



## Lessons from a review of thyroglobulin assays in the management of thyroid cancer

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### Abstract

Thyroglobulin (Tg) measurement has become increasingly an important and integral part of the follow up and management of patients with differentiated thyroid cancer. Clinicians predominantly rely on Tg for decision-making for surveillance of patients with differentiated thyroid cancer, but despite this new reliance, issues regarding Tg measurement have not been appropriately addressed especially within a local context. In the process of developing an institutional protocol we have identified that there are significant clinical and technical issues regarding Tg measurement, and surprisingly Tg assessment is currently not part of an external quality control programme. We conducted a small pilot study to specifically emphasize some of the assay issues. We aim to inform endocrinologists, pathologists and nuclear medicine physicians, the need and urgency for these issues to be addressed to improve the ongoing surveillance of differentiated thyroid cancer.

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Serum thyroglobulin (Tg) measurements are becoming an increasingly integral part of the ongoing management and follow up of patients with thyroid carcinoma.<sup>1-4</sup> Tg is a large glycoprotein (molecular weight 660 000 kDa) that acts as a prohormone in the intrathyroidal synthesis of thyroxine (T4) and triiodothyronine (T3). It is produced by normal thyroid tissue and also exists in well-differentiated epithelial thyroid cancer (DTC) cells.

Thyroid carcinoma accounts for approximately 1% of all malignancies and is the commonest endocrine malignancy.<sup>5</sup> The incidence of thyroid carcinoma is increasing worldwide and in Australia. The overall prognosis of thyroid cancer is good, but recurrences are common. The importance of long-term follow up is to detect recurrences early and therefore influence morbidity and mortality.

Current management guidelines recommend follow up of patients with DTC by Tg combined usually with either ultrasound of the neck or whole-body radio-iodine scanning, usually at 6 months post-surgery and then annually.<sup>1-4</sup> Sensitivity of Tg measurements are increased with thyroid stimulating hormone (TSH) stimulation, which also enhances the uptake of radioiodine. TSH stimulation is achieved either by T4 withdrawal or administration of recombinant human TSH (rh-TSH).

Clinical decision-making in diagnosis and management of DTC recurrences in patients who have had a total thyroidectomy and remnant ablation for DTC is based predominantly on serum Tg measurements. In this group of patients, Tg is a specific and sensitive tumour marker. Clinical guidelines have recommended that a Tg of more than 2 µg/L<sup>6</sup> or any detectable level of Tg<sup>4</sup> either after thyroid hormone withdrawal or 72 h after rh-TSH should prompt further investigation.<sup>6</sup> However, the guidelines have in part ignored the issue of variability between assay methods.

In the course of establishing an institutional protocol for the management of DTC we have become aware that there are significant clinical and laboratory issues with respect to the use of Tg measurements. The issues that need to be resolved are temporal: that is, the frequency of measurement and contextual, whether it should be measured during thyroxine suppression, withdrawal of thyroxine or stimulation with rh-TSH. In addition, there are also significant technical issues. In a recent review of Tg assays, Iervasi *et al.* identified the analytical problems with the assays which include standardization of the analytical procedure and assay comparability, long-term stability of the assay, sensitivity and precision of the assay at the low range, effect of anti-thyroglobulin antibodies and different isoforms of the Tg that may be released by cancer cells.<sup>7</sup> The authors suggested that follow up of patients should be with the same assay and results should be judged using cut-off values determined in clinical studies using the same assay.

Tg has been measured by several assay technologies that have advanced with time. Older assays were radioimmunoassays (RIA), but now most commercially available assays are immunometric assays (IMA). Several papers have shown that IMA methods are prone to underestimating Tg in the presence of anti-thyroglobulin antibodies (anti-Tg) whereas RIA methods can both overestimate or underestimate Tg in the presence of antibodies.<sup>8</sup> Therefore, detectable anti-Tg will compromise the validity of Tg result at low concentrations. Whichever technique is used, there should be clear information regarding its performance. The Royal College of Pathologists of Australia and the Australian Association of Clinical Biochemists have established several external quality assurance programmes (EQA) for a wide range of laboratory tests; however, we were surprised to find that Tg is not included in any of these programmes despite its significance in the management of patients with DTC. Therefore, currently, it would seem that there is no direct comparison of Tg results from different laboratories within Australia.

Validation of Tg assays has been focused on establishing a 'normal range;' however, except perhaps in the diagnosis of thyrotoxic factitia, this is of no clinical relevance. Consideration of the Tg measurement should be focused on detecting low concentrations of Tg and the clinical significance of these low levels in DTC. The detection limit is defined as the lowest concentration of an analyte that, with a 95% probability, can be distinguished from zero. Tg assays should have a detection limit of 1 µg/L or lower.<sup>7</sup> More recently, assays have been developed that are highly sensitive with detection limits of 0.1 µg/L and 0.03 µg/L. Using these highly sensitive assays, it has been suggested the need for rh-TSH stimulation or withdrawal of thyroxine for Tg assessment may not be required.<sup>9,10</sup> The currently available Beckman Coulter and Roche methods now claim such low detection limits of 0.1 µg/L.

To establish the relevance of this information to local clinical practice, we conducted a review of the Tg assay at our institution and also a pilot study of currently available methods in Victoria. Our aim was to determine the potential effect on patient management when sending a sample of blood to different laboratories for assessment of Tg.

Three chemical pathology laboratories in Victoria measure thyroglobulin using three different methods. The analysers used were Beckman Coulter DXI (Fullerton, CA, USA), Roche E170 (Mannheim, Germany) and Immulite 2000 (Los Angeles, CA, USA). All three manufacturers claim that their assay calibrators are traceable to the European Community Bureau of Reference CRM 457 thyroglobulin standard.

Plasma samples of known Tg concentrations and negative for anti-Tg antibodies, both measured using

Beckman Coulter DXI at our institution, were pooled into six samples with mean values from five analyses of 0.1, 0.6, 1.1, 3.4, 12.4 and 69.3 µg/L, respectively. The pooled samples were sent frozen to the two other laboratories for measurement.

To ensure the viability of the results for the final analysis, we also confirmed the stability of the above pooled Tg samples at various conditions. No significant differences were found at either room temperature or 4°C measured consecutively over 5 days. Furthermore, Tg was stable after repeat freeze-thawing up to three cycles (data not shown).

Table 1 shows the Tg results from the three laboratories in Victoria that are currently offering the test. There was a generally good agreement between these laboratories at low levels. Results of more than 5, however, become more divergent. Also, a low titre of anti-Tg antibodies was detected in pool six with a value of 23 IU/mL (values <20 IU/mL reported as negative) in the laboratory using the Immulite analyser.

To make matters even more confusing two laboratories are reporting their thyroglobulins as pmol/L whereas the third is using micrograms per litre (ng/mL). Values in picamoles per litre were converted to micrograms per litre for comparison purposes by dividing by 1.51.

Our survey of the situation with respect to Tg assays has emphasized several important caveats with regard to Tg assessment in management of patients with DTC.

- There should be caution in applying Tg cut-off values from studies conducted overseas for implementation in the local context
- Reported Tg results vary between laboratories in terms of both the absolute values and in terms of the units used to report those values
- The variable detection of Tg antibodies may further complicate comparison of results from different laboratories

**Table 1** Thyroglobulin (Tg) results (µg/L) from different laboratories in Victoria using various platforms

Instrument	Beckman Coulter DXI	Roche E170	Immulite 2000
Detection limit	0.1	0.1	0.2
Reference intervals (%)	1.0–35.0 (5–95)	1.4–78 (5–95)	1.7–55.6 (2.5–97.5)
Specimens			
Pool 1	0.1	<0.1	<0.2
Pool 2	0.6	0.4	0.4 <sup>†</sup>
Pool 3	1.1	0.8	0.8
Pool 4	3.4	5.3	3.8
Pool 5	12.4	21.8	17.3
Pool 6	69.3	84.4	92.0

<sup>†</sup>Positive anti-Tg antibodies were detected in one laboratory only in this pooled sample.

- At least until there is some objective way to assess the comparability of different assay methods patients should be followed by having all serial Tg measurements performed by the same method in the same laboratory.

The number, type and comparability of the platforms currently used to detect anti-Tg is also an area of uncertainty. Currently, there are eight laboratories in Melbourne that carry out the anti-Tg assay using five different platforms and each laboratory quotes a different reference interval.

To address these issues three things need to be done involving pathologists, endocrinologists and nuclear medicine physicians. First, there is a need to establish Tg and Tg antibody EQA programmes in Australia to provide some assurance of the performance of current assays. The programme should particularly focus on the specific area of low concentrations of Tg, which is critical to detection of DTC recurrence. Having established the stability of Tg in serum we believe this is feasible. Second, there is also a need for agreement on reporting units for Tg and finally a working party of the various stakeholders is required to establish Australian Guidelines for the management of patients with differentiated thyroid cancer taking into account, among other things, the performance of currently available Tg assays in this country.

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