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Clinical Research Article

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Impact of Thyroglobulin and Thyroglobulin Antibody Assay Performance on the Differential Classification of DTC Patients

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Abstract

Context: Measurements of thyroglobulin (Tg) and Tg antibodies are crucial in the follow-up of treated differentiated thyroid cancer (DTC) patients. Interassay differences may significantly impact follow-up.

Objective: The aim of this multicenter study was to explore the impact of Tg and Tg antibody assay performance on the differential classification of DTC patients, as described in national and international guidelines.

Design: Four commonly used Tg and Tg antibody assays were technically compared to reflect possible effects on patients with DTC follow-up. Storage stability at different storage temperatures was also investigated for LIAISON® and Kryptor assays, as this is an underexposed topic in current literature.

Results: B.R.A.H.M.S. assays yield approximately 50% lower Tg values over the whole range compared to the DiaSorin and Roche assays investigated. These differences between assays may result in potential misclassification in up to 7% of patients if fixed cutoffs (eg, 1 ng/mL) are applied. Poor correlation was also observed between the Tg antibody assays when the method-specific upper limits of normal are used as cutoffs. Storage of Tg and Tg antibodies was possible for 3 to 4 weeks at –20°C and –80°C. Calibration of the assays, however, was found to be crucial for stable results over time.

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Conclusions: Technical aspects of Tg and Tg antibody assays, including interassay differences, calibration and standardization, and cutoff values, may have a significant clinical impact on the follow-up of DTC patients.

Key Words: differentiated thyroid cancer, method comparison, thyroglobulin, thyroglobulin antibodies, thyroid carcinoma

Thyroid cancer is the most common malignancy of the endocrine system [1]. Differentiated thyroid cancer (DTC), developing from apparently normal thyroid follicular cells, accounts for almost 95% of all thyroid cancers [1]. Treatment of DTC usually consists of surgical hemi- or total thyroidectomy and/or radioactive iodine ablation. Most patients achieve excellent prognosis and a normal life expectancy after treatment [2,3]. Recurrence, however, occurs in approximately 10% to 20% of patients and depends on, for instance, initial tumor size and treatment [2]. To this end, regular follow-up is generally advised for all treated DTC patients to detect recurrences in a timely manner. Measurements of serum thyroglobulin (Tg), a large glycoprotein that has an essential role in thyroid hormone synthesis in the thyroid follicles, are an integral tool in the follow-up [4]. After treatment, the serum Tg concentration ought to be very low or undetectable, as thyroid follicular cells are the only source of Tg. Circulating Tg is thus a marker of persistent or recurrent disease in the follow-up of treated DTC patients.

National and international guidelines attribute an important role to Tg measurements for the determination of response to therapy, which impacts for instance prognostic predictions, intensity and frequency of follow-up, and additional investigations and therapies [5-7]. Typically, fixed cutoff values for Tg are indicated. For example, the 2016 American Thyroid Association guideline describes cutoffs of <0.2 ng/mL and <1.0 ng/mL for thyroid hormone suppressed Tg measurements, and cutoffs of <1.0 ng/mL and <10 ng/mL for thyroid-stimulated hormone–stimulated Tg measurements to describe excellent or indeterminate/biochemical incomplete responses to therapy, respectively [5].

In addition, guidelines advise to accompany each Tg measurement with an assessment of Tg antibodies as they may interfere with both immunometric assays (IMA) and radioimmunoassays (RIA) for Tg [5-8]. These antibodies often develop during the disease course of DTC and are detected in approximately 20% to 30% of DTC patients [9-11]. In addition, quantitative Tg antibody trends may have prognostic value in the follow-up of DTC patients [8,12]. This indicates the necessity of reliable, quantitative Tg antibody measurements to accompany the Tg assays.

Tg and Tg-antibody interassay differences received a lot of attention in the literature, as they complicate the use of these markers in the follow-up of DTC patients [13,14]. For Tg, the assay type used—RIA, IMA, or liquid chromatography-tandem mass spectrometry—significantly impacts the measured concentrations [13]. Tg interassay differences are further increased due to biological heterogeneity of Tg, varying degrees of interference by Tg antibodies, and assay sensitivity and variation in the Tg capture antibodies used in the assays. Similarly, Tg antibody assays show varying results due to differences in assay principle, assay sensitivity, degree of interference by endogenous Tg, and in vivo heterogeneity of Tg antibodies [15,16]. Although this topic has received a lot of attention in a research setting, current guidelines have not fully embraced this knowledge by, for instance, including method specific cutoff values.

An additional factor that could potentially affect measured Tg and Tg antibody levels is the storage stability of these markers. A few studies have been published on this topic more than 10 years ago, which showed that storage may have a significant effect on the Tg and Tg antibody measurement [17-19]. Studies with current generation assays have not been published, however, to the best of our knowledge. In our eyes, this topic deserves more attention since (clinical) studies and current laboratory practice often require measurements at decentral locations and/or batch-wise analysis after storage for several days to months.

The aim of this study was to explore the potential impact of Tg and Tg antibody assay performance on the differential classification of DTC patients, as described in national and international guidelines. Currently available assay generations were included that are very frequently used by laboratories in (university) hospitals to reflect current follow-up of the DTC population. More specifically, the newly released DiaSorin LIAISON® Tg II assay and the corresponding Tg antibody assay, as well as the established B.R.A.H.M.S. Kryptor hTg and anti-Tgn assays, B.R.A.H.M.S. Tg-pluS assay, Roche Cobas Elecsys Tg II and anti-Tg assays, and Phadia EliA anti-Tg assay were investigated in this study.

We discuss the impact of the stringent Tg cutoffs for the follow-up of DTC patients in relation to technical aspects of Tg assays, such as the observed interassay differences and standardization. Next, the clinical role of Tg antibodies as both an indicator for Tg assay interference and as a surrogate tumor marker is considered in relation to Tg antibody assay

characteristics and cutoffs. Finally, the impact of sample storage on Tg and Tg antibody measurements is investigated.

Materials and Methods

Ethical Approval

This study was approved by the Medical research Ethical Committees United (MEC-U) (registration no. W20.121).

Patient Samples

Samples that were included in this study were obtained in the period from 2018 to 2020 in 3 different hospitals in the Netherlands (Maasstad Hospital, Rotterdam; Erasmus Medical Centre, Rotterdam; and UMC Utrecht, Utrecht). Samples were selected based on their Tg and Tg antibody concentrations that had been measured directly after phlebotomy using the routine assays of the respective medical laboratories (see following discussion). Measurements were done in 2 additional hospitals: the IJsselland Hospital and the Spijkenisse Medical Centre (Table 1).

Method Comparisons

The selected samples were taken out of storage (2-15 months at –20°C) and analyzed using at least 1 more assay to compare to the routine assay that had been used for the initial analysis after phlebotomy in the respective laboratory (Table 1). The assays used for analysis of each sample at least comprised the DiaSorin LIAISON® Tg and Tg antibody assays, as this was the newest assay on the market that we included in this study (see following discussion). In some instances, samples were measured using 3 different assays on 3 different locations. Aliquots were always transported at –20°C, and the amount of freeze-thaw cycles were reduced to a minimum.

Stability Experiments

Samples, which had been stored for 3 to 4 weeks at –20 °C or –80 °C, were selected and taken out of storage at the Erasmus Medical Centre. The samples were measured once more using the local Kryptor assays (see following discussion) to compare to the original measurement before storage. Then, the samples were sent to the Maasstad Hospital, where Tg and Tg antibody were measured using the DiaSorin assays (see following discussion) upon arrival of the samples and after an additional storage period. Between 11 and 50 samples were included per experiment. A bias percentage of <20% after storage compared to the measurement before storage was considered acceptable.

LIAISON®Tg II and Anti-Tg Antibody Assays

The LIAISON® Tg II Gen assay (DiaSorin, Italy; RRID:AB_2894916) is a 2-step sandwich chemiluminescence immunoassay using magnetic particles coated with a mixture of mouse monoclonal antibodies and monoclonal antibodies linked to an isoluminol derivative. According to the manufacturer, the presence of Tg antibodies may affect the measured Tg by yielding false-negative results. The LIAISON® anti-Tg assay (DiaSorin, Italy; RRID:AB_2894920) is also a 2-step chemiluminescence immunoassay using magnetic particles coated with Tg and antihuman immunoglobin G antibodies linked to an isoluminol derivative.

Kryptor hTg and Anti-Tgn Assays

The Kryptor hTg sensitive assay (Thermo Fisher Scientific, Germany; RRID:AB_2894918) is a sandwich immunoassay that uses TRACE® technology: nonradiative energy transfer from a conjugated Tg capture antibody (donor) to a conjugated Tg detection antibody (acceptor) when they are in close proximity in an immunocomplex. No claim is made by the manufacturer regarding interference of endogenous Tg antibodies in the product information sheet, but they have indicated an expected interference in approximately 10% of Tg antibody-positive samples [20]. The Kryptor anti-Tgn assay (Thermo Fisher Scientific, Germany; RRID:AB_2894921) is a competitive

Table 1. Overview of the thyroglobulin and thyroglobulin antibody assays used in this study

Phlebotomy location[a]	**Local routine Tg assay**	Local routine Tg antibody assay	Location additional analyses	Tg assay	Tg antibody assay
Maasstad Hospital	LIAISON®	LIAISON®	IJsselland Hospital	Tg-PluS	Phadia EliA
Erasmus Medical Centre	Kryptor	Kryptor	Maasstad Hospital Spijkenisse Medical Centre	LIAISON® Cobas	LIAISON® Cobas
UMC Utrecht	LIAISON®	LIAISON®	Maasstad Hospital	LIAISON®	LIAISON®

Abbreviation: Tg, thyroglobulin.

[a]Location where phlebotomy took place, analysis using the local routine assays was done and samples were stored before transportation to the indicated locations for additional analyses.

immunoassay using similar TRACE® technology between conjugated Tg (donor) and the competing conjugated Tg antibody (acceptor).

Cobas Elecsys Tg II and Anti-Tg Assays

The Cobas Elecsys Tg II assay (Roche Diagnostics, Germany; RRID:AB_2894917) is a sandwich immunoassay using biotinylated and ruthenium-linked Tg antibodies and streptavidin-coated microparticles to capture sandwiched Tg. According to the manufacturer, the presence of Tg antibodies may affect the Tg measurement. The Cobas Elecsys anti-Tg assay (Roche Diagnostics, Germany; RRID:AB_2894922) is a competitive immunoassay using biotinylated Tg, ruthenium-linked Tg antibodies, and streptavidin-coated microparticles to capture and detect Tg antibodies in the sample.

Tg-PluS and Phadia EliA Anti-Tg Antibody Assays

The B.R.A.H.M.S. Tg-PluS assay (Thermo Fisher Scientific, Germany; RRID:AB_2894919) is a 2-step immunoradiometric assay using capture antibodies fixed to the inside of the reaction tube and radioactively labeled detecting antibodies. The assay is standardized against the Reference Standard CRM 457, such that a nanogram in the Tg-PluS assay is equivalent to 2 ng of CRM 457 to ensure consistency with the previous B.R.A.H.M.S. Tg-S assay [21,22]. No claim is made by the manufacturer regarding interference of endogenous Tg antibodies. The Phadia EliA anti-TG assay (Phadia GmbH, Germany; RRID:AB_2894923) is a 2-step fluoroenzymeimmunoassay using human Tg antigen and antihuman immunoglobin G antibodies linked to an enzyme.

Statistical Analysis

All results were analyzed with EP Evaluator software (version 12.0.0.11). Method comparison data were analyzed using Deming regression analysis in the alternate (quantitative) method comparison module. Nonquantitative results obtained from the assays (eg, Tg <0,1 ng/mL or Tg >500 ng/mL) were not included in the statistical analysis. Outliers were removed from the analyses using EP Evaluator outlier detection.

Results

Comparison of Tg Assays

A total of 209 residual patient materials were selected for the method comparisons, which had initially been sent to the laboratory for Tg testing. For each method comparison, the selected samples had a Tg concentration range of at least 0.5 to 45 ng/mL.

Initially, method comparisons were done on the Tg antibody-negative samples, as this is often done in literature to simplify the population (Supplementary Table 2 [23]). Since this does not do justice to the population in which the Tg assay is most commonly used; however, we then decided to include Tg antibody-positive samples in the comparisons (Supplementary Table 3 [23]). Sixteen percent to 24% of the samples was Tg antibody positive as indicated by 1 or both of the corresponding Tg antibody assays. Notably, inclusion of these samples did not result in significant changes in any of the slopes or intercepts of the linear regressions analyses of the Tg method comparisons.

The LIAISON® Tg assay showed good agreement with the Cobas Elecsys and UMCU LIAISON® Tg assays (Fig. 1A). Lower slopes were observed in the comparisons with the Tg-PluS and Kryptor Tg assays.

A total of 8 samples showed discordant results, assuming a fixed Tg cutoff of 1.0 ng/mL (Fig. 1B). This is quantitatively expressed in the table insert in Figure 1B, which indicates the number of discordant samples in relation to the amount of samples per method comparison and the percentage of discordant samples. Two out of the 8 discordant samples, both from the LIAISON®-Cobas comparison, were Tg antibody positive according to both corresponding Tg antibody assays. The other 6 samples were Tg antibody negative according to all assays used.

Comparison of Tg Antibody Assays

A total of 215 residual patient materials was selected for the method comparisons, which had initially been sent to the laboratory for Tg antibody testing (Supplementary Table 4 [23]).

The LIAISON® Tg antibody assay showed good agreement with the Cobas and UMCU LIAISON® Tg antibody assays (Fig. 2A). Poor agreement was observed in the comparisons with the Kryptor and Phadia EliA Tg antibody assays.

The 2 LIAISON® Tg antibody assays showed 100% concordance in this study, meaning that both Tg antibody assays classified each sample either as Tg antibody positive or Tg antibody negative (Fig. 2B). For the other assays, the concordance varied between 80% and 95%, using the upper limits of the reference intervals indicated by the manufacturers as the cutoff values for each assay. For example, in the comparison between the LIAISON® and Kryptor Tg antibody assays, 20 out of 156 samples showed a discordant Tg antibody result (87% concordance). Of these samples, 1 had positive Tg antibodies in the LIAISON® assay; all other samples had a positive result in the Kryptor assay.

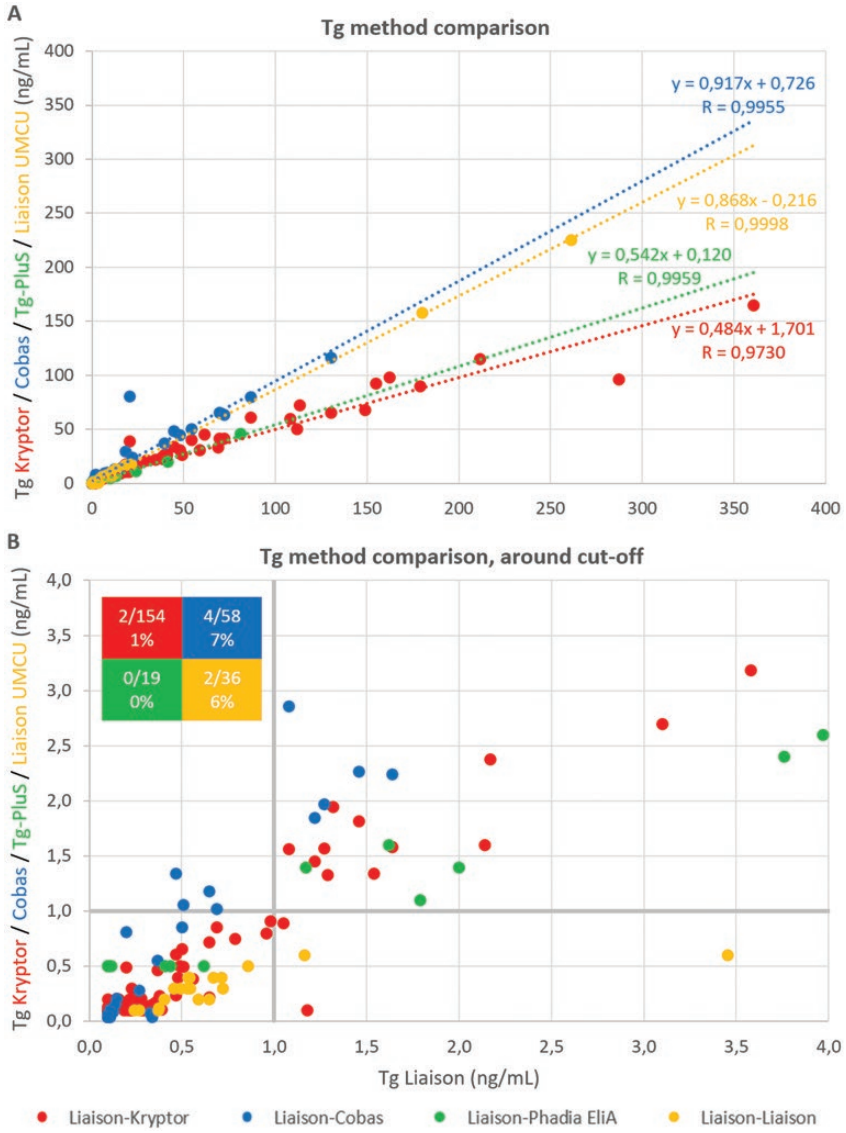

Figure 1. Results of the comparison of 4 Tg assays to the DiaSorin LIAISON® Tg assay at the Maasstad Hospital (A), including a detailed view of the result around the cutoff value of 1 ng/mL (B). The table denotes the amount and percentage of discordant results, based on the cutoff value.

Stability of Tg and Tg Antibody Upon Sample Storage

The effect of sample storage for 3 to 4 weeks at –20°C and –80°C was investigated using the LIAISON® and Kryptor Tg and Tg antibody assays. Measurements before and after storage were compared, and the percent bias was calculated (Supplementary Table 5 [23]). A bias below 20% was considered acceptable, taking into account the between-run coefficients of variation of the assays and the related reference change values.

For Tg, storage at –20°C or –80°C did not result in a significant change in either assay. The lowest bias percentages were observed at –80°C, indicating better stability at lower storage temperatures. For the LIAISON® Tg antibody assay, acceptable bias percentages were found. The Kryptor assay showed good storage stability at –80°C, but after storage at –20°C a bias of –55% was observed. This resulted in 80% of the Tg antibody positive samples becoming Tg antibody negative after storage (Fig. 3). Sometime after these experiments, experience from clinical practice made this laboratory change their recalibration protocol. Instead of recalibrating every 7 days, as suggested by the manufacturer, recalibration every 3 days was found more suitable to maintain a stable Tg antibody Kryptor assay (data not shown). After this change, the stability experiment resulted in a bias of 4% without any storage-related changes in Tg antibody positivity (Fig. 3).

Discussion

Impact of Tg Cutoff Values

Tg interassay differences are a complicating factor in the follow-up of DTC [13,14,24-26]. Even though

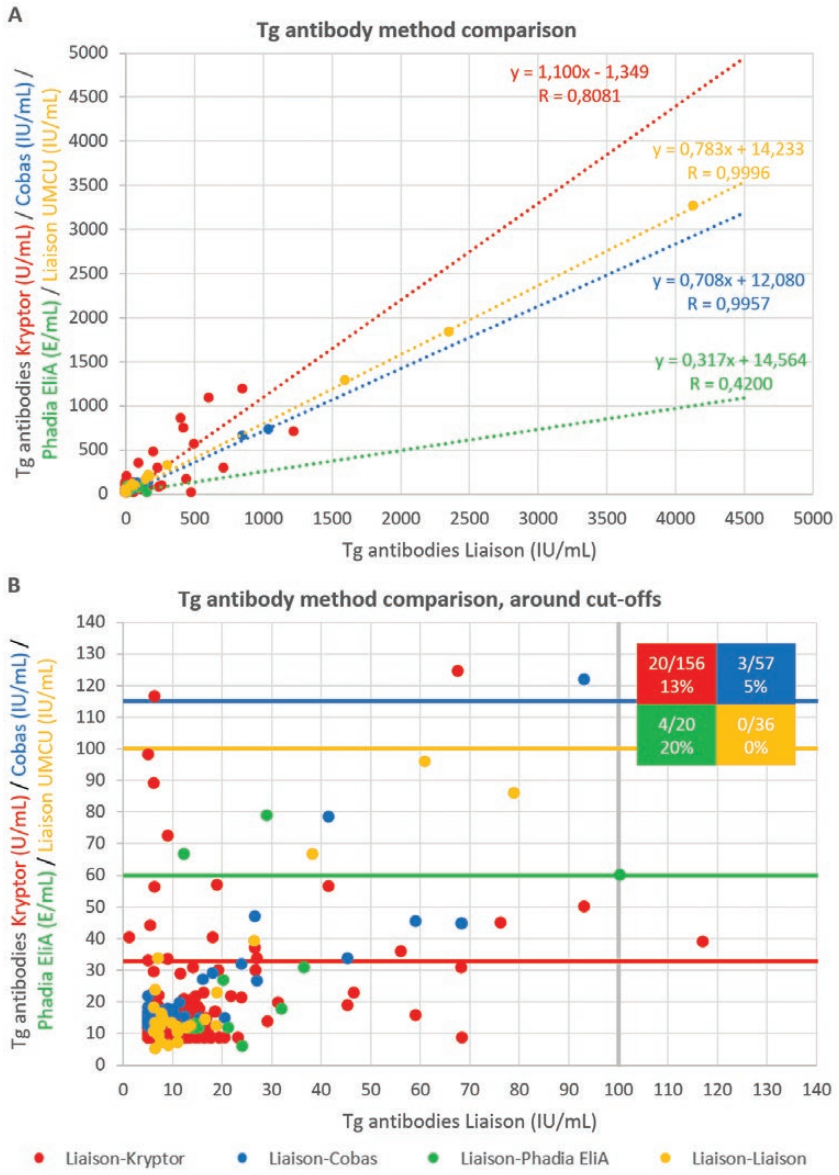

Figure 2. Results of the comparison of 4 Tg antibody assays to the DiaSorin LIAISON® Tg antibody assay at the Maasstad Hospital (A), including a detailed view of the result around the cutoff values of the respective method, as indicated by the product information sheets (B). The table denotes the amount and percentage of discordant results, based on the respective cutoff values.

assays are similarly calibrated in theory, external quality assessment scheme outcomes reveal they are not in practice. This may be due to variations in assay types (RIA, IMA, or liquid chromatography-tandem mass spectrometry), Tg capture antibodies, and in vivo processing and iodination of Tg, leading to biological heterogeneity [27-29].

In the current study, 4 Tg assays were compared that are all standardized against the BCR® 457 standard [21,22]. In this comparison, the B.R.A.H.M.S. assays (Kryptor and Tg-PluS) consistently underreported Tg concentrations by a factor of 2 when compared to the LIAISON® and Cobas Elecsys assays. A possible explanation for this effect was found in the product information sheet of the Tg-PluS assay, which states that this assay is adjusted such that 1 ng is equivalent to 2 ng of BCR® 457. Interestingly, this adjustment was not included in the product information sheet of the Kryptor assay (version R01en, 28-11-2021). Remarkably the difference between the B.R.A.H.M.S. assays on the one hand and the LIAISON® and Cobas Elecsys assays on the other hand is not well reflected by the reference ranges for these assays (Table 2). Although the lower limits of normal of the B.R.A.H.M.S. assays are approximately a factor 2 lower than that of the Cobas Elecsys assay, the lower limit of normal of the LIAISON® assay is the lowest of all.

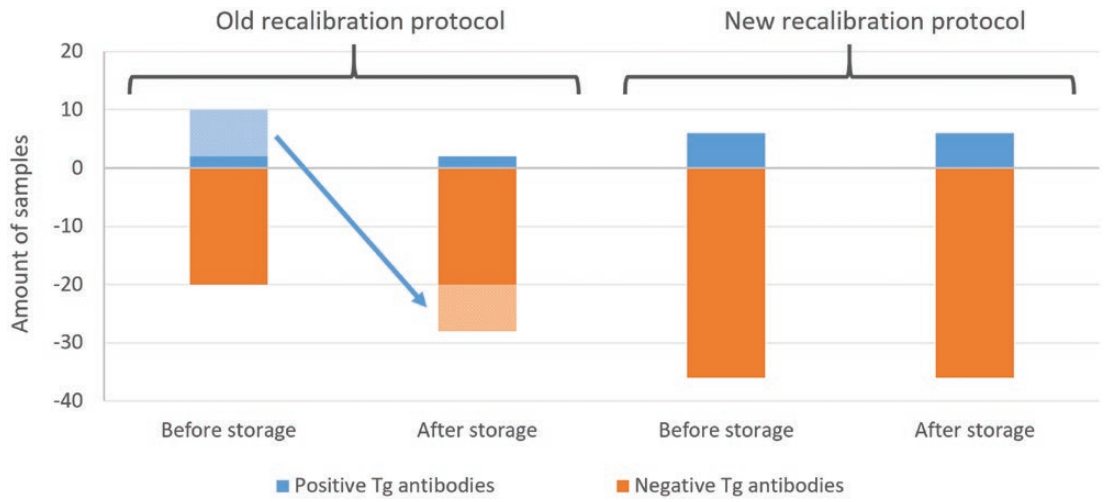

Figure 3. Impact of recalibration protocol on Tg antibody storage stability at –20 °C using the Kryptor assay.

Table 2. Overview of the thyroglobulin and thyroglobulin antibody assays used in this study

Measurand	**Assay**[a]	**Reference range**	Source reference range	Standardization	Functional sensitivity
Tg	LIAISON®	0.9-54 ng/mL	167 euthyroid subjects	CRM 457[21,22]	0.17 ng/mL
	Kryptor	1.6-61.3 ng/mL	207 healthy subjects	CRM 457[21,22]	0.15 ng/mL
	Cobas	3.5-77 ng/mL	478 healthy Caucasian subjects	CRM 457[21,22]	Not given by manufacturer
	Tg-PluS	1.7-35 ng/mL	Unknown	CRM 457[21,22]	0.2 ng/mL
Tg antibody	LIAISON®	5-100 IU/mL	193 healthy subjects	MRC 65/93	10 IU/mL
	Kryptor	≤33 U/mL	206 healthy subjects	MRC 65/93	33 U/mL
	Cobas	≤115 IU/mL	392 healthy subjects	MRC 65/93	Not given by manufacturer
	Phadia EliA	<40 IU/mL = negative >60 IU/mL = positive	Unknown	MRC 65/93	Not given by manufacturer

Abbreviation: Tg, thyroglobulin.
[a]More technical details on these assays are given in Supplementary Table 1 [23].

A number of discordant results were observed in the included patients between different Tg assays, assuming a fixed cutoff value of 1 ng/mL. Following the Dutch guideline for thyroid carcinoma as an example, a difference in Tg >1 ng/mL vs <1 ng/mL may result in additional diagnostic or therapeutic ^{131}I scintigraphy, instead of annual follow-up with a Tg measurement [7].

The discordant results cannot be explained by Tg antibody interference, as only a small part of the discordant samples were found to be Tg antibody positive. The overall trends in Tg assay method differences, as previously described, also did not correlate well with the differences in this low measurement range. For example, 4 discordant results were found in the comparison of the LIAISON® and Cobas Elecsys assays, while their overall correlation was the best of all comparisons. This illustrates how regressions are very much influenced by higher titer samples and that these may not necessarily represent correlation of a low titer cutoff. Furthermore, higher coefficients of variations are generally expected at lower concentrations.

Tg Antibodies: Interference and Surrogate Tumor Marker

Next to Tg, Tg antibodies play a very important role in the follow-up of treated DTC patients. Historically, Tg antibodies were measured either in the context of thyroid autoimmunity or as to confirm the absence of interference in Tg measurement [10,11,30-34]. Today, the role of the Tg antibody measurement is evolving to an independent risk factor for DTC recurrence due to 2 recent insights. First, the Tg antibody titer has been shown to be sensitive to changes in the mass of Tg-secreting thyroid tissue. Second, the Tg antibody epitope reactivity has been found to predict evolution of disease [35]. Therefore, antibody positivity in itself is recognized by the American Thyroid Association guideline

as an independent risk factor for biochemical incomplete response [5].

Similarly to Tg, measured Tg antibody titers are highly dependent on the assay used, even though commercially available Tg antibody assays all claim standardization against the International Reference Preparation 65/93 [15]. This poor assay correlation is most certainly partly due to a variety of external factors such as heterogeneity of Tg antibodies and their affinity for the Tg used in the assay [16].

In this current study, 4 Tg antibody assays were compared, which are all standardized against the International Reference Preparation 65/93 standard. As was expected, however, significant differences between the assays were observed. In total, 27 (10%) of 269 compared samples were discordant when using the assay-specific upper limits of normal (ULN) as defined by the manufacturers in the product information sheets.

Despite the fact that differences of up to a factor of 100 between different Tg antibody assays have been reported in the literature [15], 1 particular study showed very good correlation between the assays included in our current study when trying to establish upper reference limits for Tg antibody assays [36]. According to the latter study, the ULN for the 4 assays tested in our current study should not differ as much as they do, based on the information provided by the manufacturers (Table 1). For example, the Kryptor assay has an ULN is 33 U/mL; for the LIAISON® assay, it is 100 IU/mL, while the study by D'Aurizio et al could justify similar cutoffs [36]. If we would apply a cutoff of 100 IU/mL to both assays, this would result in 2,5% discordance instead of 13% discordance. In our eyes, this example illustrates the importance of the Tg antibody cutoff values supplied by manufacturers and applied by laboratories.

Stability of Tg and Tg Antibody Upon Sample Storage

The effect of sample storage on measured Tg and Tg antibody titers is a highly underexposed topic of discussion in our eyes. To our best of knowledge, only a few studies have been published on this topic, which are now over 10 years old and thus cover old generation assays [17-19]. Not only for long-term follow-up and clinical studies but also for the daily practice on the laboratory, it is important to know the effect of sample storage at for instance –20°C or –80°C.

The available literature has shown that storage at –20°C for even a couple of weeks can impact the Tg and Tg antibody result significantly, mostly focusing on Roche assays [17-19]. Furthermore, the available data suggest that storage stability is dependent on the type of assay used, which may be expected as each assay has a different design and uses different techniques and antibodies to capture Tg or Tg antibodies.

In our study, we evaluated the effect of sample storage at –20°C and –80°C using the LIAISON® and the Kryptor Tg and Tg antibody assays. In almost all experiments, frozen storage for 3 to 4 weeks did not result in differences of >20% in Tg or Tg antibody titers. An exception was the impact of storage at –20°C on Tg antibodies measured by the Kryptor assay. This appeared to be due to the calibration of the Kryptor Tg antibody assay, which was less stable than expected. According to the manufacturer, readjustment of the assay should be performed every 7 days. However, the negative drift associated with this long period resulted in significantly lower measurements in patients with high antibody titers, resulting in reclassification of 80% of Tg antibody samples to the Tg antibody-negative group. This drift was largely nullified by using a stricter recalibration scheme. Although this phenomenon was only observed and adjusted in 1 laboratory in our study, other laboratories using the same Tg antibody assay may run into similar findings and may benefit from stricter calibration.

Conclusion

In conclusion, we have illustrated that significant differences between new and commonly used Tg and Tg antibody assays exist, despite efforts to standardize the assays. For the included Tg assays in this study, the assay differences could have resulted in misclassification in up to 7% of patients if fixed cutoffs (eg, 1 ng/mL) are applied. Tg assay standardization may have been a major contributing factor to the observed differences, although this was not always reported by the manufacturer. For the Tg antibody assays, the importance of appropriate cutoffs has been discussed to determine Tg antibody positivity. Furthermore, we have shown the impact of frozen storage on Tg and Tg antibody assays. Because we incorporated assays that are commonly used in university or large teaching hospitals, all of these issues can have a significant impact on the clinical follow-up of individual DTC patients. It should thus be mandatory to consistently use the same Tg and Tg antibody assays in the longitudinal follow-up of thyroid patients. Whenever this is not possible, it is crucial to rebaseline patients by running the new and old assays simultaneously for a period of at least 6 months.

An important aspect that we want to comment on is the use of fixed, method-independent cutoff values in guidelines. This is not only an issue for DTC follow-up, unfortunately, but is a recurring phenomenon in guidelines. We hope this study raised awareness on how such cutoffs unnecessarily complicate the use and interpretation of laboratory tests and how results should therefore be interpreted with wisdom. If cutoff

values should be included in guidelines, we strongly advise to take interassay variability into consideration and to provide the origin and source for the chosen cutoff value. Furthermore, we believe method-independent cutoff values should not be used unless assays are properly harmonized. Second, standardization of Tg and Tg antibody assays has been a topic of discussion for several years. Although standardization may not diminish all interassay variation, the use of suitable and stable calibrators is the least that can be done from a laboratory perspective to take a step in the right direction.

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Author Contributions: L.S., M.N., H.T., S.B., and S.K. designed and performed the experiments, analyzed and interpreted the results and wrote the manuscript. C.K., C.N., W.E.V., J.G., L.B., and E.L. provided input for the manuscript.

Additional Information

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Disclosures: DiaSorin provided testing kits with which all LIAISON® Tg and Tg antibody assays were performed.

Data Availability: Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding authors on reasonable request.

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