Diagnostic performance of a new highly sensitive thyroglobulin immunoassay

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Abstract

The determination of serum thyroglobulin (Tg) is commonly used for detecting the presence of residual thyroid tissue or cancer recurrence in patients treated for differentiated thyroid cancer (DTC). The aim of the study was to evaluate the performance characteristics of a recently introduced fully automated chemiluminescent immunoassay, based on four monoclonal antibodies and which produces results in 40 min. Analytical sensitivity (0.01 µg/l) was computed from 20 replicates of the zero calibrator and of the 'Tg-free' sample pool. Functional sensitivity (0.1 µg/l at 20 coefficients of variation percent) was determined from the imprecision profile obtained by assaying ten serum pools. The reliability of the measurements in the low concentration range (Tg < 1 µg/l) has been checked by progressive dilution with the 'Tg-free' serum of a sample

pool at 5.27 µg/l; measured values were very close to the expected values (recovery 100–133%).

Cut-off at the 99th percentile in DTC stage I 'disease-free' treated patients ($n=53$) was 0.16 µg/l. Tg measurement in basal conditions during L-thyroxine suppression therapy and 5 days after recombinant human TSH stimulation was performed in 22 patients with DTC. In 80% of patients with basal Tg < 0.1 µg/l (12/15), Tg remained < 0.1 µg/l after stimulation, and in all of these Tg was < 1 µg/l.

Our results have indicated the optimal analytical and clinical performance of this Tg immunoassay and encourage further studies on larger populations of patients with DTC.

Journal of Endocrinology (2004) **182**, 287–294

Introduction

Thyroglobulin (Tg) is a large glycoprotein (molecular weight = 660 000) stored in the follicular colloid of the thyroid gland. It acts as a prohormone in the intra-thyroid synthesis of thyroxine (T4) and triiodothyronine (T3) and it is produced only by normal thyrocytes or well-differentiated thyroid cancer cells. Since the thyroid is the only source of Tg, a rise in Tg levels in patients after total thyroidectomy and radioiodine ablation of residual thyroid tissue indicates the recurrence of differentiated thyroid cancer (DTC). In this context, a strong correlation between serum Tg levels and the amount of differentiated thyroid tissue has been reported (Ozata *et al.* 1994, Duren *et al.* 1999, Pacini & Pinchera 1999, Girelli & De Vido 2000). It is recognized worldwide that the measurement of circulating Tg represents a fundamental tool in the management and follow-up of patients affected by DTC (Schlumberger 1998, Mazzaferri 1999). In addition, in a very recent Tg consensus conference (Mazzaferri *et al.* 2003), a surveillance guideline has been extended to the use of thyrotrophin (TSH)-stimulated Tg levels for

thyroidectomized patients without clinical evidence of residual tumour with Tg values below 1 µg/l during TSH suppression. The detection limit of the methods is critical to detect small amounts of Tg and to observe a change in Tg concentration. It has been proposed that only methods providing the greatest distinction between the lower limit of the euthyroid reference range and the functional sensitivity limit of the assay offer the best clinical sensitivity for detecting small amounts of thyroid tissue in the TSH-suppressed state (Spencer *et al.* 1996). On the other hand, it is noteworthy that, despite the substantial clinical use of Tg measurement, wide differences still remain between results produced in different laboratories because of poor standardization, the heterogeneity of circulating Tg, interference from anti-Tg autoantibodies (AbTg), differences in the epitope recognition by the different antibodies used in the different assays (Zucchelli *et al.* 1996) and interference from heterophile antibodies.

For these reasons, highly sensitive, precise and accurate Tg assays are needed in the follow-up of patients with DTC even if this goal is technically challenging

Figure 1 Schematic description of the Tg Access immunoassay: a simultaneous one-step immunoenzymatic (sandwich) assay. When the substrate, Lumi-Phos 530 (Lumigen Inc., Southfield, MI, USA), is added the reaction generates light which is measured with a luminometer and is directly proportional to the concentration of Tg in the sample. MAb, monoclonal antibodies; ALP, alkaline phosphatase.

(Spencer *et al.* 1996, 1997, Clark & Beckett 2002, Demers & Spencer 2002). In the last 30 years, the analytical sensitivity of Tg measurement has been greatly improved, being about 5 µg/l with the first traditional radioimmunoassays (Van Herle *et al.* 1973) and 0.01–0.02 µg/l with the recently developed immunoassay based on enzyme (Wunderlich *et al.* 2001, Zophel *et al.* 2003) or chemiluminescent tracer (Pressner *et al.* 2003). Thus, some methods can now achieve very good analytical and functional sensitivity giving reliable results in the very low concentration range. In addition, with the advent of fully automatized assays, such as the technique recently developed by Beckman Coulter on the Access analyzer, results are readily available to the clinician in less than 1 h while patients are still in the hospital.

In the present study, the analytical and clinical performance of the Tg Beckman Coulter assay on the Access analyzer were evaluated and compared with other known and substantially used immunoassays.

Materials and Methods

Assay method

The Tg Access immunoassay (Tg Access) (Beckman Coulter, Brea, CA, USA) is a chemiluminescent fully automated technique where samples can be processed at any time in a 'random access' format. The serum sample reacts with a biotinylated mixture of four monoclonal anti-Tg antibodies, streptavidin-coated paramagnetic

particles and monoclonal anti-Tg antibody conjugated with alkaline phosphatase. The biotinylated antibodies and Tg in the serum bind to the solid phase, while the conjugated antibody reacts with a different antigenic site on the Tg molecule. After incubation and washing to remove material not bound to the solid phase, immobilized Tg is detected by the addition of a chemiluminescent enzyme substrate (see Fig. 1). The light generated is measured as relative light units (RLU) with a luminometer. Tg concentration is determined by a stored multi-point response curve calibrated to CRM 457 international reference preparation using a smoothing spline mathematical model (Feldt-Rasmussen & Schlumberger 1988). The time to the first result is about 40 min; sustained throughput is 100 tests/h.

The assay system was calibrated according to the manufacturer's instructions every 56 days throughout the course of the study. The calibration curve ranged from 0 to 480 µg/l; in the range 0–1 µg/l, the analytical response (RLU) showed a good improvement from approximately 9000 RLU (standard at 0 µg/l) to approximately 45 000 RLU (standard at 0.93 µg/l), namely a ratio of 5, as reported in Fig. 2, thus indicating that the assay achieves good precision in the low concentration range.

In this study, patients with positive AbTg, measured using a two-step immunoenzymometric assay commercial kit performed on an AIA-21 analyser (Tosoh Bioscience, Tokyo, Japan), were excluded to rule out possible interference in the Tg assay; the minimal detectable

Figure 2 Representation of the first part of the calibration curves (first three points) of the Tg Access and Tg Pasteur assays. To compare the calibration curves of the two methods the ratio of the response/response at 0 $\mu\text{g/l}$ is shown on the ordinate.

concentration of AbTg was estimated to be 12 IU/ml, samples with AbTg < 12 IU/ml were considered negative.

Analytical performance

Detection limit The minimum detectable concentration (namely the detection limit or the analytical sensitivity), defined as the Tg value corresponding to a signal 3 S.D. above the mean of 20 replicates of the zero calibrator (0 $\mu\text{g/l}$ Tg; Beckman) and of a Tg-free serum from thyroidectomized patients, was determined.

Imprecision profile Ten serum pools, with different Tg concentration values, were prepared and stored at -20°C until assayed. Each pool was periodically assayed from October 2003 to May 2004 (6 months) according to the National Academy of Clinical Biochemistry (NACB) guidelines to estimate the functional sensitivity (Demers & Spencer 2002); different calibrations and different reagent lots were included and the imprecision profile (between-assay imprecision) was calculated. Functional sensitivity was determined by interpolating the imprecision profile of Tg concentration assayed with a coefficient of variation (C.V.) of 20%.

Within-assay imprecision Four serum samples were assayed in five replicates in the same run and the relative C.V. values were calculated (within-assay imprecision).

AbTg interference Five serum samples from subjects without AbTg and containing a measurable concentration

of Tg (range from 3.10 to 12.6 $\mu\text{g/l}$, mean 6.98 $\mu\text{g/l}$) were diluted with serum from a thyroidectomized and radioiodine-ablated patient (as a treatment for papillary carcinoma, stage I) who had been disease free for more than 10 years, affected by Hashimoto's thyroiditis, with Tg Access < 0.01 $\mu\text{g/l}$, but with slightly positive AbTg values (about 150 IU/ml); another five samples without AbTg and containing a measurable concentration of Tg (range from 3.94 to 23.8 $\mu\text{g/l}$, mean 12.3 $\mu\text{g/l}$) were diluted with a serum pool containing AbTg (about 300 IU/ml) and Tg < 1 $\mu\text{g/l}$. The ten diluted sera, with a final AbTg concentration of 110 IU/ml (first group) and 220 IU/ml (second group), were assayed to check for interference by AbTg in the Tg Access assay.

Dilution test The reliability of Tg measurements in the low concentration range (< 1 $\mu\text{g/l}$) was tested by progressively diluting a sample pool at 5.27 $\mu\text{g/l}$ with Tg-free serum obtained from thyroidectomized and radioiodine-ablated DTC, stage 1, disease-free patients.

Normal range Tg concentrations were measured in the sera of 120 healthy AbTg-negative euthyroid individuals (mean age 22 ± 8 years, 69 males and 51 females; TSH range 0.4–3.9 $\mu\text{U/ml}$, mean 1.67 $\mu\text{U/ml}$; free T3 range 2.1–3.2 pg/ml, mean 2.35 pg/ml; free T4 range 7.1–18.0 pg/ml, mean 12.0 pg/ml), without a personal or family history of thyroid disease and with a normal thyroid pattern at ultrasonographic examination.

Heterophile antibody interference Interference by heterophile antibodies was checked by using heterophile-blocking tubes (HTB; Scantibodies, Santee, CA, USA) according to the procedure suggested recently (Pressner *et al.* 2003) and Tg measurements were repeated after incubating 500 μl of each serum at room temperature for 1 h.

Method comparison Tg Access measurements were compared with a traditional widely used immunoradiometric assay (IRMA) produced by Pasteur (Gif-Sur-Yvette Cedex, France) based on a mixture of four monoclonal anti-Tg antibodies such as were used in the Tg Access assay.

Clinical performance

To estimate the upper reference limit (cut-off) for DTC recurrence, 53 patients (47 women and six men aged 53 ± 14 years (means $\pm$ S.D.)) were enrolled in the study. All of these patients had undergone total thyroidectomy and radioiodine ablation for primary treatment of stage I DTC, they had been 'disease-free' for more than 10 years and underwent routine follow-up at the time of the study.

Stimulation with recombinant human TSH (rhTSH) was performed for routine follow-up in 22 consecutive unselected treated patients with DTC, and Tg was measured in basal conditions during suppressive therapy and 5 days after rhTSH.

This study was approved by our Institutional Ethics review Committee. Informed consent was obtained from all patients.

Statistical analysis

Continuous variables are expressed as means $\pm$ S.D., a *P* value less than 0.05 was considered statistically significant.

Deming regression was used to calculate analytical correlation between different methods; agreement between methods was assessed by the Bland and Altman plot (Cornbleet & Gochman 1979, Bland & Altman 1986, 1999).

Results

Analytical performance

Detection limit The detection limit was found to be 0.007 $\mu\text{g/l}$ using the zero standard (0 $\mu\text{g/l}$ Tg; Beckman) and 0.012 $\mu\text{g/l}$ using Tg-free serum and therefore a detection limit of 0.01 $\mu\text{g/l}$ was assumed. For comparison, the detection limit calculated for the Pasteur IRMA using Tg-free serum was 0.54 $\mu\text{g/l}$ (declared 0.2 $\mu\text{g/l}$).

Imprecision profile The imprecision profile (C.V. values) performed using ten different serum pools was as follows: 26.8% at 0.061 $\mu\text{g/l}$, 14.4% at 0.14 $\mu\text{g/l}$, 13.8% at 0.31 $\mu\text{g/l}$, 11.3% at 0.65 $\mu\text{g/l}$, 9.6% at 0.93 $\mu\text{g/l}$, 7.6% at 3.1 $\mu\text{g/l}$, 6.7% at 9.6 $\mu\text{g/l}$, 4.3% at 36.2 $\mu\text{g/l}$, 4.3% at 64.8 $\mu\text{g/l}$, 4.3% at 168 $\mu\text{g/l}$; functional sensitivity was 0.1 $\mu\text{g/l}$ at 20% C.V.

Within-assay imprecision The C.V. values were 2.7% at 0.18 $\mu\text{g/l}$, 1.3% at 0.37 $\mu\text{g/l}$, 1.5% at 0.56 $\mu\text{g/l}$ and 2.0% at 0.75 $\mu\text{g/l}$.

AbTg interference Mean recovery, estimated after adding sera containing AbTg to sera with Tg, was $63.8 \pm 16\%$ (observed values versus expected values), thus indicating that the Tg Access assay, like other methods, was affected by interference from AbTg (Erali *et al.* 1996).

Dilution test Tg concentrations measured in samples progressively diluted with Tg-free serum were found to be very close to the expected values (recovery 100–133%) in the low concentration range and showed very good linearity (Fig. 3). The dilution test was also performed with the Tg Pasteur assay where only the first five diluted samples showed values above its detection limit (0.2 $\mu\text{g/l}$) and the bias between observed and expected values was very high.

Normal range In normal subjects, Tg ranged from 2.55 to 64.3 $\mu\text{g/l}$, with a median of 11.16 $\mu\text{g/l}$ and a 95th percentile concentration of 46.9 $\mu\text{g/l}$. This result is in agreement with the suggested reference interval (NACB guidelines approximately 3–40 $\mu\text{g/l}$; Demers & Spencer 2002).

Method comparison

Comparison between the Access assay and the Pasteur IRMA was carried out on 64 samples and Deming regression analysis gave: Tg Access = $-1.1 + 0.61$ Tg Pasteur, $r = 0.99$, $P < 0.001$ (Fig. 4).

Clinical performance

The cut-off at the 99th percentile of 53 long-term 'disease-free' patients previously treated for stage I DTC during suppressive L-T4 therapy was 0.16 $\mu\text{g/l}$; five patients had Tg values below the assay detection limit (< 0.01 $\mu\text{g/l}$), whereas 48 patients showed a measurable Tg concentration up to 0.19 $\mu\text{g/l}$ (Fig. 5).

Tg measurements before (i.e. during suppressive therapy) and after (5 days) of rhTSH stimulation was performed in 22 sera obtaining different patterns of response. The main clinical and serological data are reported in Table 1. In 68% of patients (15/22), basal Tg values were < 0.1 $\mu\text{g/l}$; in 80% of these patients (12/15) Tg remained < 0.1 $\mu\text{g/l}$ also after rhTSH stimulation and only 1/12 had a positive whole body scan (WBS), while in 20% (3/15) Tg levels showed an increment but were always < 1 $\mu\text{g/l}$ with 1/3 having a positive WBS. In the other 32% of patients (7/22), Tg levels were > 0.1 $\mu\text{g/l}$ also in basal conditions; after rhTSH stimulation Tg was always detectable but there were two different patterns of response: 57% showed an increment of Tg values (4/7), with a positive WBS in 1/4, while 43% showed a suspicious lack of response (3/7).

Interference by heterophile antibodies was tested in patients 77/F, 57/F and 46/M shown in Table 1. These three patients had detectable basal Tg (0.22, 0.79 and 2.77 $\mu\text{g/l}$ respectively) but showed a lack of rhTSH-stimulated response (0.20, 0.66 and 2.61 $\mu\text{g/l}$ respectively). In patient 77/F, Tg levels were also confirmed after the incubation of sera in HBT tubes (basal Tg 0.21 $\mu\text{g/l}$ and after rhTSH 0.28 $\mu\text{g/l}$). In the other two patients, Tg levels dropped to 0.01 $\mu\text{g/l}$ after incubation in HBT tubes, thus indicating a substantial interference by heterophile antibodies in the Tg Access assay. For comparison, further experiments in three unselected sera (Tg concentration of 0.71, 4.12 and 10.7 $\mu\text{g/l}$) did not show any interference after incubation in HBT tubes (Tg values of 0.59, 4.14 and 11.9 $\mu\text{g/l}$ respectively).

Discussion

Tg measurement is considered the best biochemical marker worldwide for detecting persistent or recurrent

Figure 3 Dilution test for Tg Access (●) and Tg Pasteur (□) performed by progressively diluting the same sample pool at 5.27 µg/l (Access) and 8.69 µg/l (Pasteur) with Tg-free AbTg-free serum obtained from thyroidectomized and radio-ablated patients for DTC stage 1 treatment (see Materials and Methods). The dashed lines indicate linear regression.

diseases in the follow-up of patients affected by DTC (Kendall-Taylor 2003); the fundamental role of this assay is indirectly reinforced by the data from several recent

Figure 4 Deming regression between Tg Access and Pasteur. Solid line, regression; dashed line, identity. (Directly represented by the freeware software Method Validator by Philippe Marquis). Unweighted Deming regression, $n=64$. Slope, 0.612 (0.532–0.692). Intercept, -1.085 (-2.601 – 0.432).

Figure 5 Distribution of 53 patients who had undergone total thyroidectomy and radioiodine ablation for primary treatment of stage I DTC and who had been disease free for more than 10 years; five patients had Tg values below the assay detection limit (0.01 µg/l) whereas 48 patients showed a Tg concentration up to 0.19 µg/l. Functional sensitivity and cut-off at the 99th percentile are indicated.

Table 1 Main clinical data and Tg measurements before and after 5 days of rhTSH in patients with DTC

		First diagnosis and treatment	TNM UICC '02*	Histology†	Tg Access basal‡ (µg/l)	Tg Access after rhTSH‡ (µg/l)	US TB	US ex TB	WBS TB§	WBS ex TB§
Patients (age/sex)										
46/F	1998		T1, N0, M0	Pap	<0.01 (<0.2)	<0.01 (<0.2)	Neg	Neg	Pos	Neg
39/F	1998		T3, N1, Mx-S1	Pap	<0.01 (<0.2)	0.01 (<0.2)	Neg	Neg	Neg	Neg
51/F	1993		T1, N0-S1	Pap	<0.01 (0.2)	<0.01 (0.2)	Neg	Neg	Neg	Neg
62/F	1999		T1, N0, Mx-S1	Pap	0.01 (<0.2)	0.01 (<0.2)	Neg	Neg	Neg	Neg
38/F	1998		T1, N0, Mx-S1	Pap, var foll	0.03 (<0.2)	0.03 (0.1)	Neg	Neg	Neg	Neg
45/F	1997		T1, N0, Mx-S1	Pap	0.04 (<0.2)	0.01 (<0.2)	Neg	Neg	Neg	Neg
49/M	2000		T1, N0, Mx-S1	Pap	0.04 (<0.2)	0.06 (<0.2)	Neg	Neg	Neg	Neg
70/F	1995		T1, N0-S1	Pap	0.04 (<0.2)	0.05 (<0.2)	Neg	Neg	Neg	Neg
73/F	1991		T1, N0, M0	Pap	0.04 (<0.2)	0.05 (<0.2)	Neg	Neg	Neg	Neg
30/F	1996		T4a, N1a, Mx-S1	Pap	0.04 (<0.2)	0.09 (<0.2)	Neg	Neg	Neg	Neg
50/M	2001		T2, N0, M0	Pap, var foll	0.04 (<0.2)	0.05 (<0.2)	Neg	Neg	Neg	Fp
48/M	1995		T1, N1, M0	Pap	0.05 (0.3)	0.38 (<0.2)	Neg	Neg	Neg	Neg
47/M	2001		T1m, N0, M0	Foll	0.07 (<0.2)	0.22 (<0.2)	Neg	Neg	Pos	Neg
32/M	1998		T1, N0, Mx-S1	Pap	0.08 (<0.2)	0.68 (<0.2)	Neg	Neg	Neg	Neg
29/F	2001		T3, N0, Mx-S1	Pap, hc	0.09 (<0.2)	0.04 (<0.2)	Neg	Neg	Neg	Neg
77/F	2001		T1, Nx, Mx-S1	Pap	0.22 (<0.2)	0.02 (0.2)	Neg	Neg	Neg	Neg
60/F	2000		T1, N1a, Mx-SIII	Pap	0.28 (<0.2)	2.27 (0.8)	Neg	Neg	Neg	Neg
53/F	1995		T1, N0, M0	Pap	0.31 (0.3)	0.57 (0.6)	Neg	Neg	Pos	Neg
50/F	1998		T1, N0, M0	Pap	0.47 (<0.2)	0.81 (0.5)	Neg	Neg	Neg	Neg
57/F	1994		T2m, N0, M0-S1	Pap	0.79 (<0.2)	0.66 (<0.2)	Neg	Neg	Neg	Neg
46/M	2001		T2, Nx, Mx-S1	Pap, var foll	2.77 (0.3)	2.61 (<0.2)	Neg	Neg	Neg	Neg
61/F	1997		T2, N1, Mx-SIII	Ins	99.2 (66.8)	334.8 (222.8)	Neg	Pos	Neg	Neg

*TNM UICC '02: tumour classification according to the International Union Against Cancer.

†Histology: Pap, papillary; var foll, variant follicular; Ins, poorly differentiated 'insular' follicular carcinoma; hc, papillary variant high cells.

‡Tg values measured by Pasteur IRMA are shown in parentheses.

|| US TB: negative (Neg) or positive (Pos) ultrasonography for thyroid remnants in the thyroid bed; US ex TB, for cervical lymph nodes.

§WBS TB: Neg or Pos pathological ¹³¹I uptake in the thyroid bed; WBS ex TB, in the extra-thyroid bed (Fp, false positive).

studies (Hardy *et al.* 1995, Schlumberger & Baudin 1998, Teppo & Hakulinen 1998, Vanderpump *et al.* 1998, Spencer *et al.* 1999, Kumar *et al.* 2001, Torens & Burch 2001).

Theoretically, an ideal biomarker should be highly sensitive and specific and should be able to be reproduced across different clinical laboratories. The inherent error in the measurement itself (C.V.) should be sufficiently low that small changes in the level of the biomarker reflect true changes in the clinical status of the patient. Finally, the assay should be relatively easy to perform and analyze so that the information is readily available to the clinician.

Tg assay performed with the Access analyzer as evaluated in our laboratory appears to satisfy most of these characteristics, providing analytical and clinical performance comparable with the most commonly used methods. This method permits a lower detection limit (0.01 µg/l), a lower functional sensitivity (0.1 µg/l) and a lower cut-off for patients with DTC (0.16 µg/l); the latter, in particular, was sixfold lower than that reported in the latest guidelines (Mazzaferri 1999, Mazzaferri *et al.* 2003). These results are even better than those found recently (Giovannella & Ceriani 2002). In addition, Tg Access is fast (40 min), feasible, simple, technically undemanding and potentially

accessible to all medical centres contrary to the previous radioactive (IRMA) methods. The clinical importance of a reliable and accurate Tg determination is even more emphasized by the recent use of Tg measurements after rhTSH stimulation. Actually, serum Tg measured during TSH stimulation (endogenous TSH or rhTSH) is more sensitive than the basal Tg value obtained during L-T4 treatment (Haugen *et al.* 1999); using this approach the presence of cancer is identified before diagnostic ¹³¹I WBS can localize the tumour (Cailleux *et al.* 2000, Mazzaferri & Kloos 2000, 2002, Pacini *et al.* 2001, 2002, Haugen *et al.* 2002, Pellegriti *et al.* 2003). Interesting clinical conclusions can be drawn from the results of our pilot study on the use of Tg Access measurements during suppressive therapy and after rhTSH stimulation. In all patients with Tg<0.1 µg/l during suppressive L-T4 therapy, Tg after rhTSH remained<1 µg/l or, in 80% of these patients, Tg levels remained<0.1 µg/l after the rhTSH. These data indicated that the Tg Access assay could also be useful in the very low concentration range (<0.1 µg/l) during suppressive L-T4 treatment, being able to discriminate low-risk patients. These preliminary findings show the high sensitivity of Tg measurements in the follow-up of low-risk patients with DTC, not only after rhTSH

stimulation but also during suppressive therapy. The Tg Access assay therefore seems to give very good clinical sensitivity (no false-negative patients) and specificity (no false-positive patients). This could be useful for choosing better and less aggressive protocols. At the moment, the commercially used Tg assays are not able to discriminate small differences in the low concentration range, Tg results below 1 µg/l are considered to be in the 'grey' zone. Tg Access measurement could then be useful in the clinical assessment of patients to be submitted to ¹³¹I WBS; in fact the usefulness of WBS after rhTSH when Tg values remain below 1 µg/l, now considered to be the gold standard for sensitivity (Torlontano *et al.* 2003, Koh *et al.* 2003), is still controversial.

Our results on AbTg interference, even though obtained in a limited number of patients, demonstrated that Tg determination was affected by the presence of AbTg using the Tg Access method. For this reason, in all samples where Tg concentration was measured in our laboratory, we also measured AbTg concentrations and when AbTg was positive we considered the Tg value to be not reliable. Besides the interference by AbTg which produced false-negative results, the assay was also interfered with by heterophile antibodies which produced false-positive results, in agreement with a previous report on Tg Access assay performance (Pressner *et al.* 2003). However, we wish to point out that the problem of antibody interference in Tg measurement is still a problem which affects any commercially available method (Demers & Spencer 2002).

In conclusion, Access Tg measurement could represent an improvement in terms of analytical sensitivity and precision when compared with the most widely employed assays. Moreover, the very good practicality of this test makes its routine application even easier. Further retrospective and prospective studies in a large series of patients treated for DTC will indicate its real usefulness in clinical practice.

Acknowledgements

We would like to thank Mrs Laura Mazza for her skilful secretarial assistance.

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Received in final form 17 May 2004

Accepted 18 May 2004