane and ethyl acetate (50:50 v/v). Combined extracts were dried under nitrogen at 45 °C and reconstituted with 250 µL methanol. SRM 972a, Vitamin D Metabolites in Frozen Human Serum, was used for quality control [13]. Extracts (3 µL injection) were analyzed on a SCIEX QTRAP 6500$^+$ LC-MS/MS system in positive mode using atmospheric-pressure chemical ionization, MRM, and an Ascentis Express F5 column at 20 °C (Supelco, Bellefonte, PA) under isocratic conditions with water:-methanol mobile phases.

VDBP was measured using the methods developed by Kilpatrick and Phinney [14]. Application to SRM 1949 are detailed in Kilpatrick et al. [15]. Briefly, serum (11 µL, n = 3 per level) were tryptic digested. Labeled peptide mixture (TSALSAK and VLEPTLK) were added to 30 µL of digest. LC-MRM analyses were performed using a Discover BIO Wide C18 column (2.1×150 mm, 3 µm, Sigma-Aldrich, St. Louis, MO) and guard column (2.1 mm×2 cm, 3 µm) at 40 °C and flow rate of 250 µL/min coupled to an Agilent 6490 triple quadrupole. SRM 1950, Metabolites in Human Plasma, with reference VDBP concentrations, was used as quality control. Calibrants and digested samples (5 and 3 µL respectively; ~1 pmol each) were injected in triplicate.

Trace elements

Concentrations of copper, selenium and zinc were determined using the inductively coupled plasma dynamic reaction cell MS (ICP-DRC-MS) method detailed in the CDC Laboratory Procedure Manual [16–18]. Replicates were separate 1:30 dilutions of serum with diluent (140 nmol/L gallium, as an IS in 2% v/v nitric acid, 5% v/v ethyl alcohol and 0.01% v/v Triton™ X-100 in≥18.2 M·Ω water). Replicates were tested by introducing an aerosol of each dilution to an ELAN DRC II ICP-MS instrument (PerkinElmer, Waltham, MA) using an SC4-DX (Elemental Scientific Inc., Omaha, NE) autosampler. External calibrators were prepared in pooled serum using SI-traceable standard SM-2107-013 (High-Purity Standards, Charleston, SC). All elements were measured in DRC mode (using ammonia gas) to eliminate polyatomic interferences. Two custom-made, characterized serum bench quality control materials were inserted at the beginning and end of each analytical run, and the multi-rule quality control system (MRQCS) developed by Caudill et al. [19], was used to determine if runs were in control.

Thyroid related proteins

Research-only grade ELISA kits were used to measure TSH (three different manufacturers) and Tg (one kit previously assessed using SRM 971). Each kit was prepared according to the manufacturer specifications. Plates were read at 450 nm absorbance using an Epoch 2 microplate reader (BioTek, Winooski, VT).

Automated IA was used to measure Tg and Tg antibodies (TgAB) using the methods described by Netzel et al. [20]. A Beckman Access (Beckman Coulter, Brea, CA) was used per the manufacturer's instructions. Tg was standardized against the certified reference material BCR 457 (European Commission Institute for Reference Materials and Measurements, Gembloux, Belgium); TgAB was standardized against the World Health Organization 65/93 International Standard (Geneva, Switzerland). Acceptance criteria required measurement within ±2 standard deviations of the established mean.

Tg was measured by LC-MS/MS using the methods described by Netzel et al. [20] (method Tg-MS-1) with slight changes. High molecular weight proteins (>80 kDa) were precipitated from 375 µL of serum with ammonium sulfate (225 µL). Pellets were re-suspended, reduced (1.875 mg of dithiothreitol) and alkylated (3 mg of iodoacetamide). Labeled (^{13}C) IS peptide was added, and specimens were trypsin-digested (0.4 mg; Worthington Biochemical Corp, Lakewood, NJ) for 16 h. Antibody coated mass spectrometric immunoassay disposable automation research tips (Thermo Fisher Scientific, Waltham, MA) were added to capture the proteotypic Tg peptide (FSPDDSAGA-SALLR), which was assayed post elution by MRM LC-MS/MS (AB SCIEX 6500). Injections were 50 µL and column temperature was 35.0 °C. Two transitions of the native peptide (704.1/587.0 and 704.1/687.6) and one transition of the IS (708.9/591.5) were monitored.

Serum density

Three vials per level were equilibrated at room temperature for 2 h. Six 1 mL replicates (23 °C) were injected into the oscillation loop of a water-calibrated digital density meter (DMA 35, Anton Paar, Graz, Austria) and allowed to stabilize before recording.

Quantitation

MS data was analyzed using ratios of analyte over IS. Values were extrapolated using simple linear regression for thyroid hormones, vitamin D, VDBP, and trace metals. Tg used weighted $1/x^2$ linear regression. Method performance data for unestablished methods can be found in Supplemental Table 2.

Statistical analysis was conducted using JMP 12 (SAS Institute, Cary, NC) to examine differences in analyte concentrations among the levels or methods. Analysis of variance (ANOVA) was conducted and if a significant difference was detected ($p \leq 0.05$) a post hoc all pairs Tukey honest significant difference was conducted. Values below the reporting limit (RL) were censored to the RL before statistical analysis.

Value assignment was based upon NIST guidelines [21]. Acceptability criteria for all SRM quality control measurements were defined as measuring the analyte within the certified or reference range.

Results

Thyroid hormones

Thyroid hormones were baseline separated and quantified (Figure 1; Supplemental Table 3). Calibrant purities determined via NMR were 97.73% for T_4, 95.83% for T_3, and 75.4% for rT_3. SRM 971 was within the certified range for both T_4 and T_3 (Supplemental Figure 1). The relative standard deviations (RSDs) for SRM 1949 quantitative product ions were ≤1.2% for T_4, ≤5.7% for T_3 and ≤14.5% for rT_3.

Statistically significant increases were detected for T_3 and T_4 from the NP to ST ($p<0.0001$; Figure 2). Only the NP level had lower concentrations of rT_3 compared to the other levels ($p \leq 0.0006$).

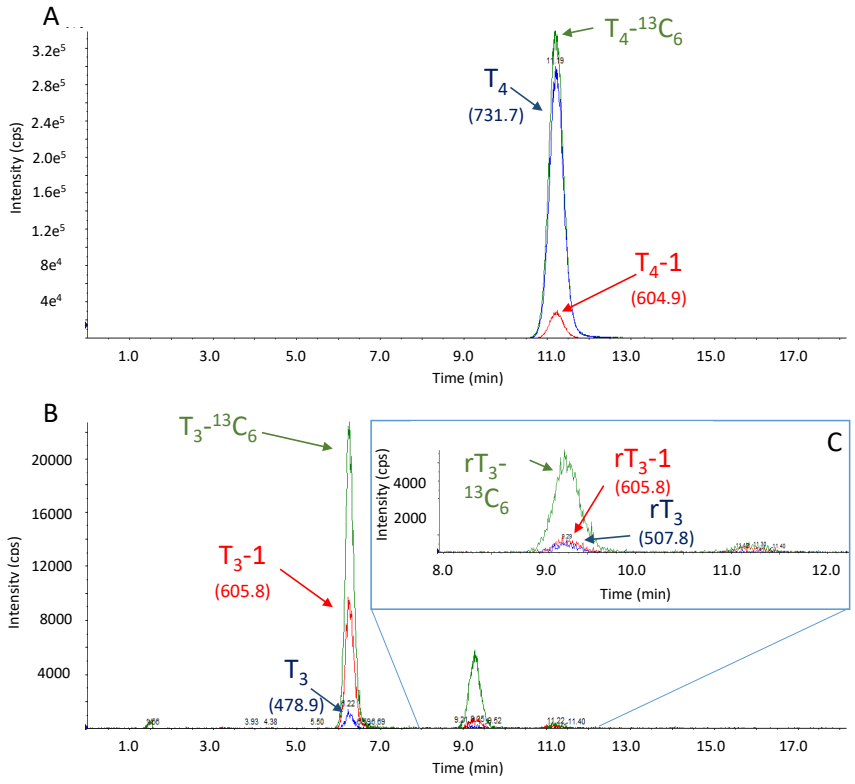

Figure 1: Representative chromatograms of thyroid hormones in SRM 971 Hormones in Frozen Human Serum (male level) (A) Thyroxine. (B) Triiodothyronine and reverse triiodothyronine. (C) Enlarged image of reverse triiodothyronine. Numbers in parentheses are product ions for the given analyte.

Vitamin D

SRM 972a values were within the certified range for all the target analytes (Supplemental Figure 2). SRM 1949 Vitamin D metabolite RSDs were ≤1% for 25(OH)D_3, <4% for 3-epi-25(OH)D_3, and <7% for 25(OH)D_2 for all levels.

Concentrations of 25(OH)D_3 and 3-epi-25(OH)D_3 increase significantly from NP to ST ($p \leq 0.0019$; Figures 3A, B). Concentrations of 25(OH)D_2 were significantly different ($p < 0.0001$) except for the NP and ST (Figure 3C).

SRM 1950 VDBP measurement was within the reference range. SRM 1949 VDBP RSDs were ≤1.5% for all levels. VDBP concentrations significantly increased from NP to TT ($p < 0.0001$; Figure 3D).

Trace elements

Quality control materials passed the MRQCS. SRM 1949 RSDs were ≤2.5% for copper, ≤3.6% for selenium and ≤7.2% for zinc.

Copper concentrations increased significantly from NP to TT ($p \leq 0.0001$). Selenium concentrations displayed a significant non-monotonic relationship ($p \leq 0.0034$). Zinc concentrations had a significant inverted-U relationship ($p < 0.0001$ for significant differences; Figure 4).

Thyroid related proteins

TSH ELISA kits generated RSDs <15% (Figure 5A); All three kits generated significantly different concentrations of TSH for the NP level (all significant differences were $p < 0.0001$). Kit 1 and Kit 3 yielded a percent difference of 41% for the NP level but agreed on concentration for the pregnancy levels (differences of <14%). Kit 2 consistently generated elevated concentrations of TSH compared to the other two kits (percent differences between 41 and 80%). All kits demonstrated a significant decrease of TSH during pregnancy when compared to the NP pool ($p < 0.0001$).

Tg measurements on SRM 1949 yielded RSDs<20% using ELISAs (Figure 5B), RSDs≤3.7% using automated IA, and RSDs≤3.4% using LC-MS/MS for all levels. There was a significant increase in Tg concentrations ($p < 0.0001$) from NP to TT regardless of measurement method ($p < 0.02$ for all ELISA comparisons; $p < 0.0001$ for automated IA except the FT to ST comparison; $p < 0.0001$ for all LC-MS/MS comparisons).

The percent difference between the Tg values generated from the 5x dilution (all values on the standard curve) and the LC-MS/MS method was 72% for the non-pregnant level with progressively greater differences (FT 155%, ST 166%, and TT 170%).

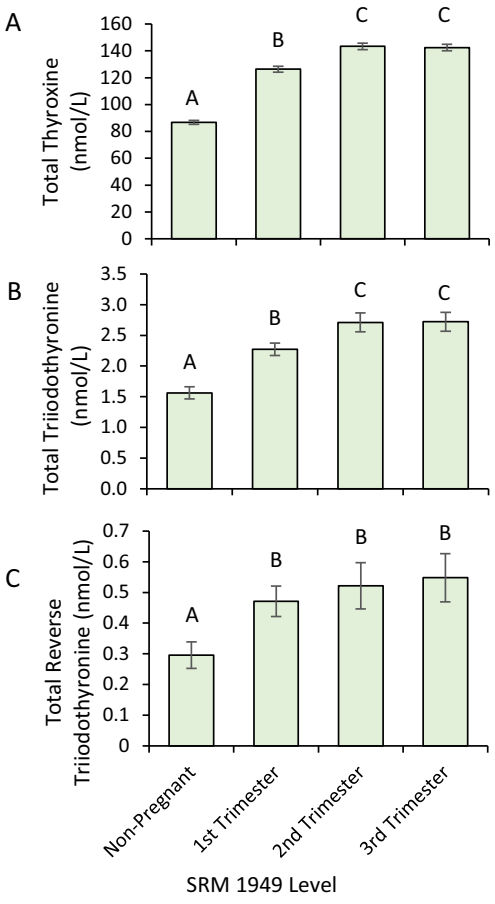

Figure 2: Differences in concentrations of thyroid hormones across the four levels of SRM 1949, Frozen Human Prenatal Serum (n=6 vials per level). ANOVA was conducted and all significant differences had p-values <0.0001.
Error bars are ± one standard deviation. Significant differences from an all pairs Tukey HSD are represented by different letters. (A) Thyroxine. (B) Triiodothyronine. (C) Reverse Triiodothyronine.

The automated IA values did not significantly differ from the LC-MS/MS values. TgAB positive levels (>1.8 kIU/L; Figure 6) had automated IA Tg values that trended lower compared to LC-MS/MS values (NP=35% and FT=14% difference respectively).

Discussion

Measurement quality resulted in certified values for total T_4 and total T_3, reference values for 25(OH)D_3, 25(OH)D_2, 3-epi-25(OH)D_3, VDBP, copper, zinc, and selenium, and information values for total rT_3, TSH, Tg, and TgAB for SRM 1949 (Supplemental Table 4). Changes in concentrations of analytes comparable to the literature and detection of method-dependent matrix interferences demonstrate the utility of this unspiked, matrix-matched reference material for measurement quality assurance during pregnancy.

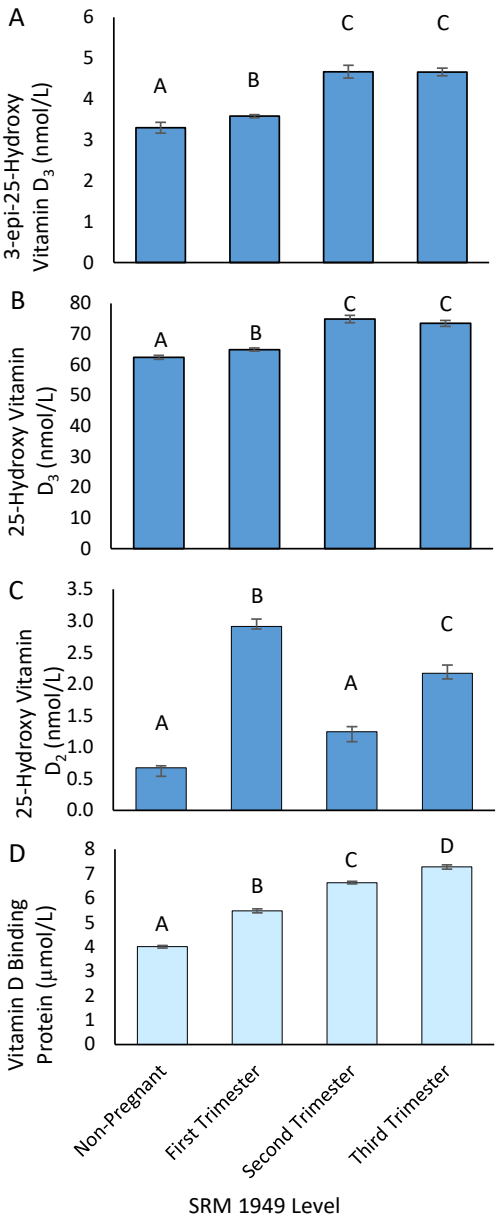

Figure 3: Differences in concentrations of vitamin D metabolites and vitamin D-binding protein across the four levels of SRM 1949 (n=3 vials per level, two preparations, two injections).
ANOVA was conducted and all significant differences had p-values ≤0.0001. Error bars are ± one standard deviation. Significant differences represented by different letters. (A) 3-epi-25-hydroxy vitamin D, (B) 25-hydroxy vitamin D_3, (C) 25-hydroxy vitamin D_2, (D) Vitamin D-binding protein.

Thyroid hormones

This new method improved upon the RMPs by quantifying all three thyroid hormone in one method. Separation of T_3 and rT_3 achieved by this method is critical for accurate quantitation as they have identical mass transitions and

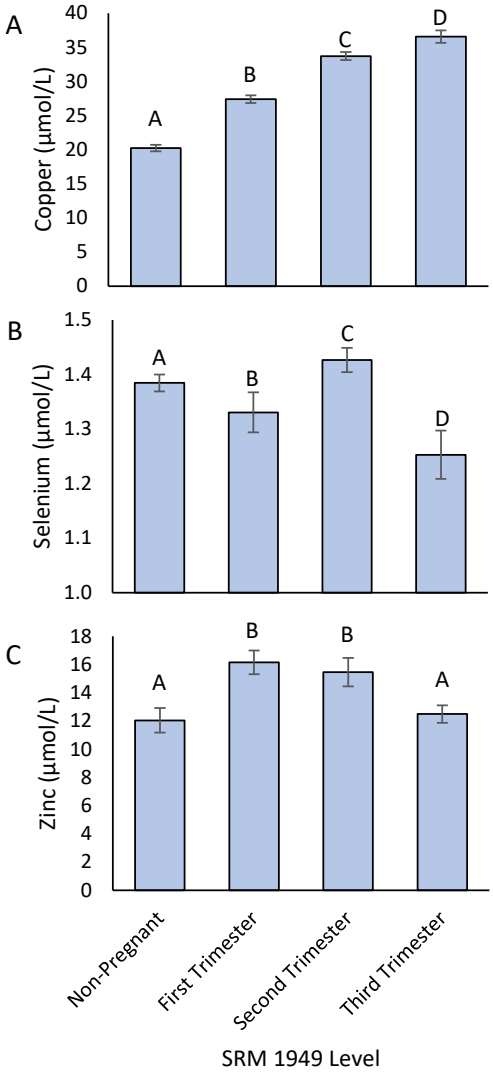

Figure 4: Differences in concentrations of trace elements across the four levels of SRM 1949, Frozen Human Prenatal Serum (n=15 per level).
ANOVA was conducted and all significant differences had p-values <0.0001. Error bars are ± one standard deviation. Significant differences from an all pairs Tukey HSD are represented by different letters. (A) Copper, (B) Selenium, (C) Zinc.

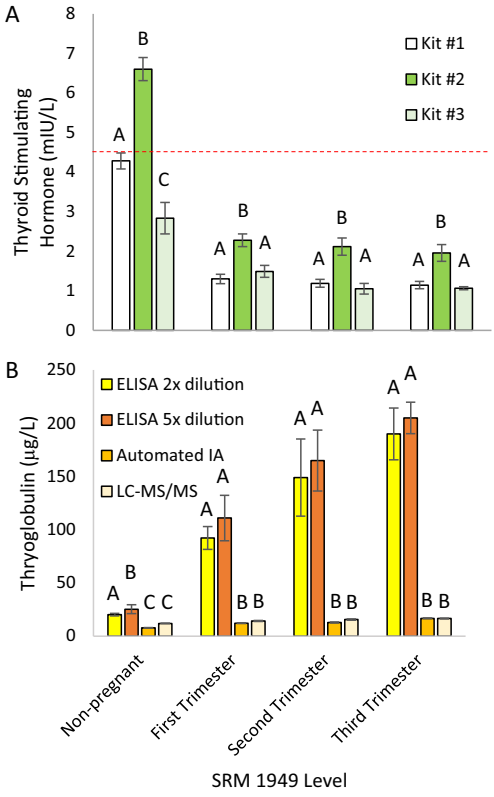

Figure 5: Differences in concentrations of thyroid related proteins in SRM 1949 (n=6 vials in triplicate).
ANOVA was conducted on each method by level and all significant differences had p-values <0.0001 (represented by different letters). Error bars are ± one standard deviation. (A) Thyroid stimulating hormone (TSH) measurements from three different kits (n=6 vials in triplicate) using undiluted serum per manufacturers recommendations. Red dotted line indicates the highest threshold for hypothyroid diagnosis. (B) Thyroglobulin using ELISA (n=6 in triplicate per level), automated immunoassay (n=3 per level), and liquid chromatography tandem mass spectrometry (n=6 per level). The 2x dilution yielded values outside the standard curve; thus a 5x dilution was conducted.

differ only by the location of one iodine. Confidence in the purity adjusted measurements of T_4 and T_3 were sufficiently accurate (within the certified range for SRM 971 and calibrated with high purity standards) and precise (RSDs<6.0%) to generate certified values for all four levels of SRM 1949. Due to the low purity of the standard, low serum concentrations, and high RSDs, rT_3 concentrations are provided only as information values. However, LC-MS/MS rT_3 measurements have comparable variance to acceptable limits in ELISA (<15%), and values generated by LC-MS/MS using purity corrections are more reliable than antibody binding techniques that are prone to interferences and nonspecific antibodies [22].

Comparison of the values generated by this analysis and the LC-MS/MS method developed by Soldin et al. [22] demonstrates similar, trimester-specific concentrations of total T_4 and total T_3 with slight variation likely due to the gestation week at sampling. According to the literature, T_4 and T_3 concentrations peak after the 16th week of pregnancy and remain elevated until parturition [1]. Because this FT pool consists of women in the 6th to 10th week of pregnancy compared to the 12th week in Soldin et al. [22], thyroid hormone concentrations are still rising but have not peaked as in the ST and TT pools. Similarity of the thyroid hormone concentrations to those in the literature

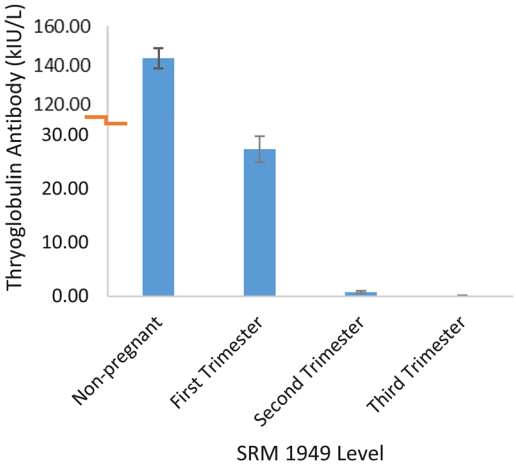

Figure 6: Mean thyroglobulin antibody values for SRM 1949 (n=3 per level).
Samples with measurements above 1.8 kIU/L are considered antibody positive. Error bars are±one standard deviation.

and confidence in the measurements make this material fit for the purpose of standardizing thyroid hormone measurements in pregnant patients.

Vitamin D

Vitamin D metabolites for all levels except 25(OH)D_2 at the NP level due to values below the RL and greater RSDs met the criteria for reference value assignment. Reference values rather than certified values were assigned due to the low number of replicates for the analysis and the lack of a secondary measurement method.

In this study, concentrations of 25(OH)D_3 and 3-epi-25(OH)D_3 increase significantly from NP to ST, while concentrations of 25(OH)D_2 had a non-monotonic pattern. Comparison to the literature is difficult due to different vitamin D supplementation regimens. One study found maternal total 25(OH)D serum concentrations to be (64±24.4) nmol/L during the FT and (75±34.4) nmol/L during the TT [23], which is comparable to the levels measured in SRM 1949. Other studies have found that 25(OH)D concentrations do not change during pregnancy unless intake or exposure to sunlight changes [24, 25]. Whether early-pregnancy increase in vitamin D is due to natural increases or supplementation of vitamin D is beyond the scope of this manuscript. However, vitamin D supplementation during pregnancy is common practice in the US. Thus, possible associated analyte increases such as those present in this material should be considered during clinical assessment.

For VDBP, measurements were precise and SRM 1950 measurements were within the reference range. However, because of low replicates and inability to fully characterize the standard purity, VDBP concentrations were provided as reference values for SRM 1949.

The increase in VDBP with pregnancy in this material concurs with the current literature. Beyond reflecting increased Vitamin D transport, VDBP concentrations are being investigated as candidate biomarkers for certain pregnancy complications and outcomes [26]. Therefore, SRM 1949 can serve the community to assist in quality control of VDBP measurements by providing a matrix-matched reference materials of relevant concentrations.

Trace elements

Patterns of trace element concentrations were consisted with the literature and were assigned reference values due to the high confidence in the measurement methods and quality control. An increase in serum copper through pregnancy consistent with these values is important for iron and red blood cell creation and neural development [27, 28]. Consistent with the literature, selenium concentrations were lowest in the third trimester [29], and zinc concentrations increased upon pregnancy, then decreased through pregnancy [30]. However, concentrations of selenium and zinc are in the higher range of previous studies and might not provide a full range of relevant concentrations for quality assurance.

Thyroid related proteins

Measured values of TSH varied greatly by kit and did not fit the current patterns in the literature. Human chorionic gonadotropin (a TSH inhibitor) decreases after the first 20 weeks of pregnancy [3]. Thus, TSH concentrations are lower in pregnancy onset but then rise again [31]. TSH remains depressed in this material indicating a departure from the literature, abnormally low TSH concentrations, or inaccurate measurement of TSH via ELISA. Therefore, a mean value of the measurements was provided only as an information value.

The current reference limit for hypothyroid diagnosis using TSH is variable (from>3.9 mIU/L to>4.5 mIU/L) [32]. Using these guidelines, values from Kits 1 and 2 would indicate potential hypothyroidism in the donor pool for the NP level, while values from Kit 3 would indicate normal thyroid regulation. Additionally, units used by clinicians, biological activity (IU: international unit), are not SI traceable units. Due to the lack of SI traceable

measurements, no primary measurement method, and cross-manufacturer ELISA kit variability, standardization and harmonization of TSH measurements should be a topmost priority for clinical measurement science.

Though the trimester specific pattern of Tg concentrations remains debated [33], this material demonstrated a significant increase from NP to TT. Tg values generated by the LC-MS/MS and automated IA methods are similar to those described in the literature, whereas the values generated using the ELISA kit methods are elevated by as much as six times [22]. Differences between the ELISA and LC-MS/MS methods increased from NP to TT suggesting that changes in the matrix associated with pregnancy increasingly interfered with the ELISA.

TgAB presence is known to decrease immunoassay measurements of Tg [20]. Therefore, TgAB could bias the automated IA values lower than the LC-MS/MS values, as was the case in the NP and FT levels. The ST level, which would be defined as Tg negative, also had depressed automated IA values compared to the LC-MS/MS values indicating there are additional interferences not accounted for in these pregnancy materials. Therefore, even when TgAB measurements are conducted to identify bias, a reference material such as SRM 1949 could aid in uncovering additional interferences.

Conclusions

The development of SRM 1949 provides the clinical community with the first serum reference material for different pregnancy trimesters. The significant changes in analyte concentrations across the trimester pools consistent with those in the literature indicate the functionality of this SRM by providing a relevant matrix-matched reference material for the different stages of pregnancy. This material can be used for quality control of clinical measurements specifically validated for pregnant patients rather than the reliance on methods developed for non-pregnant patients. Analyte concentrations are dynamic during pregnancy and the need for a matrix-specific material for each stage of development, which this material fulfills, is necessary to ensure the highest quality of patient care.

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Disclaimer: Commercial equipment, instruments, or materials are identified to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by NIST nor the CDC, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. According to NIST's Order 1801.00, "NIST does not evaluate commercial products unless such an evaluation is part of a formal agreement, usually with the manufacturer of the product." "NIST does not endorse commercial products or services; commercial products shall be neither promoted nor disparaged by NIST." Therefore, manufacturer names of commercially available ELISA kits were redacted to comply with federal regulations. This analysis was conducted not to assess manufacturer's products, but to assess the measurement technique compared to other techniques.
Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and was approved by the Institutional Review Board and NIST Human Subjects Protection Office (MML-17-0013).

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