Development of Standard Reference Materials to support assessment of iodine status for nutritional and public health purposes[1–3]

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ABSTRACT

The use of urinary iodine as an indicator of iodine status relies in part on the accuracy of the analytical measurement of iodine in urine. Likewise, the use of dietary iodine intake as an indicator of iodine status relies in part on the accuracy of the analytical measurement of iodine in dietary sources, including foods and dietary supplements. Similarly, the use of specific serum biomarkers of thyroid function to screen for both iodine deficiency and iodine excess relies in part on the accuracy of the analytical measurement of those biomarkers. The National Institute of Standards and Technology has been working with the NIH Office of Dietary Supplements for several years to develop higher-order reference measurement procedures and Standard Reference Materials to support the validation of new routine analytical methods for iodine in foods and dietary supplements, for urinary iodine, and for several serum biomarkers of thyroid function including thyroid-stimulating hormone, thyroglobulin, total and free thyroxine, and total and free triiodothyronine. These materials and methods have the potential to improve the assessment of iodine status and thyroid function in observational studies and clinical trials, thereby promoting public health efforts related to iodine nutrition. *Am J Clin Nutr* 2016;104(Suppl):902S–6S.

Keywords: clinical laboratory, iodine, reference materials, quality control, standardization, thyroid function tests

INTRODUCTION

The National Institute of Standards and Technology (NIST)[6] provides Standard Reference Materials (SRMs) for use in the determination of trace elements and certain other substances in a variety of natural matrices, including human serum, human urine, and foods. SRMs are certified reference materials issued by NIST. A certified reference material is defined as "Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures" (1). SRMs are homogenous and stable materials that have been well characterized for one or more chemical or physical properties. In the case of chemical composition, SRMs are used worldwide to assist laboratories in validating analytical measurements and to provide for routine quality assurance of chemical measurements by serving as control materials.

NIST has provided food-matrix SRMs for nearly 40 y. Very few of these food-matrix SRMs have certified values assigned for iodine content. The first food-matrix SRM to be assigned a certified value for the mass fraction of iodine (i.e., the concentration of iodine calculated on a wt:wt basis) was *SRM 1549 – Non-Fat Milk Powder*, issued in 1984. In 1998, a certified value for iodine was assigned to *SRM 1846 – Infant Formula*, thereby recognizing the importance of iodine measurements in regulating the composition of infant formulas. Generally, certified values for chemical composition are assigned to SRMs by using measurement results obtained from "2 or more independent analytical techniques" (2). That approach to assigning certified values for chemical composition is based on the assumption that if the results from 2 independent techniques are in agreement, then the possibility of biases is minimized. In addition to food-matrix SRMs, NIST has also produced a number of human-serum based SRMs, including one for metabolites of vitamin D (*SRM 972a*) (3). NIST is collaborating with the NIH Office of Dietary Supplements (ODS) to expand the number of food and biological SRMs with values assigned for iodine, an effort that supports quality assurance of analytical measurements and validation of analytical methods for global health initiatives. In the current article we discuss progress in developing SRMs and associated analytical methods for iodine in foods and dietary supplements, for urinary iodine, and for common serum biomarkers of thyroid

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[3] Certain commercial equipment, instruments, or materials are identified in this article to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment specified are necessarily the best available for the purpose.

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[6] Abbreviations used: DBS, dried blood spot; FT3, free triiodothyronine; FT4, free thyroxine; ICP-MS, inductively coupled plasma mass spectrometry; ID, isotope dilution; LC-MS, liquid chromatography–mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; NIST, National Institute of Standards and Technology; ODS, Office of Dietary Supplements; RMP, reference measurement procedure; SI, International System of Units; SRM, Standard Reference Material; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine; UIC, urinary iodine concentration.

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function. A central goal of these efforts is to foster the overall mission of the NIH Iodine Initiative and the specific aims of the 2014 ODS iodine workshops (4) by improving the assessment of iodine status and thyroid function in observational studies and in clinical studies of iodine supplementation.

SRMS FOR IODINE IN FOODS AND DIETARY SUPPLEMENTS

Assessment of dietary iodine intake relies in part on accurate assessment of the accuracy of the analytical measurement of iodine in potentially important dietary sources, including high-iodine foods not fortified with iodine, infant formulas, iodized table salt, and dietary supplements labeled with iodine content. As discussed elsewhere in this journal supplement issue, dairy products and eggs are among the foods sampled by the FDA's Total Diet Study that are high in iodine (5). A collaborative program between NIST and the ODS has led to the development of SRMs for use in measuring iodine in whole milk powder, whole egg powder, infant formula, a multi-element multivitamin supplement, and several other foods and food composites (6–10); these are listed in **Table 1**. Dietary supplements containing natural marine products such as kelp can be very high in iodine (11); accurate assessment of their iodine content may be important for preventing excessive intake. An SRM for kelp material is currently under development (*SRM 3232*).

In the United States, iodized table salt was made available as early as 1924 in response to a high incidence of goiter in the Great Lakes region (12). The FDA has approved the use of potassium iodide, but not potassium iodate, as a nutrient fortifier in table salt (11). Potassium iodate, which is more stable, is used to fortify table salt in other areas of the world, including Australia, Canada, China, India, Indonesia, and several African countries (13). In the United States, stabilizer agents consisting of sodium carbonate or sodium bicarbonate are added to iodized salt during production to prevent oxidation of the added iodide. However, the gradual loss of iodine from iodized salt still remains a serious problem, especially if the salt has been stored for months or years. *SRM 3530* is currently being developed to provide a quality assurance reference material to support measurement of iodide in table salt.

SRMS FOR URINARY IODINE

As discussed elsewhere in this supplement issue, the concentration of iodine (or more precisely, iodide) in the urine of individuals representative of a population is the most commonly used indicator of population iodine status (14). Several analytical methods are used routinely for measurement of urinary iodine concentration (UIC). The most common of the traditional methods is colorimetry based on the Sandell-Kolthoff reaction, in which iodide catalyzes the reduction of ceric ammonium sulfate by arsenious acid (15–18). The emergence of newer instrumental methods based on coupled ion chromatography and inductively coupled plasma mass spectrometry (ICP-MS) permit measurements of greater specificity and sensitivity, although at higher cost (19–22).

Two NIST human urine–matrix SRMs (**Table 2**) are available for validating analytical methods and measurements and to support proficiency testing programs and nutritional survey research programs such as NHANES. *SRM 2670a – Toxic Elements in Urine* is a freeze-dried material that was made available in 2003 through a collaborative development project between NIST and the CDC; it requires reconstitution with water and is certified for 14 elements including iodine. This is a 2-level reference material, which means that it consists of both a blank matrix (Level 1) and a fortified matrix (Level 2). However, the fortified matrix does not contain added iodine; for this reason, the iodine mass concentration is identical in both levels. Because of the nature of the production process for *SRM 2670a*, its iodine mass concentration after reconstitution is 88 μg/L, which is below the range of population median UIC values consistent with optimal iodine nutrition (14, 23); this complicates its use as a reference material. In 2011, *SRM 3668 – Mercury, Perchlorate, and Iodide in Frozen Human Urine* was developed, likewise in collaboration with the CDC, with the intent of providing a matrix of higher iodine content that does not require reconstitution and therefore is more convenient to use. This SRM consists of a base matrix that reflects a UIC value within the range consistent with "optimal" iodine nutrition and a fortified matrix that reflects a UIC value above this range.

ANALYTICAL METHODS AND REFERENCE MATERIALS FOR BIOMARKERS OF THYROID FUNCTION IN SERUM

Serum biomarkers of thyroid function are used routinely to screen for both potential iodine deficiency and potential iodine excess (24). NIST is developing higher-order reference measurement

TABLE 1
NIST SRMs for foods and dietary supplements with values for iodine[1]

SRM	Description	Iodine, mg/kg	Uncertainty,[2] mg/kg	Unit of issue	Date of issue
3280	Multi-element multivitamin tablets	132.7	6.6	5 × 30 whole tablets	January 2009
1548a	Typical diet	0.759	0.103	2 × 6.5 g	February 1998
3233	Breakfast cereal	0.04[3]	—	1 × 60 g	September 2012
1845a	Whole egg powder	3.03	0.10	5 × 10 g	September 2014
1549a	Whole milk powder	3.34	0.30	10 g	August 2013
1849a	Infant or adult nutritional formula	1.29	0.11	10 × 10 g	December 2011
2383a	Baby food composite	0.0737	0.0083	4 × 70 g	October 2012
1953/1954	Human milk[4]	—	—	5 × 5 mL, frozen	Iodine value in preparation

[1]NIST, National Institute of Standards and Technology; SRM, Standard Reference Material.
[2]Expanded uncertainty about the mean. Refer to SRM Certificate of Analysis for details.
[3]SRM Certificate of Analysis, information value.
[4]Developed for measuring contaminants.

TABLE 2
NIST SRMs for urine with values for iodine[1]

		L1			L2			
SRM	Description	Iodine, μg/L	Uncertainty,[2] μg/L	Unit of issue	Iodine, μg/L	Uncertainty,[2] μg/L	Unit of issue	Date of issue
2670a	Toxic elements in urine	88.2[3]	1.1	2 bottles	88.2[3]	1.1	2 bottles	August 2003
3668	Mercury, perchlorate, and iodide in frozen human urine	142.7[4]	1.6	5 × 1.5 mL	279.0[4]	3.9	5 × 1.5 mL	November 2011

[1]L1, Level 1; L2, Level 2; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material.
[2]Expanded uncertainty about the mean. Refer to SRM Certificate of Analysis for details.
[3]Iodine mass concentration after reconstitution with 20.00 mL water. The reference material consists of a blank matrix (Level 1) and a fortified matrix (Level 2). However, the fortified matrix does not contain added iodine and thus the iodine mass concentration is identical in Levels 1 and 2.
[4]Iodine mass concentration.

procedures (RMPs) and reference materials to support measurement of several serum biomarkers of thyroid function used in the assessment of iodine status, including thyroid-stimulating hormone (TSH), thyroglobulin, and the thyroid hormones thyroxine (T4) and triiodothyronine (T3). A higher-order method or procedure is one that has the highest metrological properties, can be completely described and understood in units defined by the International System of Units (SI), and for which a complete uncertainty statement can be defined.

To date, NIST has developed 2 RMPs for thyroid hormones in serum: one for total T4 that uses isotope dilution (ID) liquid chromatography–mass spectrometry (LC-MS) (25), and the other for total T3 that uses ID LC-tandem MS (LC-MS/MS) (26). Both procedures have been recognized by the Joint Committee for Traceability in Laboratory Medicine as higher-order RMPs.

The ID LC-MS RMP for total T4 in serum uses solid-phase extraction with deuterated T4 as an internal standard. In validation tests, recovery of added T4 (an assessment of the accuracy of measurement) ranged from 99.5% to 100.6%. The overall CV was within 1.0%, indicating excellent repeatability (25). Similarly, the ID LC-MS/MS RMP for total T3 in serum employs a ^{13}C-labeled T3 internal standard. In validation tests, recovery of added T3 ranged from 98.9% to 99.4%. Excellent repeatability was likewise obtained; the overall CV was within 2.6% (26).

Currently, NIST is validating another higher-order RMP to quantify total T4 and total T3 in serum. The new method, which uses ICP-MS for specific elemental detection of iodine at a mass-to-charge ratio of 127, will provide highly selective and sensitive detection of both thyroid hormone species. Future work at NIST on thyroid hormone analysis will involve updating the published RMP for total T4 to an LC-MS/MS method. The ICP-MS and LC-MS/MS RMPs developed at NIST as primary measurement methods will be used in combination to certify the concentrations of total T4 and total T3 in the currently available *SRM 971 – Hormones in Frozen Human Serum*. The updated *SRM 971* will offer an improvement over the most common clinical method, immunoassay, which produces inconsistent analytical results that typically do not correlate well with measured TSH values (27–31). Moreover, the updated *SRM 971* can be employed by laboratories to test the accuracy of their methods, calibrators, and controls, and also to establish SI traceability of measurements.

Only a small fraction of the total amounts of circulating T4 and T3 are free (i.e., unbound to serum proteins) (32–34). The concentrations of free T4 (FT4) and free T3 (FT3) are considered biologically relevant because they are available to interact with target cells (35). Although the above-described RMPs and certified measurements will be critical for standardizing measurement of total T4 and total T3 in serum, they cannot be used for standardizing measurement of FT4 and FT3. Because of the inaccuracy of current methods for measuring FT4 and FT3 in serum, development of more accurate RMPs would most likely facilitate their use in the clinical assessment of thyroid function (31, 36). At NIST, the ICP-MS methodology for total T4 and total T3 in serum is also being applied to the measurement of FT4 and FT3 in serum, with the aim of validating these measurements for inclusion in an ICP-MS RMP. In addition, the higher-order ICP-MS RMP will allow for the analysis of serum inorganic iodine, which is simultaneously measured during the analysis of free thyroid hormones. Application of a related method for plasma inorganic iodine to plasma from neonatal alligators revealed a robust positive correlation ($R^2 = 0.96$, $P = 0.003$) with plasma total T3 (37). Future studies will use the ICP-MS RMP to investigate whether a correlation between inorganic iodine and thyroid hormones is present in human serum.

To support clinical measurement of thyroid function biomarkers for iodine status assessment and other diagnostic and research uses, NIST is dedicated to producing relevant SRMs by adding certified values to current SRMs and developing new SRMs. As discussed above, SRMs with certified values for UIC are currently available. SRM Certificate of Analysis reference values for TSH and thyroglobulin will be provided for *SRM 971*; these will be determined at NIST with the use of commercially available immunoassay kits combined with external clinical laboratory measurements. Values for total T4 and total T3 will be determined with the established LC-MS and the recently developed ICP-MS RMPs that will be certified on *SRM 971*. Once the ICP-MS method for FT4 and FT3 is validated, values for these free hormones will be added to *SRM 971*.

The assays developed to measure serum biomarkers of thyroid function in men and nonpregnant women are often unreliable in pregnant women because they are affected by pregnancy-associated changes in the concentrations of circulating proteins, including T4-binding proteins (38). With this in mind, NIST will explore the development of an SRM for T4 and T3 with 4 different materials: serum obtained from reproductive-age, nonpregnant women and serum obtained from pregnant women midway through each trimester of pregnancy. Potentially, this SRM could be certified for other important biomarkers of health and disease for which changes in serum during pregnancy can affect

measurement results. We anticipate that this SRM would be used for quality assurance, for calibration of secondary standards, and to provide SI traceability for clinical assays.

REFERENCE MATERIALS FOR BIOMARKERS OF THYROID FUNCTION IN DRIED BLOOD

Dried blood spots (DBSs) are of particular interest to the research community because they offer several advantages over conventional blood draws: no phlebotomist is required, collection times can be optimized, cards containing DBSs are easily transported and stored, and collection of DBSs is less expensive than collection of blood (39). DBSs have been used in worldwide studies to evaluate iodine deficiency in school-age children (40, 41). In iodine-deficient areas of the world, intervention studies have used DBSs to monitor the effect of introducing iodized salt to school-age children (42). The research community is currently showing interest in monitoring thyroglobulin, a protein sensitive to the intake of iodine (40–43). NIST is investigating the production of a human whole-blood-material SRM that researchers could spot onto a DBS card directly after collecting a subject's blood. The goal is certification of this whole blood material for important biomarkers of thyroid function, including TSH, thyroglobulin, total T4, and total T3, thereby allowing for the standardization of DBS measurements.

One potential drawback is the reliability of the thyroglobulin measurements, which are currently derived from immunoassays. A challenge with this measurement approach is that 10% of patients typically have natural antibodies for thyroglobulin that interfere with the assay; in addition, thyroglobulin is a highly modified protein with a large degree of polymorphism (44, 45). For NIST to certify a thyroglobulin value on an SRM, a higher-order RMP is needed. To date, one primary method exists for quantifying thyroglobulin. That method, which uses digestion followed by peptide immunoprecipitation and analysis by LC-MS/MS (46, 47), is dependent on an antibody interaction for accuracy. Development of an SRM with certified values for thyroglobulin in blood would allow for the standardization of immunoassays and LC-MS/MS methods across laboratories.

CONCLUDING REMARKS

Reliable assessment of iodine status is critically underpinned by accurate analytical measurements of dietary iodine, urinary iodine, and biomarkers of thyroid function. In cooperation with the ODS, NIST is providing a new generation of matrix reference materials to support quality assurance and proficiency testing programs for assessing iodine status and thyroid function and to provide useful tools for the development and validation of new routine analytical methods. As part of this effort, NIST has been working with the ODS to provide benchmark quality assurance programs and proficiency testing for the materials and methods developed. Development of these materials, methods, and programs is on track to improve the assessment of iodine status and thyroid function by the international public health community and to foster the specific aims of the 2014 ODS iodine workshops as described by Ershow et al. (4) in this supplement issue.

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