Current Thyroglobulin Autoantibody (TgAb) Assays Often Fail to Detect Interfering TgAb that Can Result in the Reporting of Falsely Low/Undetectable Serum Tg IMA Values for Patients with Differentiated Thyroid Cancer

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Context: Specimens have thyroglobulin antibody (TgAb) measured prior to thyroglobulin (Tg) testing because the qualitative TgAb status (positive or negative) determines risk for Tg assay interference, and the quantitative TgAb concentration serves as a surrogate tumor marker for differentiated thyroid cancer.

Objective: This study assessed the reliability of four TgAb methods to detect interfering TgAb [as judged from abnormally low Tg immunometric assay (IMA) to Tg RIA ratios] and determine whether between-method conversion factors might prevent a change in method from disrupting TgAb monitoring.

Methods: Sera from selected and unselected TgAb-negative and TgAb-positive differentiated thyroid cancer patients had serum Tg measured by both IMA and RIA and TgAb measured by a reference method and three additional methods.

Results: The Tg IMA and Tg RIA values were concordant when TgAb was absent. Tg IMA to Tg RIA ratios below 75% were considered to indicate TgAb interference. Manufacturer-recommended cutoffs were set in the detectable range, and when used to determine the presence of TgAb misclassified many specimens displaying Tg interference as TgAb negative. False-negative misclassifications were virtually eliminated for two of four methods by using the analytical sensitivity (AS) as the detection limit for TgAb. Relationships between values for different specimens were too variable to establish between-method conversion factors.

Conclusions: Many specimens with interfering TgAb were misclassified as TgAb negative using manufacturer-recommended cutoffs. It is recommended that assay AS limits be used to detect TgAb to minimize false-negative misclassifications. However, for two of four assays, AS limits failed to detect interfering TgAb in 20–30% of cases. TgAb methods were too qualitatively and quantitatively variable to establish conversion factors that would allow a change in method without disrupting serial TgAb monitoring. **(*J Clin Endocrinol Metab* 96: 1283–1291, 2011)**

Serum thyroglobulin (Tg) is primarily measured as a postoperative tumor marker test for patients with differentiated thyroid cancer (DTC) (1). Unfortunately, the Tg immunometric assay (IMA) methodology used by most laboratories is prone to interferences, from both human antimouse antibodies (HAMA) (2, 3) and Tg autoantibodies (TgAb) (4–7). HAMA interference usually results in a falsely high serum Tg that may prompt unnecessary investigations for disease (2, 3). In contrast, TgAb interference causes a falsely low or undetectable serum Tg that can have

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Abbreviations: AS, Analytical sensitivity; DTC, differentiated thyroid cancer; HAMA, human antimouse antibody; IMA, immunometric assay; MC, manufacturer-recommended cutoff; Tg, thyroglobulin; TgAb, Tg autoantibody; WHO, World Health Organization.

more serious consequences because it can mask the presence of disease (5, 8–10). Although IMAs routinely include blocker reagents to minimize HAMA interference (~0.5%) (11, 12), there are currently no effective measures to overcome TgAb interference with Tg IMA tests. The reliable detection of TgAb is critical because the prevalence of TgAb in DTC patients is high (10–30%), and the presence of TgAb appears to convey an increased cancer risk for thyroid nodules, and when persistent after surgery, an increased risk for recurrent disease (4, 5, 7, 9, 10, 13).

Serum TgAb measurement is made on all specimens requesting Tg testing to determine the risk for Tg assay interference and because TgAb concentrations *per se* can be used as a surrogate tumor marker (1, 4, 8–10, 14–16). Current TgAb methods are based on competitive immunoassay or noncompetitive IMA methodology (6, 14, 17). Although assays are calibrated against the same International Reference Preparation [World Health Organization (WHO) first international reference preparation 65/93], the values reported by different methods are highly variable because of TgAb heterogeneity among patients, compounded by assay sensitivity and specificity differences (4, 6, 17–21). Over recent years it has become apparent that serial TgAb concentrations can be monitored as a surrogate tumor marker (4, 9, 10, 15, 22–29). Because TgAb concentrations reported by different methods can vary 100-fold for the same specimen, it is critical to use the same method to monitor changes in TgAb concentrations (6, 17, 19, 22). Not infrequently these methodological differences result in specimens being classified as TgAb positive by one method but TgAb negative by another (6, 20, 22). False-negative misclassifications increase the risk of reporting an inappropriately low or undetectable serum Tg IMA value for patients with disease (5, 6, 9, 10).

Because there is no reliable TgAb reference method, it is necessary to use an independent parameter to assess the presence of interfering TgAb. Previous studies have suggested that Tg RIA methodology is more resistant to TgAb interference than IMA, although interference causing falsely low or high serum Tg RIA values can occur in some TgAb-positive patients (1, 6, 23, 24). Irrespective of whether the Tg IMA or RIA result is valid for any given serum, one recognized hallmark of TgAb interference is discordance between the Tg IMA *vs.* Tg RIA values (low/undetectable Tg IMA *vs.* higher/detectable Tg RIA) (4, 6, 25, 26). The current study used a low Tg IMA to Tg RIA ratio (below the 2.5th percentile for TgAb negative controls) to indicate the presence of TgAb interference.

The goals of this study were: 1) to assess whether the manufacturer-recommended cutoffs (MCs) of four different TgAb methods reliably identified TgAb-containing specimens, using a low serum Tg IMA to Tg RIA ratio to indicate the presence of interfering TgAb, and 2) to evaluate whether fixed factors could be used to convert the TgAb values reported by different methods so that a change in TgAb method could be made without disrupting the serial monitoring pattern.

Materials and Methods

Assays

Tg assays

Both Tg assays (IMA and RIA) were standardized 1:1 against the International Reference Preparation CRM-457.

Tg RIA. The Tg RIA was developed by the University of Southern California Endocrine Laboratories, Keck School of Medicine (Los Angeles, CA) as previously described (6, 30). The assay had first-generation functional sensitivity [0.5 ng/ml (micrograms per liter)] established using the NACB protocol (14). Between-run precision assessed over a 3-yr period was 17.8, 8.3, 9.7, and 11.9% for serum Tg concentrations of 0.78, 2.0, 11.1, and 31.7 ng/ml (micrograms per liter), respectively. Within-run precision was 7.1, 1.5, and 5.3% at concentrations of 2.0, 16.1, and 31.6 ng/mL (micrograms per liter), respectively.

Tg IMA. The Tg IMA was the Access immunochemiluminometric method (Beckman Coulter, Fullerton, CA). This assay had second-generation functional sensitivity [0.1 ng/ml (micrograms per liter)] (14). The between-run precision assessed over a 14-month period was 11.1, 5.1, 4.9, and 4.3% for serum Tg concentrations of 0.16, 0.58, 7.0, and 348 ng/ml (micrograms per liter), respectively. Within-run precision was 4.1, 3.2, 1.7, and 1.9% at concentrations of 0.15, 0.76, 7.0, and 106 ng/ml (micrograms per liter), respectively.

TgAb assays

The four TgAb assays were all standardized against the WHO reference serum 65/93 and were performed according to the manufacturer's instructions.

Assay 1 (reference method). This assay was a semiautomated radioassay (Kronus, Boise, ID, also known as RSR, Cardiff, UK). This method uses I^{125} labeled Tg to bind TgAb in a diluted (1:21) serum specimen. The TgAb/Tg I^{125} labeled complex is precipitated using protein A. Within-run precision was 0, 2.7, and 4.1% at concentrations of 0.8, 2.7, and 14.1 kIU/liter, respectively. Between-run precision assessed over a 13-month period was 8.7, 6.2, and 9.3% for concentrations of 0.8, 2.6, and 14.5 kIU/liter, respectively. Analytical sensitivity (AS) [within-run precision of the assay matrix (31)] and the MC for detecting TgAb were 0.3 and 1.0 kIU/liter, respectively. The manufacturer considered values between AS and MC (0.3–1.0 kIU/liter) as indeterminate and were treated as borderline positive for the purposes of this study.

Assay 2. Assay 2 was an automated Elecsys 2010 electrochemiluminescent immunometric assay method (Roche Diagnostics, Indianapolis, IN). In this competitive method, TgAb from the serum specimen competes for biotinylated human Tg with ru-

thenium-labeled TgAb. The Tg-TgAb complexes formed are bound to streptavidin-coated microparticles and magnetically captured onto the surface of an electrode. Within-run precision was 4.9, 5.1, and 5.6% at concentrations of 63, 115, and 2894 kIU/liter, respectively. Between-run precision assessed over a 16-month period was 14.5, 8.9, and 16.7% for concentrations of 87, 146, and 1329 kIU/liter, respectively. The AS and MC were 10 and 115 kIU/liter, respectively. The values between the AS and MC (10–115 kIU/liter) were considered as borderline positive for the purposes of this study.

Assay 3. This assay was an automated Access chemiluminescent immunometric assay method (Beckman Coulter). In this two-step sandwich assay, TgAb from patient sample is bound to solid-phase Tg and Tg labeled with enzyme. Within-run precision was 6.2, 5.8, 5.2, and 3.7% at concentrations of 2.3, 13.3, 39.1, and 190.1 kIU/liter, respectively. Between-run precision assessed over a 12-month period was 11.1, 7.0, 7.0, and 5.9% for concentrations of 2.8, 14.6, 41.6, and 197.3 kIU/liter, respectively. AS and MC were 0.9 and 4.0 kIU/liter, respectively. Values between the AS and MC (0.9–4.0 kIU/liter) were considered borderline positive for the purposes of this study.

Assay 4. This assay was an automated Immulite chemiluminescent immunometric assay method (Siemens Corp., Los Angeles, CA) in which the TgAb present in diluted patient sera is captured by solid-phase Tg and enzyme-labeled murine antihuman IgG. Within-run precision was 5.3, 2.3, and 4.9% at concentrations of 31, 66, and 461 kIU/liter, respectively. Between-run precision assessed over a 3-yr period was 11.2 and 15.4% for concentrations of 32 and 526 kIU/liter, respectively. The AS and MC were 20 and 40 kIU/liter, respectively. Values between AS and MC (20–40 kIU/liter) were considered as borderline positive for the purposes of this study.

Serum specimens

Specimens from patients with DTC having follow-up serum Tg testing were divided into three groups for the study.

Group A

A total of 785 consecutive specimens were used the assess TgAb prevalence using the reference assay 1 and adopting the MC of 1.0 kIU/liter. Group A consisted of 143 TgAb-positive specimens (using the MC of assay 1) with sufficient volume for TgAb to be analyzed by the other three assays and used to evaluate the ranking of values between methods and to qualitatively compare the concordance of classifying specimens as TgAb positive according to the assay MC.

Group B

This group consisted of 413 specimens with unequivocally detectable serum Tg RIA [≥1.0 ng/ml (micrograms per liter)] and sufficient specimen for Tg IMA measurement. Specimens also had TgAb determined by each of the four methods. The relative value of using the MC *vs.* the AS for assigning a positive TgAb status was evaluated relative to the ability of that cutoff to appropriately classify specimens as positive when they exhibited Tg assay interference as evidenced by a low Tg IMA to Tg RIA ratio.

Group C

Group C consisted of 24 specimens from group B that were considered to be unequivocally devoid of TgAb on the basis of having values below the AS limit of each of the four methods. These specimens were used to establish the relationship between serum Tg IMA and Tg RIA values and the range of Tg IMA to Tg RIA ratios characteristic of specimens devoid of TgAb.

TgAb interference with serum Tg measurement

The relationship between serum Tg IMA and Tg RIA measurements made on the unequivocally TgAb-negative group C specimens was used as the benchmark for detecting interfering TgAb. Group C had comparable serum Tg IMA and Tg RIA values [mean 9.1, range 0.9–31.1 *vs.* 10.2, range 1.2–30.4 ng/ml (micrograms per liter), IMA *vs.* RIA, respectively]. Mean Tg IMA to Tg RIA ratio was 90 ± 10 (SD)%, 95% confidence interval was 75–110% and Tg IMA to Tg RIA ratios below the 2.5th percentile value of 75% were considered indicative of TgAb interference. Severe interference was considered to be present when the Tg IMA was undetectable [<0.1 ng/ml (micrograms per liter)] and yet the Tg RIA was unequivocally detectable [≥1.0 ng/ml (micrograms per liter)]. No specimen had serum Tg IMA above 1.0 ng/ml (micrograms per liter) and yet an undetectable (<0.5 ng/ml (micrograms per liter)] Tg RIA.

Statistics

Comparison of Tg IMA to RIA ratios between groups was performed using the Student *t* test (version 13.0; SPSS, Chicago, IL), and $P < 0.05$ was statistically significant.

Results

The prevalence of TgAb in 785 consecutive DTC specimens was 23.8% (187 of 785) when measured using the reference TgAb assay (assay 1) and the MC of 1.0 kIU/liter. As shown in Fig. 1, many of the 143 group A specimens that were classified as containing TgAb according to the MC of assay 1 were classified as TgAb negative according to the MC limits of the other assays (44.1, 35.0, and 62.2%, assays 2–4, respectively). This was thought to reflect false-negative misclassifications because in 134 of 143 of cases (93.7%); the Tg IMA to Tg RIA ratio was low (<75%), indicative of TgAb interference. This interference was severe [Tg IMA < 0.1 ng/ml (micrograms per liter) to Tg RIA ≥ 1.0 ng/ml (micrograms per liter)] in the case of 80 of 143 specimens (55.9%).

The high frequency of apparent false-negative TgAb misclassifications associated with using MCs prompted the study of the 413 group B specimens in which the MC *vs.* the AS cutoffs were compared for classifying TgAb status (data summarized in Table 1 and shown in Fig. 2). Using MCs, significantly more specimens were classified as TgAb positive using assay 1 as compared with the other methods [181 of 413 (43.8%) *vs.* 123 of 413 (29.8%), 110 of 413 (26.6%), and 87 of 413 (21.1%), assay 1 *vs.* assays

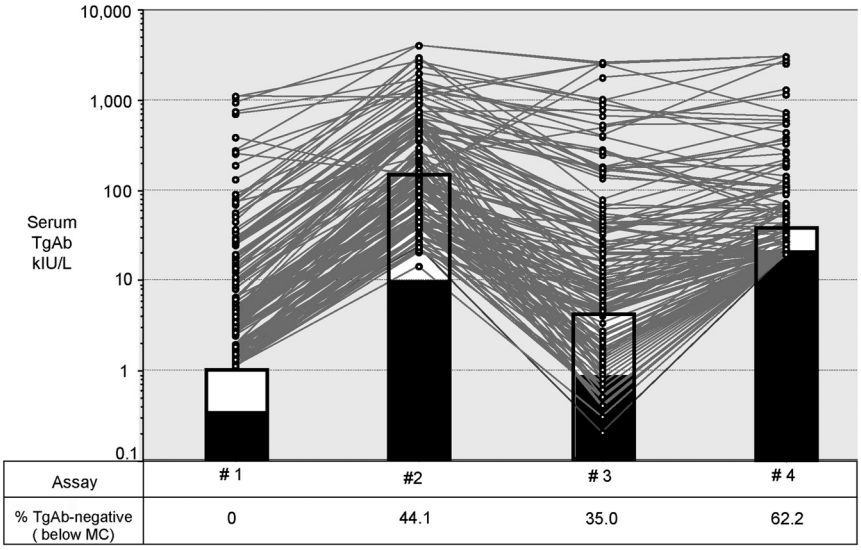

FIG. 1. Relative serum TgAb concentrations reported by each of the four TgAb methods for the 143 group A specimens with TgAb above the MC of the reference assay (assay 1). The *open bars* show the assay MC limits and the *solid bars* the AS limits.

2–4, respectively, $P < 0.05$]. Regardless of the method, greater than 90% of specimens with TgAb above the MC displayed interference [173 of 181 (95.6%), 118 of 123 (95.9%), 100 of 110 (90.9%), and 79 of 87 (90.8%), assays 1–4, respectively]. It was striking to see that a high percentage of specimens that were classified as TgAb negative according to MCs exhibited interference [86 of 413 (20.8%), 141 of 413 (34.1%), 159 of 413 (38.5%), and 180 of 413 (43.6%), assays 1–4, respectively]. This interference was severe in many cases [18 of 413 (4.4%), 36 of 413 (8.7%), 26 of 413 (6.3%), and 43 of 413 (10.4%), assays 1–4, respectively]. As shown in Fig. 1, the MCs were set in the detectable range (*i.e.* above the AS limit) for all assays. This led to a reanalysis of false-negative misclassifications using AS limits to assign a positive TgAb status. By adopting the AS limits for assays 1 or 2, virtually all of the false-negative misclassifications were eliminated (≤1%), and no specimen displaying severe interference was misclassified as TgAb negative. In contrast, when AS cutoffs were adopted for assays 3 or 4, the high number of false-negative misclassifications remained [90 of 413 (21.9%) and 142 of 413 (34.3%) cases, respectively], and a significant number of these misclassified specimens displayed severe interference [14 of 413 (3.3%) and 33 of 413 (7.9%), assays 3 and 4, respectively]. When the TgAb was borderline positive (between AS and MC), the percent of specimens exhibiting interference and/or severe interference was intermediate between the AS and MC groups for that assay.

Each of the four TgAb assays claimed to be standardized against the International Reference Preparation Medical Research Council 65/93 and yet MCs, as well as the absolute values reported for the same specimen, were quantitatively very different, as shown in Figs. 1 and 3. Despite the general ranking between assay values evident in Fig. 1, Fig. 3 analysis of group B found that for any individual serum, the relationship between the reference (assay 1) value and the TgAb value reported by the other methods was too highly variable to allow calculation of a conversion factor that could be used to switch between methods without disrupting the pattern of serial TgAb monitoring. Specifically, mean ratios between the reference assay 1 and the other three methods were 59.5 ± 31.0 (SD), range 0.3–153; 4.6 ± 3.6, range 0.3–18.5 and 7.6 ± 6.3, range 2.2–34.7, assay 1 *vs.* assays 2–4, respectively. Analyses using each of the other assays in turn as the reference showed equal between-method variability (data not shown).

Discussion

Guidelines mandate that TgAb should be measured in all specimens sent for serum Tg testing because the qualitative TgAb status (positive or negative) determines the risk for Tg assay interference, and serial quantitative TgAb measurements can be used as a surrogate tumor marker (1, 14, 32). This study of four different TgAb methods confirmed that current assays are qualitatively and quantitatively variable and cannot be used interchangeably (4, 6, 17–19, 22). Using a low Tg IMA to Tg RIA ratio as an indicator for Tg assay interference, the study found that when the MCs were used to determine the presence of TgAb, many specimens displaying interference were falsely classified as TgAb negative, and in some cases, these misclassified specimens exhibited severe Tg assay interference with the potential to influence patient management (undetectable Tg IMA/detectable Tg RIA). Lowering the cutoff for TgAb detection to the assay AS limit virtually eliminated the false-negative misclassifications for two of the four methods, indicating that current MCs are set too high for reliably detecting interfering TgAb. Thus, the reliability of TgAb measurement was clearly both assay and cutoff dependent, with some specimens being classified as TgAb positive by one or more method(s) but TgAb negative by others (Figs. 1 and 3). Given that it is not unusual for laboratories to change TgAb methods without giving physicians the opportunity to re-baseline patients, it would be useful if between-method conversion factors could be established that might allow a change in method without a

TABLE 1. Tg interference detected using different TgAb assays and different cutoff values

TgAb method	Cutoff (kIU/liter)	n (%)	Tg RIA ng/mL (μg/L) median (range)	Tg IMA ng/mL (μg/L) median (range)	n (%) interference with serum Tg[a]	n (%) with severe[b] interference
Assay 1	AS (<0.3)	76 (18.4)	8.6 (1.0–33.1)	7.1 (0.6–32.8)	4 (5.3)	0 (0)
	Borderline (0.3–1.0)	156 (37.8)	3.5 (1.0–38.5)	2.8 (<0.1 to 45.0)	82 (52.5)	18 (11.5)
	MC (>1)	181 (43.8)	3.6 (1.0–36.9)	0.3 (<0.1 to 32.4)	173 (95.6)	70 (38.7)
Assay 2	AS (<10)	26 (6.3)	10.0 (1.2–30.4)	9.2 (0.9–31.1)	0 (0)	0 (0)
	Borderline (10–115)	264 (63.9)	3.3 (1.0–38.5)	2.6 (<0.1 to 45.0)	141 (53.4)	36 (13.6)
	MC (>115)	123 (29.8)[b]	6.0 (1.1–36.9)	0.3 (<0.1 to 32.4)	118 (95.9)	52 (42.3)
Assay 3	AS (<0.9)	200 (48.4)	6.4 (1.0–35.5)	4.5 (<0.1 to 45.0)	90 (45.0)	14 (7.0)
	Borderline (0.9–4.0)	103 (24.9)	3.4 (1.0–38.5)	2.4 (<0.1 to 40.0)	69 (67.0)	12 (11.7)
	MC (>1)	110 (26.6)[b]	3.6 (1.0–32.1)	<0.1 (<0.1 to 43.0)	100 (90.9)	62 (56.4)
Assay 4	AS (<20)	279 (67.6)	4.7 (1.0–38.5)	3.2 (<0.1 to 45.0)	142 (50.8)	33 (11.8)
	Borderline (20–40)	47 (11.4)	2.9 (1.0–36.9)	1.9 (<0.1 to 27.1)	38 (80.9)	10 (21.3)
	MC (>40)	87 (21.1)[b]	5.3 (1.0–31.0)	<0.1 (<0.1 to 32.4)	79 (90.8)	45 (51.7)

AS, Analytical sensitivity; MC, manufacturers cut-off, borderline values between AS and MC.

[a] Tg IMA to Tg RIA ratio below control group C (<75%).

[b] Tg IMA < 0.1 with Tg RIA ≥ 1.0 ng/mL (μg/L).

[c] $P < 0.05$ *vs.* assay 1.

disruption in serial TgAb monitoring. Despite the weak ranking of specimen values between methods evident in Fig. 1, the relationships between TgAb values reported by the different methods were specimen dependent and varied 100-fold (Fig. 3). Between-method relationships were too disparate to allow the calculation of conversion factors that might offset a disruption of serial TgAb monitoring should a change in method become necessary.

The presence of TgAb in patients with thyroid malignancies does not appear to be merely a manifestation of thyroid autoimmunity. Specifically, whereas thyroid peroxidase antibodies appear to be the more pathological

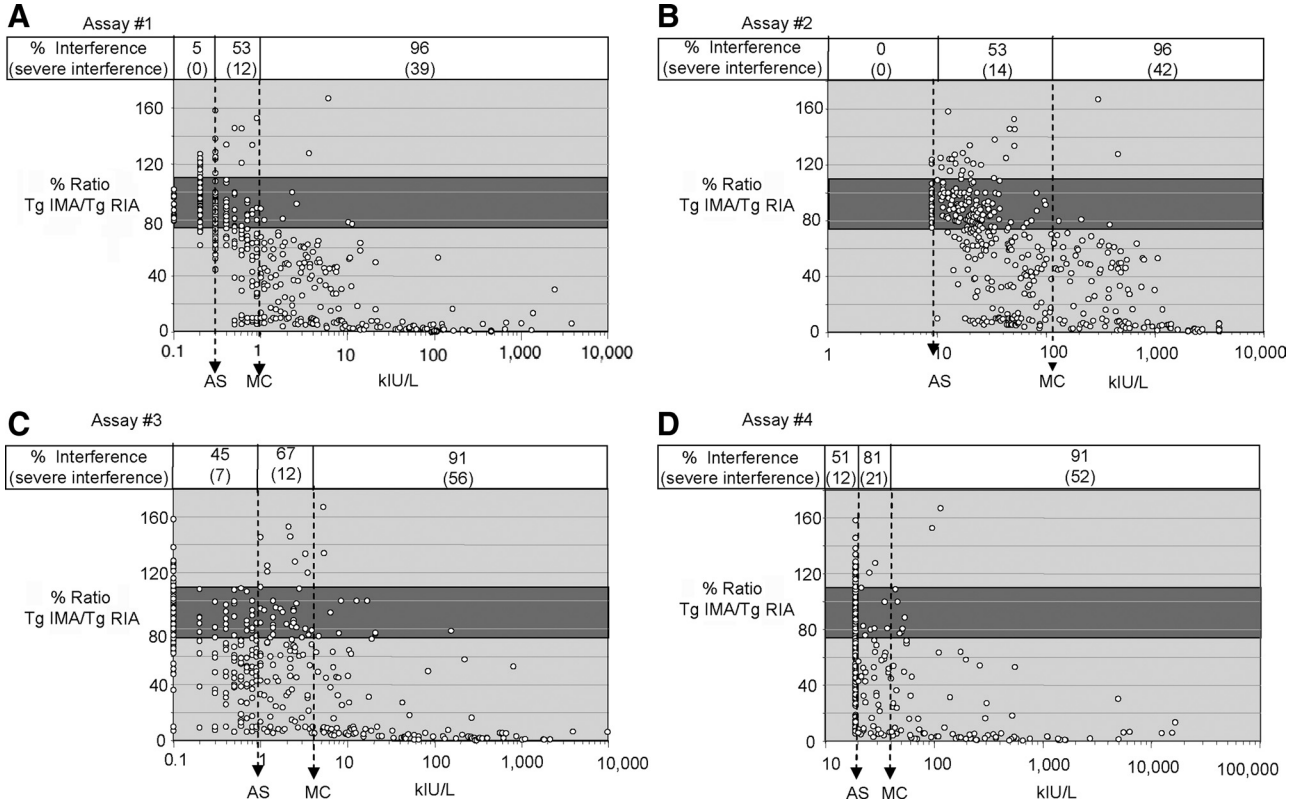

FIG. 2. The relationships between TgAb concentrations (on abscissae) measured by each of the TgAb methods and the presence of TgAb interference with serum Tg measurements, as judged from the presence of a low (<75%) serum Tg IMA to Tg RIA ratio. The *dark shading* represents the 95% confidence limits (75–110%) established from the group C specimens that had unequivocally absent TgAb (below the analytic sensitivity (AS) limits of all assays). The manufacturer cut-off for detecting TgAb is indicated by MC.

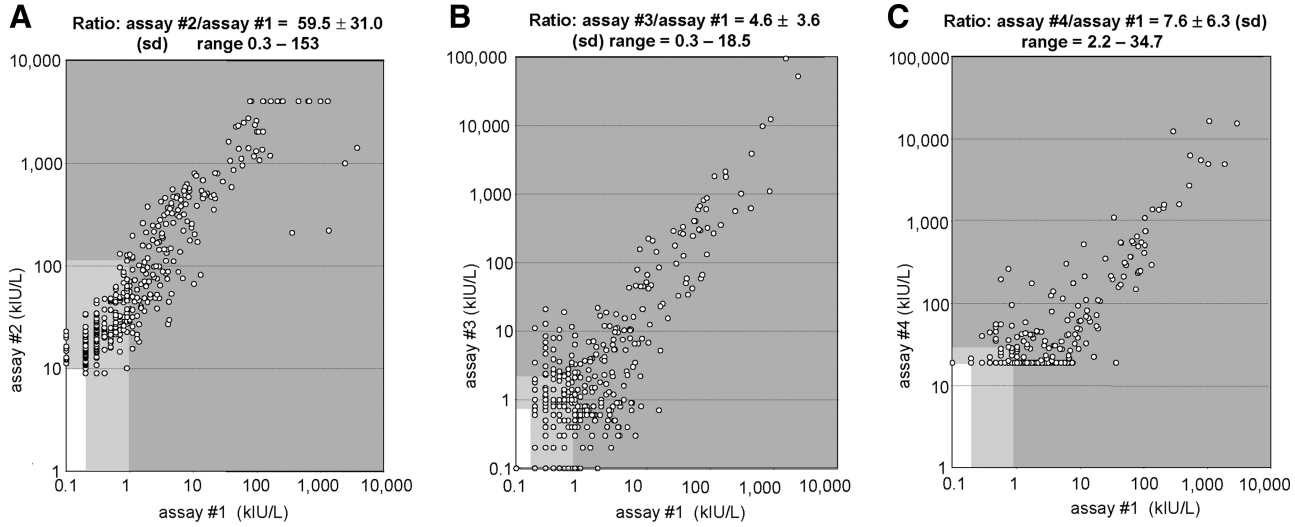

FIG. 3. This shows the relationships between assay 2, 3, and 4 values reported for the 413 Group B specimens (on the ordinates) relative to the reference assay (assay 1) shown on the abscissae. The correlations only included specimens with TgAb concentration(s) above the analytic sensitivity limits (AS) of one or both assays. The *dark shading* represents the range above the manufacturer cut-off (MC). *Lighter shading* represents the borderline values (between the AS and MC). *No shading* represents the range below the AS limit.

antibody marker for thyroid autoimmunity, TgAb concentrations appear to be an independent risk factor for malignancy in thyroid nodules and respond more rapidly to changes in thyroid tissue mass than thyroid peroxidase antibodies (13, 15, 29). Because the immune system is sensitive to the circulating Tg antigen, serial TgAb concentrations appear to act as a surrogate tumor marker for TgAb-positive DTC patients during long-term follow-up monitoring for recurrence (1, 4, 5, 8–10, 15, 33). Specifically, when patients are rendered disease free by thyroidectomy and remnant ablation, removal of the antigenic stimulus has been shown to reduce TgAb concentrations 50% by 6–12 months, with eventual disappearance of TgAb with a median time of 3 yr (8, 9, 15). Conversely, TgAb concentrations rise in response to increases in antigen concentrations after second surgeries, fine-needle aspiration biopsy, or radioiodine therapy (4, 34, 35). Although each of the four TgAb methods was found to have excellent between-run precision over the typical clinical interval used to monitor DTC patients (6–12 months), the value of serial TgAb measurements would be lost and serum Tg measurement would be unreliable for specimens misclassified as TgAb negative. The discordant relationships between TgAb values reported for the same specimen using different methods (Fig. 3) likely reflect serum TgAb heterogeneity compounded by differences in the specificity of circulating TgAb for Tg antigen, assay reagents, and the standards used by the various methods (4, 16, 20, 29, 36–38). This heterogeneity explains why high TgAb concentrations do not always interfere with serum Tg measurements, whereas in some cases very low TgAb concentrations can exhibit profound interference (4, 6, 21, 26). Standardization differences also likely contribute to between-method variability. All of the TgAb methods used secondary serum standards that claimed to be calibrated against the International Reference Preparation (WHO first international reference preparation 65/93). However, this reference material is a serum pool more than 50 yr old and more representative of TgAb arising from thyroid autoimmunity than DTC (4, 13, 16, 36, 37). Whatever the factors responsible for the different methods reporting different values, this study emphasized that a change in TgAb method would severely disrupt the use of TgAb concentrations as a surrogate tumor marker and that this problem could not be overcome by deriving between-method conversion factors (1, 4, 9, 15).

Given the high prevalence of TgAb in DTC found in this (23.8%) and other studies, it is critical to ensure that specimens do not contain TgAb before authenticating a serum Tg result because even low levels of TgAb can interfere with Tg measurement (4, 6, 21, 26). Direct TgAb measurement is generally recommended in preference to an exogenous Tg recovery test because studies have shown that Tg recoveries do not reliably detect the presence of interfering TgAb (1, 6, 8, 14, 26, 37, 39). Interference with Tg measurement caused by TgAb is the most serious problem that currently limits the clinical utility of serum Tg used as the tumor marker for DTC (1, 32). When Tg is measured using IMA methodology, interfering TgAb typically causes falsely low or undetectable serum Tg concentrations with the potential to mask the presence of disease. This underestimation is illustrated by studies showing that thyroidectomized DTC patients with unequivocal evidence of persistent/recurrent disease, as well as euthyroid control subjects with intact thyroid glands, may have inappropriately low or undetectable serum Tg

values when TgAb is present (4–10, 15, 33). Tg underestimation is an especially serious problem, given the reports that TgAb-positive patients tend to have a higher stage of disease and the persistence of TgAb after thyroidectomy conveys a higher risk of recurrence (5, 7, 9, 10, 33). Although RIA methodology is considered more resistant to TgAb interference than IMA, either under- or overestimated serum Tg RIA results can occur, depending on the interaction between the assay reagents and the specificity of the patient's TgAb (4, 6, 23, 24). One recognized characteristic of TgAb interference has been a discrepancy between Tg measured by IMA *vs.* RIA methodology (low/undetectable Tg IMA *vs.* higher Tg RIA) (4, 6, 24, 25). The current study used a low Tg IMA to Tg RIA ratio as an independent parameter to assess the presence of TgAb and showed that all of the TgAb methods tested were prone to falsely classify specimens exhibiting evidence of interfering TgAb as TgAb negative when the MC was used.

Assay MCs are typically set above the AS limit in the detectable range and represent the 97.5th percentile for young biochemically euthyroid male subjects who are considered least likely to have thyroid autoimmunity that would skew the range (14, 17, 40). Why some apparently euthyroid normal subjects exhibit detectable TgAb is currently unclear and may represent the measurement of natural antibodies (17, 41, 42) or early thyroid autoimmunity or may merely be nonspecific matrix effects. The problem of false-negative misclassifications was dramatically reduced by adopting the AS limit to classify specimens as TgAb positive. In the case of two assays (assays 1 and 2), the use of the AS limit eliminated all false-negative misclassifications of specimens displaying severe interference (undetectable Tg IMA/detectable Tg RIA), showing that assay MCs are typically set too high (17). In clinical practice most laboratories adopt the MC limit without independent evaluation and without recognizing that manufacturers set the MC limit for diagnosing thyroid autoimmunity and not for detecting interfering TgAb (6, 18, 19). However, even when diagnosing thyroid autoimmunity, assays show variable concordance when MCs are used as the detection limit for TgAb (17–19). It appeared significant that it was the two assays based on competitive assay principles (assays 1 and 2) that had superior ability to detect interfering TgAb as compared with the assays that used noncompetitive IMA or sandwich assay principles (assays 3 and 4). This might reflect steric inhibition of the formation or detection of TgAb-Tg reagent complexes, perhaps a similar mechanism to that responsible for TgAb interfering and causing low serum Tg IMA measurements (6).

This study of four different TgAb methods found that the use of the MC to determine the presence of TgAb frequently led to false-negative misclassifications of specimens displaying Tg interference, as judged from the presence of discordance between Tg IMA *vs.* Tg RIA measurements. In many of these cases, the interference was severe and had the potential to influence clinical management (undetectable [$<$ 0.1 ng/ml (micrograms per liter)] Tg IMA/detectable [$\leq$ 1.0 ng/ml (micrograms per liter)] Tg RIA). When all values detected above the AS limit were considered as TgAb positive, false-negative specimen misclassifications were greatly reduced and all misclassifications of specimens displaying severe interference were eliminated for two of the four methods (assays 1 and 2). However, with methods 3 and 4, even the use of the AS limit failed to detect interfering TgAb in a high percentage of cases (21.9 and 34.3%, respectively). Although there was a general ranking of values between assays, there were wide disparities between the absolute values reported by the different methods for the same specimen and disparate relationships between the values reported for different specimens. This study suggests that TgAb assay AS limits should be used to detect TgAb in order to minimize false-negative misclassifications. Furthermore, it is probably not possible to harmonize the TgAb values reported by different assays or calculate between-method conversion factors that would allow changing methods without disrupting serial TgAb monitoring used as a surrogate DTC tumor marker.

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