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Assessment of Iodine Status Using Dried Blood Spot Thyroglobulin: Development of Reference Material and Establishment of an International Reference Range in Iodine-Sufficient Children

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Context: Thyroglobulin (Tg) may be a valuable indicator of improving thyroid function in children after salt iodization. A recently developed Tg assay for use on dried whole blood spots (DBS) makes sampling practical, even in remote areas.

Objective: The study aim was to develop a reference standard for DBS-Tg, establish an international reference range for DBS-Tg in iodine-sufficient children, and test the standardized DBS-Tg assay in an intervention trial.

Design, Participants, and Interventions: Serum Tg reference material of the European Community Bureau of Reference (CRM-457) was adapted for DBS and its stability tested over 1 yr. DBS-Tg was determined in an international sample of 5- to 14-yr-old children (n = 700) who were euthyroid, anti-Tg antibody negative, and residing in areas of long-term iodine sufficiency. In a 10-month trial in iodine-deficient children, DBS-Tg and other indicators of iodine status were measured before and after introduction of iodized salt.

Results: Stability of the CRM-457 Tg reference standard on DBS over 1 yr of storage at −20 and −50 C was acceptable. In the international sample of children, the third and 97th percentiles of DBS-Tg were 4 and 40 μg/liter, respectively. In the intervention, before introduction of iodized salt, median DBS-Tg was 49 μg/liter, and more than two thirds of children had DBS-Tg values greater than 40 μg/liter. After 5 and 10 months of iodized salt use, median DBS-Tg decreased to 13 and 8 μg/liter, respectively, and only 7 and 3% of children, respectively, had values greater than 40 μg/liter. DBS-Tg correlated well at baseline and 5 months with urinary iodine and thyroid volume.

Conclusions: The availability of reference material and an international reference range facilitates the use of DBS-Tg for monitoring of iodine nutrition in school-age children. **(*J Clin Endocrinol Metab* 91: 4881–4887, 2006)**

DESPITE SIGNIFICANT GLOBAL progress against the iodine-deficiency disorders (IDDs), one in three school-age children remain iodine deficient (1). Iodine deficiency is the single most important preventable cause of mental retardation worldwide (2). Three measures, urinary iodine (UI), goiter rate, and serum TSH, are recommended for assessment of iodine nutrition in populations (2), but each has limitations. UI is an indicator of recent iodine intake but not thyroid function. Because thyroid size decreases only slowly after iodine repletion, the goiter rate may remain high for several years after introduction of iodized salt (2, 3). TSH is a sensitive measure of iodine status only in the newborn period (2, 4). Thus, an additional indicator of thyroid function, sensitive to recent changes in iodine nutrition and applicable in children, would be valuable in monitoring iodine status in populations.

Thyroglobulin (Tg), a thyroid-specific protein that is a precursor in the synthesis of thyroid hormone, has no known physiological role outside the thyroid (5, 6). Stimulated by TSH, transcytosis of Tg-containing endosomes across the thyrocyte results in small amounts of Tg being released into the blood (7). If a sensitive assay is used, Tg can be detected in the serum of all healthy individuals (7, 8). In the absence of thyroid damage, the major determinants of serum Tg are thyroid cell mass and TSH stimulation (8). Thus, serum Tg is elevated in iodine-deficient areas due to TSH hyperstimulation and thyroid hyperplasia. In 1994, the World Health

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Abbreviations: BSA, Body surface area; CRM, Community Bureau of Reference; CV, coefficient of variation; Delfia, dissociation enhanced lanthanide fluorescent immunoassay; DTC, differentiated thyroid cancer; IDD, iodine-deficiency disorder; DBS, dried whole blood spots; Tg, thyroglobulin; Tg-Ab, Tg antibodies; T_{vol}, thyroid gland volume; UI, urinary iodine; WHO, World Health Organization.

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Organization (WHO) recommended using serum Tg to assess iodine nutrition and proposed that a median Tg concentration of less than 10 μg/liter in a population indicated iodine sufficiency (9). However, data to support this Tg cutoff value were limited, and the recommendation was not included in the revised 2001 WHO guidelines (2).

We recently adapted and validated a widely used sandwich fluoroimmunometric serum Tg assay for use on dried whole blood spots (DBS) (10). In an intervention study that measured DBS-Tg in children before and after the introduction of iodized salt, the assay was a sensitive indicator of improving thyroid function after iodine repletion. However, use of Tg for monitoring iodine status is limited by large interassay variability and lack of reference data for Tg in healthy, iodine-sufficient school-age children. The development of an international serum Tg reference standard [Community Bureau of Reference (CRM)-457] has led to the restandardization of many Tg assays and reduced intermethod variability (11, 12). When CRM-457 standardization is used, the normal serum Tg reference range in adults is approximately 3–40 μg/liter (12).

Our study aims were: 1) to develop standard reference material for the DBS-Tg assay using the CRM-457 Tg reference preparation; 2) using this material, to establish an international reference range for DBS-Tg in iodine-sufficient children that could be used for monitoring iodine nutrition; and 3) to evaluate the standardized DBS-Tg assay and reference range in a longitudinal study of goitrous children before and after introduction of iodized salt.

Subjects and Methods

As described previously (10), a two-site dissociation enhanced lanthanide fluorescent immunoassay (Delfia) serum Tg assay (PerkinElmer Life Sciences, Wallac, Turku, Finland) was adapted for DBS and validated in Swiss children. An advantage of two-site Tg assays is their lower cross-reactivity and improved specificity, compared with one-site assays (5).

Development and stability testing of the DBS-Tg reference standard

The lyophilized Tg reference preparation of the Community Bureau of Reference of the Commission of the European Communities (CRM-457) (11, 12) was provided by C. Profilis (BCR, Brussels, Belgium). The reference material was reconstituted by adding 1 ml distilled water to the ampule, mixing gently for 5 min, and again intermittently to maintain dissolution. The reconstituted standard was stored for no longer than 24 h at 4 C before use. Varying concentrations of the CRM-457 standard (1, 10, 100, 500, and 1000 μg/liter) were prepared using as the diluent the 0 standard from the Delfia kit (PerkinElmer Life Sciences).

Calibrators for the DBS-Tg assay were then prepared using both the CRM-457 reference standard and the calibration material provided in the Delfia kit. Whole blood obtained from the local blood bank was centrifuged at 1529 × *g* for 10 min at 18 C (Rotanta; Hettich Zentrifugen, Tuttlingen, Germany); the serum was removed, 0.9% NaCl was added, the solution rotated in a blood mixer until homogeneous, and then recentrifuged at 1529 × *g* for 10 min at 18 C, and the supernatant fluid was removed. This step was repeated three times. Calibration solutions prepared from the Delfia serum Tg assay kit and the CRM-457 reference preparation were then added to the washed erythrocytes, rotated in a blood mixer for 10 min, dropped onto filter paper (grade 903; Schleicher & Schuell, Einbeck, Germany), and air dried for 24 h at 20 C (10). The calibration curves were constructed by using duplicate measurements at 0, 1, 10, 100, 500, and 1000 μg/liter and smoothed by fitting a third-order polynomial with the use of a smoothed spline technique (Multicalc Program; PerkinElmer Life Sciences).

To assess the stability of the CRM-457 on DBS, a 1-yr stability study was done comparing the CRM-457 reference preparation on DBS with the Tg calibration material from the Delfia kit (prepared as described above) at concentrations of 100 and 20 μg/liter and stored at 25, 4, −20, and −70 C. DBS-Tg concentrations were measured in duplicate every 2 wk for the first two months and at monthly intervals thereafter.

Establishing an international reference range for DBS-Tg in iodine-sufficient children

The subjects were healthy children living in areas of long-term iodine sufficiency (13) in South America, Central Europe, the Eastern Mediterranean, Africa, and the Western Pacific. Recruitment was from primary schools at the middle to lower socioeconomic level. The sample included children from five major ethnic groups: Lima, Peru (Hispanic); Zürich, Switzerland (white); Manama, Bahrain (Arabic); Cape Town, South Africa (black); and Dalian, China (Asian). Preanalytic exclusion criteria were: 1) age younger than 5 yr or older than 14 yr; 2) personal or immediate family history of thyroid disease; 3) cigarette smoking; or 4) pregnancy. Both cigarette smoking and pregnancy may be associated with higher serum Tg values (12). Postanalytic exclusion criteria were: 1) abnormal serum TSH and/or T_4; and 2) detectable anti-Tg antibodies (Tg-Ab). With the relative precision for the 97th percentile for DBS-Tg specified at 3–5% of the total length of the 95% reference range, and the estimated SD of DBS-Tg taken as 2.1 μg/liter (based on a small study in iodine-sufficient Swiss children), it was estimated that a sample size of approximately 500 children was required to obtain the required precision level (14). However, because of uncertainty on the variability of DBS-Tg in children due to the small amount of available data, roughly 700 children were finally enrolled.

Ethical committees approved the protocol at each local institution involved in the study. Informed written consent was obtained from the parents and oral assent from the participating children. Height and weight were measured using standard anthropometric technique (15). For the measurements, children removed their shoes, emptied their pockets, and wore light indoor clothing. Heights were recorded to the nearest mm and weights to the nearest 100 g. Pubertal staging was not done. Whole blood from a finger stick was spotted onto filter paper (grade 903; Schleicher & Schuell), allowed to dry at room temperature (~20 C), and stored at 4 C in sealed low-density polyethylene bags until analysis. Spot urine samples were collected and aliquots were stored at −20 C until analysis.

Intervention study

The study was done in a rural village in the Brikcha Commune, an area of endemic goiter in northern Morocco (16). Per-capita salt intakes in school-age children in this region are 7–12 g/d (16). A local cooperative supplies nearly all salt in this region; the salt is produced in drying ponds using water from a salty spring. Although Morocco legislated mandatory salt iodization in 1997, it is estimated that only approximately 45% of the population has access to iodized salt (Chaouki, N., personal communication, 2002). Due to financial constraints, this small local cooperative had not yet begun iodization. To prepare the iodized salt, iodine was added as reagent-grade potassium iodate (Sigma & Aldrich, Buchs, Switzerland) at a level of 25 μg iodine per gram of salt, using an electric rotating drum mixer (ELTE 650; Engelsmann, Ludwigshafen, Germany) as described previously (16).

The subjects were primary school children, aged 5–14 yr (n = 86). Informed oral consent was obtained from parents of the children and oral assent from the children. The Swiss Federal Institute of Technology in Zürich and the Ministry of Health in Rabat gave ethical approval for the study. At baseline, height and weight were measured using standard anthropometric technique (15). Whole blood from a finger stick was spotted directly onto filter paper (grade 903; Schleicher & Schuell), allowed to dry at room temperature (~20 C), and stored at 4 C in sealed low-density polyethylene bags until analysis. To check for the influence of sampling on the DBS-Tg assay, whole blood was obtained from a subsample of children (n = 20; mean ± SD age, 9.2 ± 2.1 yr) by both

TABLE 1. Age, gender ratio, anthropometric measurements, and UI concentration in an international sample of 5- to 14-yr-old children from areas of long-term iodine sufficiency and with normal thyroid function, by site and combined

Site	n	Age (yr)[a]	Male to female ratio	Height (cm)[b]	Weight (kg)[b]	UI (μg/liter)[a]
Bahrain	142	10.1 (5.9–14.1)	1.03	137 ± 11	33.5 ± 12.5	177 (43–701)
Peru	125	10.0 (5.0–12.0)	1.66	133 ± 11	32.4 ± 9.2	161 (15–860)
South Africa	127	9.7 (6.0–13.1)	1.02	135 ± 14	34.7 ± 13.9	266 (38–758)
China	230	9.0 (6.0–12.0)	1.05	138 ± 13	32.5 ± 11.1	234 (0–672)
Switzerland	86	10.0 (7.0–14.0)	1.53	143 ± 11	36.4 ± 9.8	130 (6–390)
Total	710	9.6 (5.0–14.1)	1.18	137 ± 13	33.5 ± 11.6	198 (0–860)

[a] Data shown as median (range).
[b] Data shown as mean ± SD.

venipuncture of a forearm vein and finger prick. The blood from venipuncture was collected, and then spotted onto filter paper. Spot urine samples were collected, and aliquots were stored at −20 C until analysis. Thyroid gland volume (T_{vol}) was measured using a portable Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5-MHz linear transducer (17). Each household was then provided 2 kg salt to supply all household needs at the beginning of each month for 10 months. Salt aliquots were taken (n = 6) after each mixing for determination of iodine content. The salt was dispensed directly to the head of the household from a central supply at the local health center, and it was explained to the participating families that the new salt should be used for all cooking and food preparation as well as at the table. At 5 and 10 months, the baseline measurements were repeated.

Laboratory analyses

UI was measured using the Pino modification of the Sandell-Kolthoff reaction (18). At UI concentrations of 47 and 79 μg/liter, the interrun coefficient of variation (CV) of this assay at the Human Nutrition Laboratory at the Swiss Federal Institute of Technology, Zürich, our laboratory, is 10.3 and 12.7%, respectively. The limit of detection is 2 μg/liter; samples below this limit were assigned a value of 0. Salt iodine concentration was measured using a modification of the Sandell-Kolthoff reaction, after dissolution of salt aliquots in distilled water. At iodine concentrations of 30 μg/g salt, the interrun CV of this assay at the Human Nutrition Laboratory is 7%. T_{vol} was calculated using the method of Brunn *et al.* (19). M.B.Z. performed all ultrasound measurements during the study. To estimate intraobserver variability, duplicate T_{vol} measurements were done in 10 children at the 5- and 10-month time points; the mean (SD) variability was 3.9 (2.1)%. Goiter was defined using sex- and body surface area (BSA)-specific reference criteria for T_{vol} (17). DBS-Tg analyses were done in Zürich at the Proteinhormone Laboratory at the University Children's Hospital (10). DBS were analyzed for TSH (Delfia NeoTSH) (20) and total T_4 (Delfia Neonatal T_4 kit, PerkinElmer Life Sciences). Normal reference values are whole blood TSH, 0.2–3.7 mU/liter; and serum total T_4, 65–165 nmol/liter. Tg-Ab were measured by RIA (RIA Tg-Ab; RSR, Cardiff, UK) adapted in our laboratory for measurement on DBS. For the Tg-Ab assay, between- and within-assay CV is 10.1 and 2.5%, respectively (n = 145). Tg-Ab status was classified as: detectable, Tg-Ab greater than 0.3 U/ml; and elevated, Tg-Ab greater than 10 U/ml.

Statistical analyses

Data processing and statistics were done using SPLUS-2000 (Insightful Corp., Seattle, WA) and Excel (Enterprise Edition, Microsoft, Seattle, WA). Distribution of Tg in the sample exhibited a long-tailed-positive skewness and kurtosis. Log transformation removed most of the skewness and kurtosis, leaving a nearly Gaussian distribution at all ages and BSAs, for both sexes. Transformed data were used to calculate percentiles based on the Gaussian distribution, which were then transformed back to the linear scale. ANOVA was used to test differences between the six sites modeling age, BSA, and sex dependence. Simultaneous 95% confidence intervals for the differences in DBS-Tg between sites were done using the Tukey method of multiple comparisons (21). For the ANOVA, data from 12 subjects (two from Bahrain, three from China, one from Peru, and six from South Africa) of 710 were excluded as outliers in the relation of DBS-Tg to age and would possibly have had an undue influence on the calculations. In the intervention study, one-way ANOVA was done to compare changes in urinary iodine, TSH, T_4, Tg, Tg-Ab, and thyroid volume. Tukey's test was used for *post hoc* comparisons. Variables not normally distributed were logarithmically transformed before analysis. To evaluate the relation between Tg and other IDD indicators, Pearson's correlation coefficients were calculated as well as linear regression models with Tg as the dependent variable. For the regression, to reduce the influence of age and sex on thyroid volume, SD scores were obtained using current reference values (17) as previously described (10). Significance was set at $P < 0.05$.

Results

Stability of the CRM-457 Tg reference standard on DBS

Regression analysis of the results of the 1-yr stability study showed that the slopes for both CRM and Delfia material were not significantly different from 0. The intercepts for the 100- and 20-μg/liter samples were: 109.7 and 17.0 μg/liter (Delfia) and 115.1 and 18.4 μg/liter (CRM), respectively. The stability study showed that both reference materials (CRM-457 and kit calibrator) were stable in DBS for 1 yr when stored at least at −20 C. At 4 C, both preparations were stable for 2 months. At higher storage temperatures, Tg degraded sig-

TABLE 2. Whole blood TSH, serum total T_4 (TT4), and anti-Tg-Ab in an international sample of 5- to 14-yr-old euthyroid children from areas of long-term iodine sufficiency, by site and combined

Site	n	TSH (mU/liter) blood[a]	TT4 (pmol/liter)[b]	Tg-Ab (U/ml)[a]
Bahrain	142	1.1 (0.3–3.6)	102 ± 17	0.3 (0.3–6.1)
Peru	125	1.0 (0.5–3.4)	97 ± 16	0.3 (0.3–7.8)
South Africa	127	0.9 (0.5–2.8)	108 ± 22	0.3 (0.3–2.9)
China	230	1.4 (0.4–3.7)	114 ± 19	0.3 (0.3–9.0)
Switzerland	86	0.6 (0.2–1.2)	90 ± 22	0.3 (0.3–7.3)
Total	710	1.1 (0.2–3.7)	105 ± 21	0.3 (0.3–9.0)

[a] Data shown as median (range).
[b] Data shown as mean ± SD.

TABLE 3. DBS Tg on the linear scale and log(10) transformed, by site, showing the median and 97th percentiles in an international sample of 5- to 14-yr-old euthyroid children from areas of long-term iodine sufficiency, by site

Site	n	Tg (μg/liter)		log(10) Tg	
		Median (25th, 75th percentile)	97th percentile	Median (25th, 75th percentile)	97th percentile
Bahrain	140	19.3 (12.6, 27.6)	48.9	1.28 (1.10, 1.44)	1.69
Peru	124	11.6 (7.0, 19.2)	36.1	1.06 (0.85, 1.28)	1.56
South Africa	121	18.4 (13.2, 26.0)	40.7	1.27 (1.12, 1.42)	1.61
China	227	13.3 (8.9, 21.2)	35.6	1.12 (0.95, 1.33)	1.55
Switzerland	86	11.2 (7.0, 15.9)	24.8	1.05 (0.85, 1.20)	1.39
Total	698	14.5 (9.4, 22.7)	40.2	1.16 (0.97, 1.36)	1.61

nificantly. Recovery of CRM-457 material when measured against the Delfia reference was 87%. There was excellent correlation between the CRM-457 and Delfia results in the international sample of iodine sufficient children (described below) ($r = 0.967$, $P < 0.0001$).

Reference range for DBS-Tg in iodine-sufficient children

Table 1 shows the age, gender ratio, anthropometric measurements, and UI concentration in the study sample by site and combined. All sites were iodine sufficient as defined by a median UI between 100 and 300 μg/liter (1). Overall, mean age ± SD was 9.6 (5.0–14.1), with the whole sample nearly equally divided between boys and girls. Table 2 shows the whole blood TSH, serum total T_4, and anti-Tg-Ab, in the study sample, by site and combined.

Table 3 shows DBS-Tg on the linear scale and log(10) transformed, by site, showing the median and 97th percentiles. Pooling the data, the median (third, 97th percentiles) log(10) DBS-Tg was 1.16 (0.57, 1.61). Transformed back to the linear scale, the median (third, 97th percentiles) for DBS-Tg was 14.5 (3.7, 40.3). The nonparametric 95% confidence intervals for the median, third, and 97th percentiles were 13.4, 15.5; 3.1, 4.3; and 37.8, 45.6, respectively. There were complex and significant differences for DBS-Tg between sites in the means and slope with respect to age but no significant gender differences at any of the sites. The differences in DBS-Tg between sites after controlling for age are shown in Fig. 1.

There was no clear relationship between DBS-Tg and UI; within a country, the correlation coefficients ranged between −0.14 (Bahrain) and 0.084 (China), and none were statistically significant. The correlation coefficients of DBS-Tg and TSH, within country, were: Bahrain (0.077, NS); Peru (0.035, NS); South Africa (0.21, $P = 0.02$); China (0.18, $P = 0.007$); and Switzerland (0.34; $P = 0.001$); and for the pooled sample (0.13, $P = 0.0004$). The differences between countries were statistically significant in level but not slope (no significant interaction). The correlation coefficients of DBS-Tg and total T_4, within country, were: Bahrain (0.071, NS); Peru (0.30, $P = 0.0006$); South Africa (0.13, NS); China (0.14, NS); and Switzerland (0.072; NS); and for the pooled sample (0.15, $P < 0.0001$). The differences between countries were statistically significant in level but not in slope (no significant interaction).

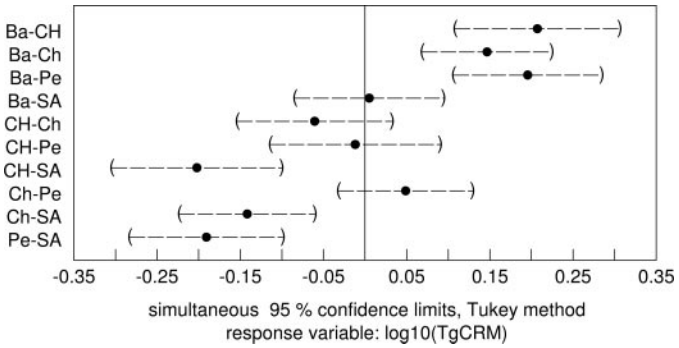

FIG. 1. Simultaneous 95% confidence intervals for the differences in DBS-Tg between sites, using the Tukey method of multiple comparisons (17). The differences are adjusted for age and are significant if the confidence interval does not include zero. Ba, Bahrain; Pe, Peru; SA, South Africa; Ch, China; CH, Switzerland.

Intervention study

Of the 86 children who began the study, 83 completed it; three children moved away or were absent from school on the measurement days. Characteristics of the children at baseline were as follows: mean ± SD age was 10.6 ± 2.4 yr; the gender ratio was 40 females to 43 males; mean ± SD weight was 31.2 ± 9.9 kg; mean ± SD height was 1.34 ± 0.14 m. In the monitoring aliquots of iodized salt taken at the monthly mixings, the mean iodine concentration (± SD) was 22.8 ± 5.1 μg/g. As shown in Table 4, median UI at 5 and 10 months was significantly increased, compared with baseline ($P < 0.001$). At 10 months, median UI had increased to near the WHO/International Council for the Control of Iodine Deficiency Disorders cutoff value (100 μg/liter) for risk of iodine deficiency (2). Mean T_{vol} and median TSH at 10 months were significantly decreased, compared with baseline ($P < 0.05$). Median (range) DBS-Tg was 49 (1–862) μg/liter at baseline and fell rapidly after introduction of iodized salt to 13 (1–208) and 8 (1–95) μg/liter at 5 and 10 months ($P < 0.001$). Tg-Abs were not measured in the children. Using the 97th percentile of the reference range for DBS-Tg reported above, 68, 7, and 3% of children had elevated DBS-Tg concentrations at baseline and 5 and 10 months, respectively.

DBS-Tg correlated well with the other major response variables during the study. At baseline and at 5 months, DBS-Tg was negatively correlated with urinary iodine [correlation coefficients −0.41 ($P < 0.001$) and −0.19 ($P < 0.05$), respectively] and was positively correlated with thyroid volume [correlation coefficients 0.47 ($P < 0.001$) and 0.18 ($P < 0.05$), respectively]. DBS-Tg was significantly correlated with TSH at baseline only [correlation coefficient 0.32 ($P < 0.001$)]. The regression of DBS-Tg on urinary iodine, TSH, T_4, and thyroid volume was done at each time point. The regression

TABLE 4. UI concentration, T_{vol}, and goiter rate measured using ultrasound, serum total T_4 (TT4), whole blood TSH, and DBS-Tg in Moroccan schoolchildren (n = 83) before (0 months) and 5 and 10 months after introduction of iodized salt

	Baseline	5 months	12 months
UI (μg/liter)[1,3]	12 (2–70)[a]	74 (2–239)[b]	104 (22–1784)[b]
T_{vol} (ml)[2,3]	8.3 ± 3.5[a]	7.8 ± 3.1[a]	6.9 ± 2.2[b]
TT4 (nmol/liter)[2]	98 ± 18	111 ± 21	101 ± 19
TSH (mU/liter blood)[1,3]	1.3 (0.3–7.4)[a]	0.7 (0.3–3.0)[b]	0.6 (0.2–4.4)[b]
DBS-Tg (μg/liter)[1,3]	49 (1–862)[a]	13 (1–208)[b]	8 (1–95)[c]

Values in the same row with *different superscript letters* are significantly different, $P < 0.05$ (Tukey's test for *post hoc* comparisons).
[1] Data shown as median (range).
[2] $P < 0.0001$ (ANOVA).
[3] Data shown as mean ± SD.

of UI and thyroid volume on DBS-Tg was significant ($P < 0.001$) at baseline and at 5 months ($P < 0.01$). Comparing the DBS-Tg assay on the parallel venipuncture and finger-stick samples (n = 20), geometric mean (range) DBS-Tg was 8.4 (1.8–94.9) and 7.8 (2.1–115.7) μg/liter (NS).

Discussion

In our international sample of iodine-sufficient children, there were no significant gender differences in DBS-Tg. In adults, serum Tg levels are slightly higher in females, but this is not clinically informative, and Tg reference ranges for adults are not gender specific (12). Data on the effects of chronological age on serum Tg are scarce. In healthy term infants, serum Tg increases in the first few days after birth, presumably due to the postnatal surge in TSH (22). Serum Tg then decreases by 50% over the next few months and then more slowly declines throughout childhood to reach adult levels at puberty (23). In our sample, although there were statistically significant differences in DBS-Tg between younger and older children, these differences were small and varied by site. Overall, our data suggest age-, site-, or gender-adjusted reference ranges for DBS-Tg are unnecessary for children in the age range of 5–14 yr. We would therefore recommend use of a single reference range for screening and monitoring of iodine nutrition in this age group. Based on our data (Table 3), the DBS-Tg reference interval for iodine-sufficient, Tg-Ab-negative, euthyroid school-age children, using CRM-457-standardization, is 4–40 μg/liter.

This proposed reference range is nearly the same as the usual adult reference range for serum Tg when CRM-457 standardization is used, *i.e.* approximately 3–40 μg/liter (12). The close similarity of the child and adult reference ranges for Tg parallels that of serum concentrations of TSH and T_4: although age-specific reference limits for TSH and T_4 are needed for young children, by age 5 yr, the child to adult ratio for TSH and T_4 are approximately 1 (24). The long-term intraindividual variability in serum Tg in adults is low (CV ~14%); there appears to be no significant diurnal or seasonal variability in serum Tg concentrations in moderately iodine-deficient populations (22), but this has not been studied in severe iodine deficiency. Using a different serum Tg assay, Vanderschueren-Lodeweyckx (25) proposed normal mean values and ranges for serum Tg in children aged 1–10 and 11–20 yr of 35 (2–65) and 18 (2–36) μg/liter, respectively. Previous studies measuring serum Tg in children from iodine-deficient areas have reported median values ranging from 27–214 μg/liter (26–29). In Chinese adults in regions of mild iodine deficiency and iodine excess, serum Tg values were in the range of 6.0–11.7 ng/ml, with no significant differences between regions (30). Further investigation in other iodine-deficient populations to determine whether DBS-Tg is elevated relative to the degree of iodine deficiency would be useful.

Even with CRM-457 standardization, there is significant technical variability in serum Tg assays, presumably due to epitope specificity differences that cause interassay biases independent of standardization (12). The primary clinical use of serum Tg measurements is a marker for patients carrying a diagnosis of differentiated thyroid cancer (DTC). Interassay variability has precluded the use of serial serum Tg measurements by different laboratories for following individuals after surgery for DTC (12). However, for the purposes of diagnostic testing, *i.e.* distinguishing iodine-deficient from iodine-sufficient populations using a normal reference interval, assay bias and imprecision goals need not be as stringent as for serial measurements for DTC follow-up. Therefore, although use of our DBS CRM Tg standard will not eliminate DBS-Tg interassay variability, it may facilitate the calibration of assays and allow the interchangeable use of different DBS Tg assays to characterize iodine status in a population. More research is needed on this issue.

A potential limitation to the use of a DBS-Tg assay for IDD monitoring is interference from Tg-Ab. Even trace amounts of Tg-Ab in a specimen may interfere with Tg measurement, and this is a common source of Tg assay error in adults followed up for thyroid cancer (12). Detectable Tg-Ab are found in approximately 10% of the general adult population (31). It is unclear how prevalent Tg-Ab are in iodine-deficient children or whether they are precipitated by iodine prophylaxis (32). Several studies (33, 34) reported high prevalences, ranging from 7 to 69%, in iodine-deficient children and during iodine prophylaxis. In contrast, data from our group and others (35–37) suggest elevated Tg antibodies in IDD-affected children are rare (0–2% prevalence). In our international sample of children, only 25 (3.3% of the total sample) had detectable (2.4%) or elevated (0.9%) Tg-Ab. These data suggest screening for Tg-Ab may not be necessary when using a DBS-Tg assay in children to classify population iodine status. Further research is needed to address the question of whether simultaneous measurement of anti-Tg antibodies is necessary.

Cross-sectional studies have reported a negative correla-

tion between Tg and UI and positive correlation between Tg and thyroid volume and TSH (26, 38, 39). Intervention studies suggested that Tg is a more sensitive indicator than TSH or T_4 in measuring response to iodized oil (26–28) and potassium iodide (29). In the present study, before introduction of iodized salt, median DBS-Tg was high (49 μg/liter), and more than two thirds of children had DBS-Tg values greater than 40 μg/liter. After 5 months of iodized salt use, the median had decreased to 13 μg/liter, well within the normal range, and only 7% of children had a value greater than 40 μg/liter. During the intervention study, DBS-Tg correlated well at both baseline and 5-month time points with UI and thyroid volume, the two other major response variables used to measure impact of iodized salt in children. Thus, the data suggest that Tg, used in conjunction with UI to measure recent iodine intake and thyroid volume to assess long-term anatomic response, may be a useful biological indicator for monitoring thyroid function in children after introduction of iodized salt. The DBS assay makes sampling practical even in remote areas.

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