



# Performance Evaluation of the Serum Thyroglobulin Assays With Immunochemiluminometric Assay and Immunoradiometric Assay for Differentiated Thyroid Cancer

Yoon Young Cho, M.D.<sup>1,\*</sup>, Sejong Chun, M.D.<sup>2,\*</sup>, Soo-Youn Lee, M.D.<sup>2</sup>, Jae Hoon Chung, M.D.<sup>1</sup>, Hyung-Doo Park, M.D.<sup>2</sup>, and Sun Wook Kim, M.D.<sup>1</sup>

Division of Endocrinology & Metabolism<sup>1</sup>, Department of Medicine, Thyroid Center; Department of Laboratory Medicine & Genetics<sup>2</sup>, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

**Background:** Measurement of postoperative serum thyroglobulin (Tg) is important for detecting persistent or recurrent differentiated thyroid cancer. We evaluated the analytic performance of the Dxl 800 assay (Beckman Coulter, USA) for serum Tg and anti-thyroglobulin antibodies (TgAbs) in comparison with that of the GAMMA-10 assay (Shinjin Medics Inc., Korea) for serum Tg and RIA-MAT 280 assay (Stratec, Germany) for TgAb.

**Methods:** We prospectively collected blood samples from 99 patients thyroidectomized for thyroid cancer. The functional sensitivity was investigated in standards and human serum. Precision and linearity were evaluated according to the guidelines of the Clinical and Laboratory Standards Institute. The correlation between the two assays was assessed in samples with different Tg ranges.

**Results:** The functional sensitivity of the Dxl 800 assay for serum Tg was between 0.0313 and 0.0625 ng/mL. The total CV was 3.9-5.6% for serum Tg and 5.3-6.9% for serum TgAb. The coefficient of determination ( $R^2$ ) was 1.0 and 0.99 for serum Tg and TgAb, respectively. The cut-offs for serum TgAb were 4.0 IU/mL (Dxl 800) and 60.0 IU/mL (RIA-MAT 280), and the overall agreement was 68.7%. The correlation between the two assays was excellent; the correlation coefficient was 0.99 and 0.88 for serum Tg and TgAb, respectively.

**Conclusions:** The Dxl 800 is a sensitive assay for serum Tg and TgAb, and the results correlated well with those from the immunoradiometric assays (IRMA). This assay has several advantages over the IRMA and could be considered an alternative test for Tg measurement.

**Key Words:** Dxl 800, Immunochemiluminometric assay, Evaluation, Performance, Thyroglobulin

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**Corresponding author:** Sun Wook Kim  
Division of Endocrinology & Metabolism,  
Department of Medicine, Thyroid Center,  
Samsung Medical Center, Sungkyunkwan  
University School of Medicine, 81 Irwon-ro,  
Gangnam-gu, Seoul 06351, Korea  
Tel: +82-10-9933-1653  
Fax: +82-2-3410-3849  
E-mail: swkimmd@skku.edu

**Co-corresponding author:** Hyung-Doo Park  
Department of Laboratory Medicine &  
Genetics, Samsung Medical Center,  
Sungkyunkwan University School of  
Medicine, 81 Irwon-ro, Gangnam-gu, Seoul  
06351, Korea  
Tel: +82-10-2064-0290  
Fax: +82-2-3410-2719  
E-mail: nayadoo@hanmail.net

\*Yoon Young Cho and Sejong Chun equally contributed as first authors.

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## INTRODUCTION

Thyroglobulin (Tg) is a useful post-operative tumor-marker for

differentiated thyroid carcinomas because it is produced only within the thyroid tissue [1]. Undetectable levels of serum Tg (stimulated Tg values less than 1-2 ng/mL) are expected after

total thyroidectomy and radioactive iodine ablation. However, detectable serum Tg levels require additional work-up for recurrent or persistent disease [2]. Because Tg is an important prognostic factor, the American Thyroid Association (ATA) recommends regular measurements of serum Tg levels [3].

Assays for measuring serum Tg have improved since the first-generation RIA [4], although accurate measurement of serum Tg is still challenging. RIA has longer history of clinical use and specific methodology, but is less sensitive than immunometric assay, requires the use of radioactive tracers, and is labor-intensive [5]. Currently, non-competitive immunometric assay (IMA) is commonly used in most laboratories with the advantages of shorter incubation time, full automation, and higher sensitivity compared with the RIA [5, 6]. Moreover, the IMA does not produce radioactive byproducts, and reagents for IMA are stable over time with appropriate inventory, whereas reagents for RIA have a shorter half-life because of the property of radioactive nuclides [5, 6].

The presence of anti-thyroglobulin antibodies (TgAbs) leads to over- or underestimation of Tg concentrations with different degrees among assays [5, 7]. The prevalence of TgAbs is higher in patients with thyroid cancer than in the general population (25-30% vs 10%) [8, 9]. The IMA is prone to more interference from TgAbs, but the interference is unidirectional and more predictable, while the RIA is relatively resistant to interference from TgAbs, serum Tg might be over- or underestimated [5, 7]. Thus, none of the commercially available assays is free from TgAb interference. Although liquid chromatography-tandem mass spectrometry (LC-MS/MS), which recently emerged, is expected to overcome this interference, it requires further validation [10].

As a second-generation Tg assay, there are several IMA variations. Among them, the immunochemiluminometric assay (ICMA) is fully automated, non-radioactive, and presents high sensitivity (a detection limit of 0.05 µg/L) and short processing time [11]. While many comparative studies of Tg assays have been conducted in North America [5, 12], validation of Tg assays is scarce in laboratories in Korea [13]. We previously reported a good correlation among three different immunoradiometric assays (IRMAs) that covered more than 90% of all IRMAs performed in Korea [13]. However, the clinical utility of the ICMA has not been tested by using samples from a Korean population.

Thus, we aimed to evaluate the sensitivity, precision, and linearity of ICMA with the Dxl 800 device (Beckman Coulter, Fullerton, CA, USA) for detecting serum Tg and to demonstrate that ICMA is equivalent to IRMA with the BRAHMS Tg plus as-

say (Thermo Scientific, Waltham, MA, USA) using samples from patients with differentiated thyroid cancer during radioactive iodine therapy (RAIT).

## METHODS

### 1. Samples and reagents

For the evaluation of precision and linearity, control materials and human pooled serum prepared as per the guidelines provided by the CLSI EP5-A2 and EP6-A were used [14, 15]. Control materials, calibrators, and diluents were supplied by Beckman-Coulter (Access Thyroglobulin Calibrator, Thyroglobulin Antibody II Calibrator, and Thyroglobulin Sample Diluent).

For the comparison study, blood samples were drawn at admission for RAIT. We prospectively and consecutively collected serum samples from patients who underwent thyroid surgery for differentiated thyroid cancer and were admitted for subsequent RAIT between December 2013 and April 2014 at Samsung Medical Center, a tertiary medical center in Korea. The final set included 100 samples from 100 patients. Among them, one sample was not sufficient to perform two assays, thus 99 samples were utilized for the analysis.

Among the 99 patients, 50 patients (51%) were treated with recombinant human thyroid stimulating hormone, and the others (n=49, 49%) underwent thyroid hormone withdrawal before RAIT. Most of them (n=84, 85%) received RAIT for the first time at enrollment. Eighty patients (81%) showed limited uptake in the thyroid bed and/or local lymph nodes on their post-treatment scans, three patients (3%) showed uptake on the bone (n=3) with one of them on the bone and lung (n=1), and the remaining 16 patients showed no uptake. From the 80 patients for whom localized uptake was seen on scans, recurred lesions were noted on postoperative ultrasonography for four patients. For seven patients, no distant metastatic lesions were detected on scans. Metastatic sites were mainly microscopic lung metastases.

Medical records were reviewed in cases with discrepant Tg results between the two assays, which were determined considering the clinical implications of Tg concentration. A postoperative serum Tg less than 1-2 ng/mL is a strong predictor of remission, while Tg levels more than 10-30 ng/mL are associated with persistent or recurrent disease [2, 16]. We identified cases presenting different ranges of Tg concentrations (<1 ng/mL, 1-10 ng/mL, and >10 ng/mL) measured by the two assays. Informed consent was obtained from each participant. The Institutional Review Board of Samsung Medical Center reviewed and ap-

proved this study.

## 2. Precision

We used three concentrations of serum Tg and two concentrations of TgAb to assess precision according to the guidelines provided by the CLSI EP5-A2. The three concentrations were obtained by mixing Tg calibrator (ref. 33865, Beckman Coulter) and thyroglobulin sample diluent (ref. 33866, Beckman Coulter). Two concentrations of TgAb were obtained by mixing thyroglobulin antibody II calibrator (ref. A36920, Beckman Coulter) and thyroglobulin sample diluent. The calibrator used in precision testing was retrieved from a different lot from that used for the initial calibration of the Dxl 800 device and the material used for linearity testing described below. The intended concentrations for serum Tg were 32.5, 250, and 400 ng/mL. The two concentrations of TgAb were 4.5 and 500 IU/mL. In addition, pooled serum from patients was used for the assessment of serum Tg and TgAb. Each run was performed in duplicate for 20 days and separated by a minimum of two hours. Within run precision, between-day precision, and total precision were evaluated.

## 3. Linearity

For the evaluation of linearity, five concentrations of standards obtained by mixing calibrators and the diluent were used for both serum Tg and TgAb. Calibrators were retrieved from a lot different from the initial calibrator of the Dxl 800 device and the precision testing described above. The intended concentrations of serum Tg were 0, 94, 188, 282, 376, and 470 ng/mL. The intended concentrations of serum TgAb were 0, 480, 960, 1,440, 1,920, and 2,400 IU/mL. All assessments were performed in duplicate four times. Regression analysis was performed to compare the intended concentrations and actual levels of serum Tg and TgAb. The square of the coefficient of determination ( $R^2$ ) was calculated.

## 4. Method comparison

Serum Tg measured by using the Dxl 800 and the GAMMA-10 (Shinjin Medics Inc., Seoul, Korea) methods was compared by using 99 serum samples. Analytical measurement ranges (AMR) of serum Tg were from 0.1 to 500 ng/mL for the Dxl 800 and 0.16 to 500 ng/mL for the GAMMA-10. GAMMA-10 has a clinical reportable range (CRR) of 0.16 to 500,000 ng/mL. The BRAHMS Tg plus assay (Thermo Scientific), which is used on the GAMMA-10, is calibrated by the Certified Reference Material 457, an international Tg reference material, and its measurement values were multiplied by two before analysis accord-

ing to the manufacturer's instructions. The Pearson product-moment correlation coefficient ( $R$ ) was calculated to determine the relationship between the results of the two assays.

For serum TgAb, the agreement between results obtained on the Dxl 800 and the RIA-MAT 280 (Stratec, Birkenfeld, Germany) was evaluated as categorical variables divided by the cut-offs for the TgAb positivity. The cut-offs for serum TgAb were 4.0 IU/mL for the Dxl 800 and 60.0 IU/mL for the RIA-MAT 280. We also evaluated the correlation between the two methods; AMR was 0.9 to 2,500 IU/mL and 5.2 to 200 IU/mL, respectively. RIA-MAT's CRR can be expandable to 5.2 to 200,000 IU/mL.

## 5. Functional sensitivity

Functional sensitivity of the Tg assays was determined by using the 20% CV between-run precision in compliance with the guidelines of the National Academy of Clinical Biochemistry (NACB) [7]. We evaluated the precision at low levels of serum Tg. Control materials were diluted to the concentrations of 0.2, 0.1, 0.075, and 0.05 ng/mL. Each run was conducted in duplicate for 20 days as described above.

Additionally, we collected blood samples from 20 healthy individuals who visited the health promotion center for regular health checkups without any clinical symptoms or signs of illnesses. Informed consent was received from all participating subjects. Blood samples were pooled to create a specimen with a serum Tg concentration of 2.0 ng/mL using the serum of healthy subjects which had Tg levels between 0.5 to 5.0 ng/mL. This specimen was serially diluted to 2.0, 1.5, 1.0, 0.75, 0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.0156 ng/mL. Each specimen was measured 20 times.

# RESULTS

## 1. Precision and linearity

The mean, standard deviation, and CV of each analyte and its respective levels were tabulated (Table 1). The total CV was within 10% in all assays, meeting the manufacturers' claimed performance. The total CV of serum Tg was 3.9% for pooled serum from healthy subjects, with a better precision than low, middle, and high levels of analytes manufactured from control materials (total CV of 4.4-5.6%). The CVs of serum TgAb were also within acceptable levels (total CV of 5.3%). Little difference was observed in CV levels for all levels of analytes.

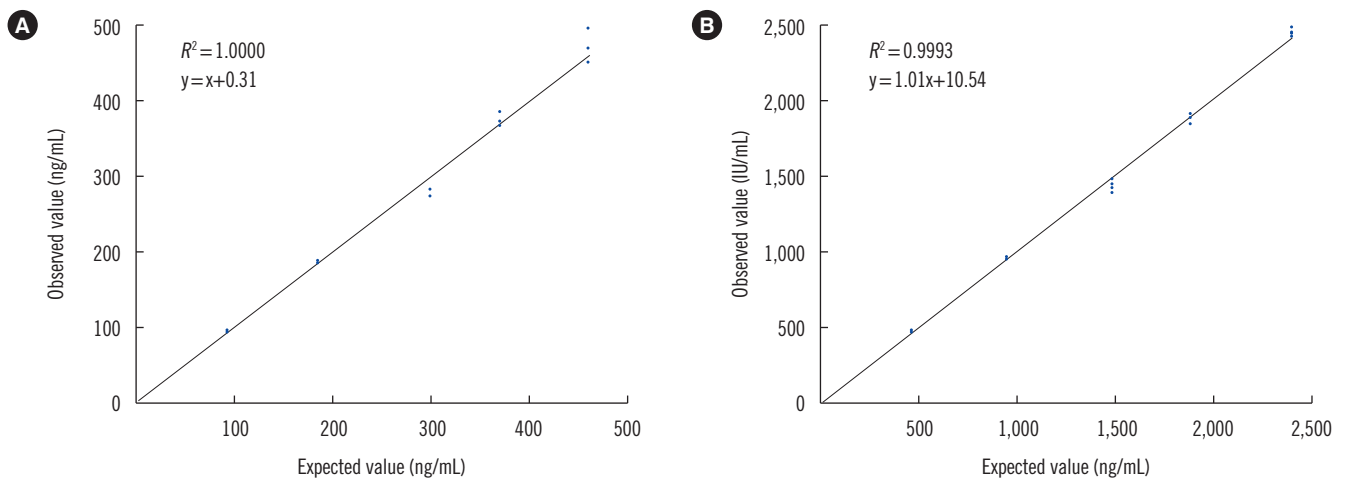
Linearity was observed in both the serum Tg and TgAb at the tested concentrations.  $R^2$  was 1.0 for serum Tg and 0.99 for serum TgAb (Fig. 1).

**Table 1.** Precision profile of the Dxl 800 assay for serum thyroglobulin and anti-thyroglobulin antibody

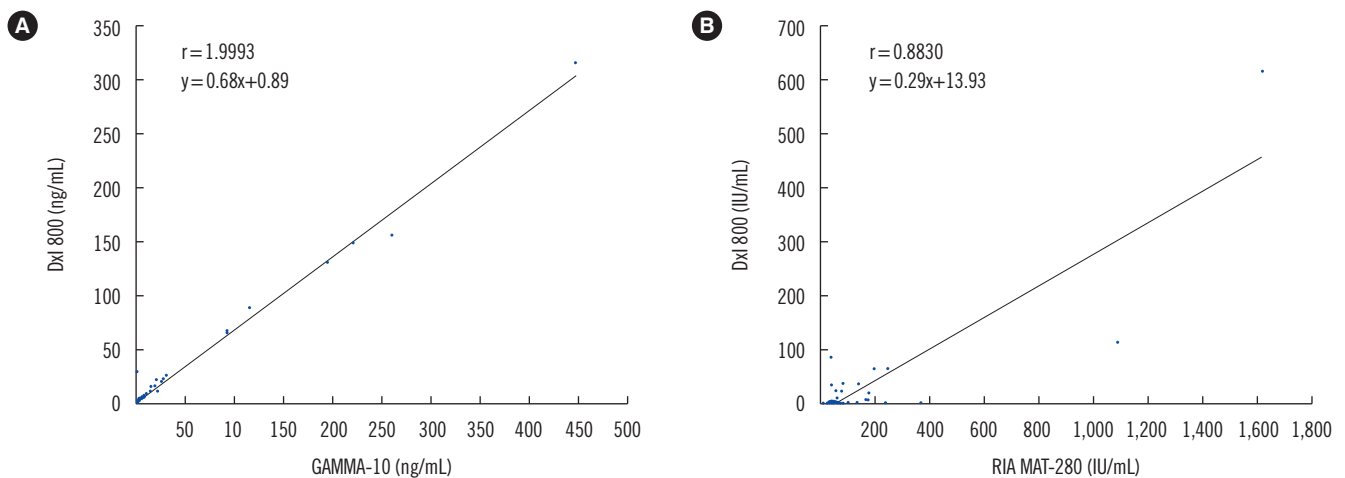
Analyte	Calibration level	Mean	SD	CV (%)			
				Within run	Between run	Between day	Total
Tg (ng/mL)	Pooled serum	170.6	6.7	2.9	1.7	1.9	3.9
	Low level	31.7	1.4	3.9	0	2.2	4.4
	Middle level	120.6	5.8	4.2	1.0	2.1	4.8
	High level	380.0	21.4	4.0	3.9	1.0	5.6
TgAb (IU/mL)	Pooled serum	80.4	4.3	3.5	2.5	3.2	5.3
	Low level	4.2	0.3	6.2	3.1	0	6.9
	High level	475.5	32.6	4.5	5.1	1.2	6.9

For serum Tg, control materials were manufactured with three concentrations: 32.5 ng/mL (low level calibrator), 250 ng/mL (middle level calibrator), and 400 ng/mL (high level calibrator). For serum TgAb, control materials were manufactured with two concentrations: 4.5 IU/mL (low level calibrator) and 500 IU/mL (high level calibrator).

Abbreviations: Tg, thyroglobulin; TgAb, anti-thyroglobulin antibody.



**Fig. 1.** Linearity profile of the Dxl 800 assay for serum thyroglobulin (Tg) and anti-thyroglobulin antibody (TgAb). For the evaluation of linearity, five concentrations of control materials were used for both Tg and TgAb. The intended concentrations for serum Tg were 0, 94, 188, 282, 376, and 470 ng/mL (A), and 0, 480, 960, 1,440, 1,920, and 2,400 IU/mL for serum TgAb (B).



**Fig. 2.** Correlation of serum thyroglobulin (A) and anti-thyroglobulin antibody (B) concentrations measured by the two assays (results within analytical measurement ranges).

## 2. Correlation of serum Tg and TgAb concentrations between the two assays

Some samples exceeded the AMR range of Dxl 800. Three samples exceeded 500 ng/mL for Tg, and one sample exceeded 2,500 IU/mL for TgAb. Correlation between each method was evaluated after excluding these results. *R* was 0.99 and 0.88 for serum Tg and TgAb, respectively. The equation were  $Dxl\ 800 = 0.68 \times GAMMA-10 + 0.89$  (serum Tg) and  $Dxl\ 800 = 0.29 \times RIA-MAT\ 280 + 13.93$  (serum TgAb) (Fig. 2).

When serum Tg levels were categorized into <1 ng/mL, 1-10 ng/mL, and >10 ng/mL, the weighted Kappa statistic value for Tg was 0.884 (Table 2, top). Serum TgAb levels were compared based on the cut-off value for each assay, resulting in an 84.8% (56/66) negative agreement and 36.4% (12/33) positive agreement, with a total agreement of 68.7% (Cohen's Kappa statistic, 0.231; Table 2, bottom).

## 3. Functional sensitivity

In analytes containing low levels of serum Tg (0.2-0.05 ng/mL) manufactured with calibrators, CV values were less than 10% at a

**Table 2.** Agreement of serum thyroglobulin and anti-thyroglobulin antibody between the two assays

Tg (ng/mL)*		GAMMA-10			Total
		<1	1-10	>10	
Dxl 800	<1	37	5	0	42
	1-10	0	37	2	39
	>10	0	1	17	18
Total		37	43	19	99
TgAb†		RIA-MAT 280		Total	
		Negative	Positive‡		
Dxl 800	Negative	56	21	77	
	Positive‡	10	12	22	
Total		66	33	99	

Values are presented with numbers of subjects.

\*Weighted Cohen's Kappa statistics was 0.884 for serum Tg; †Cohen's Kappa statistics was 0.231 for TgAb, and the agreement rate was 68.7%; ‡The cut-offs for positivity of serum TgAb were 4.0 IU/mL for the Dxl 800 and 60.0 IU/mL for the RIA-MAT 280.

Abbreviations: Tg, thyroglobulin; TgAb, anti-thyroglobulin antibody.

concentration  $\leq 0.075$  ng/mL. The CV exceeded 20% (28.5%) in the analyte presenting a 0.05 ng/mL Tg concentration (Table 3).

To assess the functional sensitivity in diluted sera from healthy subjects, CV values ranged from 3.8 to 14.5% at Tg concentrations of 0.0625-2.0 ng/mL. When the level was diluted to 0.0313 ng/mL, the CV value exceeded 20% (21.3%). The functional sensitivity was estimated to be between 0.0313 and 0.0625 ng/mL (Table 3).

## 4. A case with discrepant Tg results between the two assays

The case with discrepant results between the two assays was a 34-yr-old male patient who underwent total thyroidectomy for classic papillary thyroid cancer (T3N1aM0) and received subsequent RAIT (50 mCi). The serum Tg level measured using the GAMMA-10 was 1.2 ng/mL, while it was 29.37 ng/mL when measured with the Dxl 800. Serum TgAb level was 0.91 IU/mL with the Dxl 800 and 69.9 IU/mL with the RIA-MAT 280. One year later, a diagnostic scan (<sup>125</sup>I) was performed; serum Tg and TgAb levels declined to 0.2 ng/mL with the GAMMA-10 and 16.0 IU/mL with the RIA-MAT 280, respectively. There was no evidence of disease during the 2-yr observation period.

## DISCUSSION

We tested the analytic performance of the Dxl 800 assay, an ICMA, for serum Tg, which yielded a functional sensitivity between 0.0313 and 0.0625 ng/mL. The results from the Dxl 800 for serum Tg and TgAb correlated well with those obtained by using the IRMAs, the GAMMA-10 assay for serum Tg and the RIA-MAT 280 assay for serum TgAb, which are commonly used in Korea.

The advantages of the ICMA method include good functional sensitivity and short processing time. In the present study, the functional sensitivity was expected to be between 0.0313 and 0.0625 ng/mL, which satisfies the criterion suggested by an expert panel, indicating that the Tg assay requires a functional sensitivity of at least 1 ng/mL [17]. Generally, IMAs show a higher sensitivity than RIAs, although some degrees of variation

**Table 3.** Functional sensitivity of low levels of serum thyroglobulin manufactured from calibrators and samples from healthy subjects

Target (ng/mL)	Calibrators				Healthy subject samples								
	0.2	0.1	0.075	0.05	2	1.5	1	0.75	0.5	0.25	0.125	0.0625	0.0313
Mean (ng/mL)	0.195	0.100	0.072	0.039	2.065	1.489	0.878	0.773	0.504	0.209	0.114	0.062	0.040
SD (ng/mL)	0.013	0.008	0.007	0.011	0.078	0.071	0.041	0.032	0.034	0.012	0.008	0.009	0.008
CV (%)	6.5	7.6	9.3	28.5	3.8	4.7	4.6	4.1	6.7	5.6	6.9	14.5	21.3

in analytic performance exist [5, 6]. Persoon *et al* [11] reported a superior detection limit of 0.05 ng/mL and functional sensitivity of 0.6 ng/mL with the ICMA, as compared with RIA and IRMA. Recently developed mass spectrometry-based Tg assays have improved functional sensitivity ( $\leq 0.5$  ng/mL). However, false-negative results have been reported for Tg concentrations between 0.1 and 0.5 ng/mL [10].

The correlation of serum Tg between the Dxl 800 and the GAMMA-10 methods was excellent. A previous study reported that serum Tg levels measured by using the ICMA, IRMA, and RIA were highly correlated using TgAb-negative samples ( $R=0.992$ ,  $R=0.999$ , respectively) [11]. However, a major concern with the ICMA is the TgAb interference, which often results in falsely low or undetectable Tg concentrations [5, 9] in *in vitro* and clinical studies [8, 9, 19]. Endogenous TgAbs bind to free Tg and conceal the Tg epitopes needed for recognition by signal monoclonal antibody reagents [18]. Nevertheless, we observed a good correlation between the two Tg assays using TgAb-positive and -negative samples, although both assays may not fundamentally overcome the TgAb interference.

For the ICMA, a 2- or 3-fold difference has been reported between some assays [5, 11]. This might result from differences in specificity for the capture and/or signal monoclonal antibody reagents or standardization, although the ICMA is standardized against the CRM-457 international standard material [5, 11]. Because of high variability and low interchangeability among assays [5], the current guidelines for differentiated thyroid cancer developed by the ATA recommend serial measurement of serum Tg using the same assay in individual patients [20]. Changes in Tg assays, even if it is an ICMA, may result in abrupt serum Tg fluctuation and need to be cautiously considered for adequate decision making.

Eight cases presented discrepant results in term of Tg levels (Table 2). Among them, only subtle variations in Tg concentrations were observed in seven cases (i.e. 10.8 ng/mL using the IRMA vs 9.56 ng/mL using the ICMA). These differences were clinically acceptable changes and may not alter the clinical implication. However, one case showed a considerable elevation of serum Tg concentration on the Dxl 800 when compared with the results obtained by using the GAMMA-10 (29.37 ng/mL vs 1.2 ng/mL). Based on clinical information, there was no evidence of remnant or recurrent disease on two instances of thyroid ultrasonography with a decreasing trend of subsequent stimulated Tg. Thus, the value measured by the Dxl 800 might have been falsely elevated. Serum TgAb was positive, even with a slight elevation in this patient. However, the presence of TgAb

always interferes with serum Tg levels, resulting in an underestimation in the ICMA [5]. Therefore, overestimation of serum Tg by the Dxl 800 might derive from another type of interference. Human anti-mouse antibody (HAMA) also affects serum Tg results, mainly in terms of false elevation, even though this rarely leads to underestimation of Tg concentrations [21]. This antibody can bind to animal antigens and form a complex between antibodies, which produces a false elevation of serum Tg results [21]. As the prevalence of HAMA ranges 1.5-3% in automated Tg assays, HAMA interference should be suspected in cases showing Tg results discordant with the clinical situation [21].

This study has several limitations. Neither the ICMA nor IRMA tested in this study was a gold standard, although they are commonly recommended for Tg measurement. As described above, all IMA methodologies are affected by the interference from TgAbs as well as HAMA. This interference is not as evident as in other assays, including RIA and the LC-MS method. However, they also do not fully overcome it yet. In the present study, we identified one case showing a falsely elevated serum Tg result with the Dxl 800, which should be interpreted in the clinical context. The proportion of discordant results (1/99) seems to be acceptable because the prevalence of HAMA has been reported to be up to 3% in IMAs. Even though the IRMA may not serve as the gold standard test, as the most prevalent assay for serum Tg in current laboratories, including South Korea, we sought to validate the excellent discrimination (linear correlation) between the two assays and to identify whether the ICMA would be a viable alternative to IRMA. Considering the good correlation between the two assays, irrespective of various titers of TgAb, and the better analytic performance of the ICMA, the ICMA could be considered as an alternative test for the Tg measurement.

The Dxl 800 method is a sensitive assay for Tg measurement, and the results were highly correlated with those obtained with the GAMMA-10. Analytic performance and clinical convenience make this assay a good substitute for the IRMA.

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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