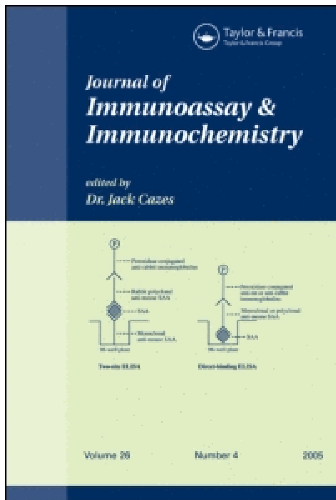
This article was downloaded by:
On: *16 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Correlation of Thyroglobulin Concentrations Measured by Radioimmunoassay and Immunoradiometric Assay and the Influence of Thyroglobulin Antibody

Marijana Stanojević[a]; Svetlana Savin[b]; Dubravka Cvejić[b]; Aleksandar Đukić[a]; Snežana Živančević Simonović[a]
[a] School of Medicine, University of Kragujevac, Serbia [b] Institute for the Application of Nuclear Energy INEP, Zemun, Serbia

To cite this Article Stanojević, Marijana , Savin, Svetlana , Cvejić, Dubravka , Đukić, Aleksandar and Živančević Simonović, Snežana(2009) 'Correlation of Thyroglobulin Concentrations Measured by Radioimmunoassay and Immunoradiometric Assay and the Influence of Thyroglobulin Antibody', Journal of Immunoassay and Immunochemistry, 30: 2, 197 — 207

To link to this Article: DOI: 10.1080/15321810902782897

URL: http://dx.doi.org/10.1080/15321810902782897

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Immunoassay and Immunochemistry®, 30: 197–207, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 1532-1819 print/1532-4230 online
DOI: 10.1080/15321810902782897

Taylor & Francis
Taylor & Francis Group

Correlation of Thyroglobulin Concentrations Measured by Radioimmunoassay and Immunoradiometric Assay and the Influence of Thyroglobulin Antibody

Marijana Stanojević,[1] Svetlana Savin,[2] Dubravka Cvejić,[2] Aleksandar Đukić,[1] and Snežana Živančević Simonović[1]
[1]School of Medicine, University of Kragujevac, Serbia
[2]Institute for the Application of Nuclear Energy INEP, Zemun, Serbia

Downloaded At: 09:57 16 January 2011

Abstract: There are a large number of commercial diagnostic assays for measuring thyroglobulin (Tg) concentration in human serum. The assay principle, as well as the potential presence of antithyroglobulin autoantibody (TgAb) in patient's serum, could influence the measured amount of Tg. Our objective was to determine the concentration of Tg by radioimmunoassay and immunoradiometric assay, to compare the values obtained and to investigate the influence of TgAbs on those results. Analysis of serum specimens ($n = 58$) showed close correlation between the investigated assays, regardless of the presence of TgAb in some samples. The mean value for Tg concentration, determined by radioimmunoassay, was 25% lower than that obtained by immunoradiometric assay. However, this ratio was not uniform for the whole population because the differences were more prominent for high values of Tg. The significant difference between these two methods was confirmed by Student's t-test, which indicated that patients must be monitored in continuity only by one selected method.

Keywords: Antithyroglobulin autoantibodies, Immunoradiometric assay, Radioimmunoassay, Thyroglobulin

Address correspondence to Snežana Živančević Simonović, Medical Faculty in Kragujevac, Svetozara Markovića 69, 34000 Kragujevac. E-mail: simonov@EUnet.yu

INTRODUCTION

Thyroglobulin (Tg) is a glycoprotein of molecular weight 660 kDa, which is synthesized in the thyroid gland, and is detectable in the serum of most normal individuals when a sensitive method is used. Serum concentrations of Tg can be increased by endogenous and exogenous stimulation of the thyroid gland or due to disturbed integrity of its follicular structure, most frequently in patients with differentiated thyroid carcinomas (papillary and follicular) and autoimmune diseases of the thyroid gland (Graves disease and Hashimoto's thyroiditis).

In addition, measurement of Tg concentration is also necessary in differential diagnosis of nodular changes detected in the thyroid gland region, differential diagnosis of agenesis and ectopia of the thyroid gland and differential diagnosis of thyrotoxicosis. Regarding autoimmune thyroid diseases, as well as differentiated thyroid carcinomas, increased concentrations of antithyroglobulin antibodies (TgAb) can sometimes be detected in patient's serum, in addition to increased concentrations of Tg. When TgAbs are present in the serum, part of the Tg molecule is exposed, and part of it is covered in complexes with specific antibodies (Tg-antiTg complexes).

Measurement of serum Tg encounters some methodological problems which can diminish its clinical importance.[1] There are many commercial diagnostic sets, which can be divided into two groups according to the detection principle [competition between labelled and serum Tg for binding limited number of TgAb (radioimmunoassay – RIA) or serum Tg captured in a sandwich between labelled and unlabelled antibodies (immunoradiometric assay – IRMA)]. The assay principle, as well as the potential presence of TgAb in the examinee's serum,[2–4] could influence the measured concentration of Tg. This may depend on the affinity and capacity of the antibodies on the one hand, and on the assay procedure itself on the other hand.[5–7] It has been generally accepted that RIA is less sensitive to the presence of TgAb than IRMA,[5] giving sometimes lower and sometimes higher values than the real ones.[8,9] Nowadays, many laboratories use immunometric assays because they have certain technical advantages, such as shorter incubation time and process automatization.[1,10,11] Literature data indicate that in the presence of TgAb in IRMA and similar assays, the measured values of Tg are usually lower than the real values[5] even if the concentrations of antiTg autoantibodies are very low.[6,12]

This paper presents the results for Tg concentration measured by radioimmunoassay, (INEP, Zemun, Serbia) and immunoradiometric assay (CIS Bio-international, France), their correlation and the influence of TgAb on the values for Tg (in particular specimens) obtained by these two methods.

Downloaded At: 09:57 16 January 2011

EXPERIMENTAL

Blood samples (5 mL) for measurement of Tg and TgAb concentrations were collected in the Department for *In Vitro* Diagnostics, Centre for Nuclear Medicine, The Clinical Centre Kragujevac during a three-month period, from Sept. 18 to Dec. 18, 2006. After allowing the sample to clot, the serum was separated by centrifugation at 3,000 rpm for 15 minutes, decanted and preserved at −20°C until used. The total number of specimens was 58. The concentrations of Tg and TgAb were measured at the same time, regardless of whether the patients had previously been diagnosed with malignant or autoimmune disease of the thyroid gland or were without previously diagnosed thyroid gland disease.

Downloaded At: 09:57 16 January 2011

Assays for Tg

The concentration of Tg was measured by radioimmunoassay (RIA Tg, PEG, INEP, Serbia) and immunoradiometric assay (IRMA, THYRO, CIS Bio-international, France).

a) RIA Tg (PEG)

The principle of RIA is based on the competitive binding of serum Tg and a fixed amount of radioactively labelled Tg (^{125}I-Tg) for a limited number of determinants on specific TgAb. The formed immune complexes are precipitated with immunoadsorbent (secondary antibodies in buffer solution) and separated out. The amount of radioactivity, measured in a gamma scintillation counter, is inversely proportional to the concentration of Tg in the examined specimen.

b) IRMA Tg

The principle of IRMA is based on binding serum Tg to an excess of TgAbs fixed to the test tube wall (four specific monoclonal TgAbs selected for their properties of avidity and complementarity). A fifth monoclonal antibody labelled with ^{125}I and specific for a different part of the Tg molecule then binds with Tg on the test tube wall forming a "sandwich" of Tg between the unlabelled and labelled Ab. After aspiration and rinsing, the amount of radioactivity, measured in a gamma scintillation counter, is directly proportional to the concentration of Tg in the examined specimen.

Some detailed characteristics of both assays, according to producers' manuals, are given in Table 1.

Table 1. The characteristics of RIA (INEP) and IRMA (CIS) according to the manufacturers

Parameters	RIA Tg (PEG)-INEP	IRMA Tg (CIS)
Principle of the assay	RIA, PEG	CT, IRMA
Tracer	^{125}I-Tg CRM 457	^{125}I-MoAbTg
Antibodies	polyclonal	4 monoclonal
Measuring range	5–320 µg/L	0.2–500 µg/L
Reference material	CRM 457[a]	CRM 457
Detection limit	4 µg/L	0.2 µg/L (0.7 µg/L)[b]
Intra-assay reproducibility	6.2–8.7%	1.8–7.7%
Inter-assay reproducibility	3.8–8.4%	2.0–16.7%
Hook-effect	/	>800 000 µg/L

[a]1 µg/L CRM 457 equals 1 µg/L in RIA Tg (PEG)-INEP.
[b]Functional sensitivity.

Downloaded At: 09:57 16 January 2011

Assay for TgAb

Concentrations of antiTg autoantibodies in human sera were measured by RIA (TGAB ONE STEP, CIS Bio-international, France). The assay is based on the competitive binding of a fixed amount of monoclonal TgAb and TgAbs from the examined sample with a particular, limited number of specific points on ^{125}I-labelled Tg molecules. Unbound molecules of labelled Tg are removed by aspiration and lavage. Radioactivity was measured in a gamma scintillation counter. The extent of binding is inversely proportional to the concentration of TgAb in the examined specimen.

The characteristics of this assay according to the manufacturer: detection limit 6 IU/mL, detection range 6-2000 IU/mL.

Statistical Procedures

The results obtained were analyzed by the following statistical methods: linear regression, variance analysis, Student's t-test and non-linear correlation using STATGRAPHICS (version 4.2) and the MSEXCEL programme.

RESULTS

The values for serum Tg concentrations obtained for 58 serum specimens by RIA and IRMA were compared by regression analysis and the following equation was obtained ($y = 5.069\ X^{0.599}$; correlation coefficient

r = 0.92). Although the relation was not linear, the results indicated that there was a close correlation between these assays (Figure 1). Thus, whether TgAb were found or not, determination of Tg with the INEP RIA set was in highly positive correlation with the results obtained using the CIS IRMA set for all the samples. However, the overall mean value for Tg measured by RIA was 25% lower than that measured by IRMA (statistical regression analysis). This ratio was not uniform for all samples as low values tended to be closer, while the absolute and relative differences were often much greater in the range of high Tg values. The difference between the two methods was statistically significant (Student's t-test t_a and t_b differed from t_{tab} at a probability level of 0.05, data not shown), which indicated that patients must be monitored in continuity only by one selected method.

Concerning the influence of TgAbs on the results, Figure 2 gives a comparative display of the Tg values obtained by RIA and IRMA and the concentrations of TgAbs in 58 specimens of serum. It seems that compatibility of the Tg results obtained with the two methods varied between particular specimens, independently of the presence of TgAbs. Thus, large differences were found in some specimens which gave negative results for TgAbs (Figure 2), while the values were similar in many

Downloaded At: 09:57 16 January 2011

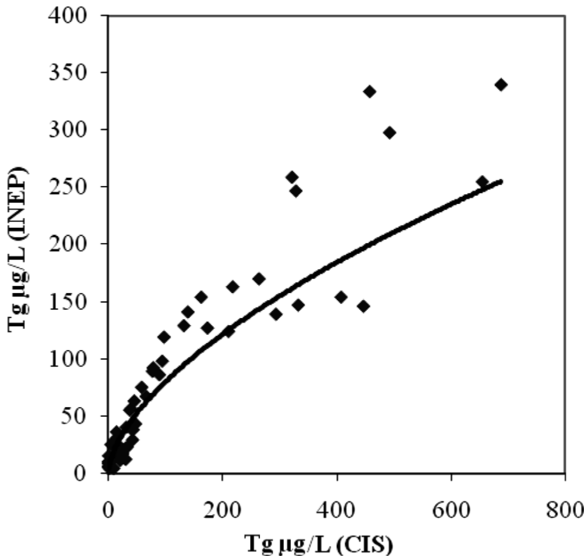


Figure 1. Correlation between IRMA Tg (CIS) and INEP RIA Tg (PEG) assay. The points on the curve are mean values for two replicates. The correlation equation: $y = 5.069\ X^{0.599}$ gave a very high correlation coefficient (r = 0.92), with $R^2 = 0.856$, which indicates that the equation describes 85.6% of the variance between the values.

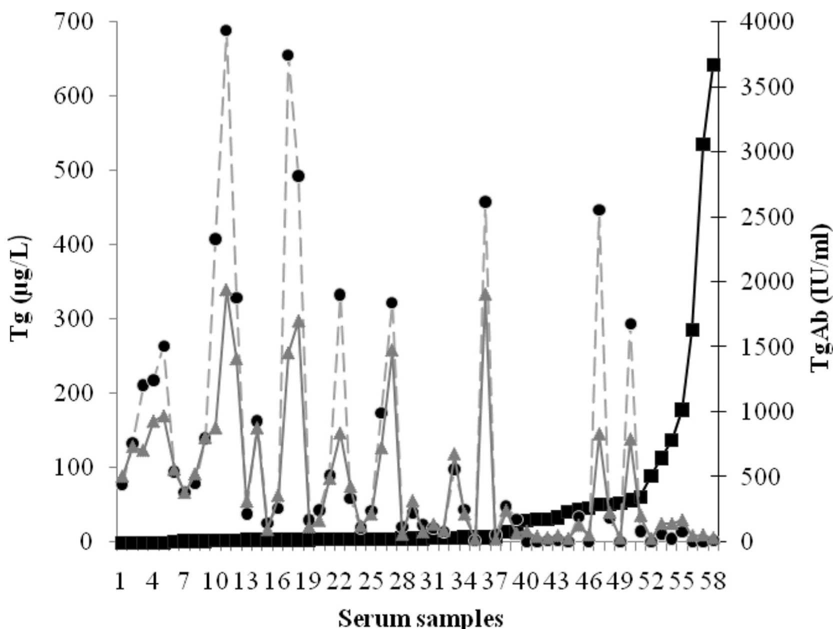


Figure 2. Comparative display of values for TgAb and Tg in serum samples from 58 patients. Squares represent the values for TgAb, black circles Tg concentrations obtained with IRMA (CIS) and grey triangles Tg concentrations obtained with RIA (INEP). The concentration of TgAb in specimens 1 to 34 was very low (<40 IU/ml), while in the remaining 24 specimens (35 do 58) it ranged from 41 to 3663 IU/ml.

Downloaded At: 09:57 16 January 2011

specimens containing endogenous TgAbs. Thus, the absolute values for particular Tg samples obtained with both sets, differed by less than 10% (which is below the allowed limit of absolute measuring error) in 10 out of 34 patients with concentrations of TgAbs $<$ 40 IU/ml, but only in 2 patients out of 24 patients with TgAbs $>$ 40 IU/mL. The values for Tg measured by RIA were higher than those obtained by IRMA in 16/24 (66%) of patients with TgAb concentrations above 40 IU/mL. Contrary to that, in the group of 34 patients with serum TgAb $<$ 40 IU/ml, only 8 patients (23%) had higher values of Tg with RIA. In this group, twice as many patients had higher values with IRMA.

DISCUSSION

Differences between the concentrations of Tg found here could be the consequence of methodological differences between the two assays

(RIA and IRMA) from separate producers (INEP, Serbia and CIS Bio international, France). There are many factors which can cause differences in the results obtained for serum concentration of Tg: different reference materials, discrepancy between standards regarding the same reference material, specific properties of the primary and secondary antibodies for different antigenic determinants on Tg and different binding affinities of these antibody epitopes, as well as interference by serum factors (TgAb in the first place) with the primary and secondary Tg antibodies from the set.

Due to inter-method differences, a European project, supported by the Committee for Reference Materials of the European Union Committee, was initiated for the isolation and characterisation of a reference preparation of Tg. The resulting reference material, CRM 457,[13,14] is now employed directly or indirectly for calibrating most assays. Both commercial sets, RIA Tg (PEG) and IRMA (THYRO) used here were calibrated according to CRM 457 in the same way, so differences in the measured values were not caused by discrepancy in standardization.

Therefore, variability between the different assays could reflect differences in methodological procedure and specific properties of isoforms of circulating Tg,[1,15–17] together with interference with the antibodies in the kits by serum autoantibodies. The individual characteristics of different assays for some isoforms of Tg which can be found in patients' sera, are determined by the properties of the antibodies (labelled and unlabelled) contained in assay sets. In the applied IRMA, there were four monoclonal antiTg antibodies fixed to the test tube wall and a fifth monoclonal TgAb labelled with ^{125}I, while in the RIA there was a sheep antiTg antiserum, which contains polyclonal antiTg antibodies. Since Tg is a large glycoprotein molecule, which expresses a large number of epitopes, it is possible that there are differences in the binding of antibodies specific for Tg between RIA and IRMA, and this may account for the differences in measured Tg concentrations in those sera where no TgAb was found.

It is known that endogenous serum TgAbs interfere in Tg measurement in a method-dependant way.[6,8,18] It is generally accepted that the concentrations of Tg determined by IRMA in the presence of serum TgAb are lower than the real values, while in RIA apparently lower or higher values of Tg can be obtained.[6,10,18,19] Thus, in these cases the values of Tg measured by RIA are often higher than those obtained by IRMA,[6,20] which our results confirm. However, currently there is no method which can measure the exact concentration of Tg in the presence of TgAbs. Thus, we cannot say with certainty whether the Tg concentrations found in our study by IRMA are lower or the values obtained by RIA are

Downloaded At: 09:57 16 January 2011

higher than the actual concentrations of Tg in any specimens which contain TgAbs. The influence of TgAbs on the measured concentrations of Tg by RIA and IRMA, can be estimated by an additional recovery test (i.e. determining the increase in Tg concentration in the specimen after a suitable known concentration of Tg has been added). Even though it is not ideal, the recovery test can indicate whether the measured values of Tg are lower or higher than the real value.

Considering that our group of examinees included patients with differentiated thyroid carcinomas, the interpretation of undetectable or low values of Tg in the presence of TgAbs (obtained by IRMA) must be very careful,[11] especially as TgAbs are considered to be an additional tumour marker indicating the presence of thyroid tissue.[6,21] However, serial measurement of TgAb also requires the application of an identical assay, because commercial assays differ in sensitivity, specific properties and absolute values, regardless of standardization according to the international reference preparation MRC 65/93,[6,7,11,22,23] most probably due to differences in the specific properties of assays for conformational epitopes typical for TgAb in serum.[6,7,21,22,23]

The lower values for Tg obtained by RIA in comparison to IRMA, when the Tg concentration was above 300 μg/L, could be the consequence of the somewhat smaller capacity of the antibodies in the RIA to bind the total amount of Tg.

Since RIA (INEP) and IRMA (CIS) are employed in the diagnosis of thyroid gland disease in many laboratories, and since the patients are not always examined in the same health care institution, it is important to point out the clinical implications of our results. Those results which demonstrated good compatibility between the assays, whether the sera contained TgAbs or not, can be considered sufficiently accurate and acceptable for the clinicians. Differences between the obtained results for sera without autoantibodies most probably occurred due to inter-method differences. During monitoring of patients (especially those with differentiated thyroid carcinoma) it is important that the particular individual is always controlled by the same method (RIA or IRMA). If TgAbs are present, and undetectable or low concentrations of Tg (especially by IRMA assay) are found, this should definitely be interpreted in the context of positive TgAbs. Naturally, serum concentration of Tg is just one parameter for estimating pathological processes in the thyroid gland. If there are differences in the results obtained with two methodologically different assays, the clinician should conclude that either one or both results are not accurate and therefore a more detailed clinical examination should be undertaken.

Downloaded At: 09:57 16 January 2011

CONCLUSIONS

Comparing the concentrations of Tg in individual human serum specimens obtained using RIA (INEP) and IRMA (CIS Bio International) showed a high degree of correlation. However, differences in Tg concentrations measured by these assays were not clearly related to the concentrations of detected TgAb. Since the values for Tg concentrations differed significantly in a large number of specimens, we suggest that patients should be monitored in continuity only by one selected method.

ACKNOWLEDGMENTS

This paper forms part of projects no. 143039 and no. 145065 financed by the Ministry of Science, Republic of Serbia.

Downloaded At: 09:57 16 January 2011

REFERENCES

1. Spencer, C.A.; Bergoglio, L.M.; Kazarosyan, M.; Fatemi, S.; LoPresti, J.S. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J. Clin. Endocrinol. Metab. **2005**, *90*(10), 5566–5575.
2. Black, E.G.; Hoffenberg, R. Should one measure serum thyroglobulin in the presence of anti-thyroglobulin antibodies? Clin. Endocrinol. **1983**, *19*(5), 597–601.
3. Mariotti, S.; Barbesino, G.; Caturegli, P.; Marino, M.; Manetti, L.; Pacini, F.; Centoni, R.; Pinchera, A. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: an unobtainable goal? J. Clin. Endocrinol. Metab. **1995**, *80*, 468–472.
4. Massart, C.; Maugendre, D. Importance of the detection method for thyroglobulin antibodies for the validity of thyroglobulin measurements in sera from patients with Graves' disease. Clin. Chem. **2002**, *48*, 102–107.
5. Spencer, C.A. J Editorial: Challenges of serum thyroglobulin (Tg) measurement in the presence of Tg autoantibodies. Clin. Endocrinol. Metab. **2004**, *89*(8), 3702–3704.
6. Spencer, C.A.; Wang, C.; Fatemi, S.; Guttler, R.B.; Takeuchi, M.; Kazarosyan, M. Serum thyroglobulin autoantibodies: Prevalence, influence on serum thyroglobulin measurement and prognostic significance in patients with differentiated thyroid carcinoma. J. Clin. Endocrinol. Metab. **1998**, *83*(4), 1121–1127.
7. Benvenga, S.; Burek, C.L.; Talor, M.; Rose, N.R.; Trimarchi, F. Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in Hashimoto's thyroiditis and non-autoimmune thyroid diseases. J. Endocrinol. Invest. **2002**, *25*(11), 977–982.

8. Schneider, A.B.; Pervos, R. Radioimmunoassay of human thyroglobulin: effect of antithyroglobulin autoantibodies. J. Clin. Endocrinol. Metab. **1978**, *47*, 126–137.
9. Feldt-Rasmussen, U.; Rasmussen, A.K. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J. Endocrinol. Invest. **1985**, *8*(6), 571–576.
10. Spencer, C.A.; Takeuchi, M.; Kazarosyan, M. Current status and performance goals for serum thyroglobulin assays. Clin. Chem. **1996**, *42*, 164–173.
11. Baloch, Z.; Carayon, P.; Conte-Devolx, B.; Demers, L.M.; Feldt-Rasmussen, U.; Henry, J.F.; LiVosly, V.A.; Niccoli-Sire, P.; John, R.; Ruf, J.; Smyth, P.; Spencer, C.A.; Stockigt, J.R. Guidelines committee, national academy of clinical biochemistry. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid **2003**, *13*(1), 3–126.
12. Cubero, J.M.; Rodriques-Espinosa, J.; Gelpi, C.; Estorch, M.; Corcoy, R. Thyroglobulin autoantibody levels below the cut-off positivity can interfere with thyroglobulin measurement. Thyroid **2003**, *13*(7), 659–661.
13. Feldt-Rasmussen, U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin and thyrotropin receptor. Clin. Chem. **1996**, *42*, 160–163.
14. Feldt-Rasmussen, U.; Profilis, C.; Colinet, E.; Black, E.; Bornet, H.; Bourdoux, P. et al. Human thyroglobulin reference material (CRM 457) 2nd part: Physicochemical characterization and certification. Ann. Biol. Clin. **1996**, *54*(10–11), 343–348.
15. Spencer, C.A. Recoveries cannot be used to authenticate thyroglobulin (Tg) measurements when sera contain Tg autoantibodies. Clin. Chem. **1996**, *42*(5), 661–663.
16. Ferrari, L.; Biancolini, D.; Seregni, E.; Aliberti, G.; Martinetti, A.; Villano, C.; Palloti, F.; Chiesa, C.; Bobardieri, E. Critical aspects of immunoradiometric thyroglobulin assays. Tumours **2003**, *89*(5), 537–539.
17. Schultz, R.; Bethauser, H.; Stempka, L.; Heilig, B.; Moll, A.; Hufner, M. Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. J. Clin. Invest. **1989**, *19*(5), 459–463.
18. Schaadt, B.; Feldt-Rasmusen, U.; Rasmusson, B.; Torring, H.; Foder, B.; Jorgensen, K.; Hansen, H.S. Assessment of the influence of thyroglobulin (Tg) autoantibodies and other interfering factors on the use of serum Tg as tumour marker in differentiated thyroid carcinoma. Thyroid **1995**, *5*(3), 165–170.
19. Feldt-Rasmussen, U.; Schlumberger, M. European interlaboratory comparison of serum thyroglobulin measurement. J. Endocrinol. Invest. **1988**, *11*(3), 175–181.
20. Spencer, C.A. New insights for using serum thyroglobulin (Tg) measurement for managing patients with differentiated thyroid carcinomas. Thyroid Intl. **2003**, *4*, 1–14.

Downloaded At: 09:57 16 January 2011

21. Pacini, F.; Mariotti, S.; Formica, N.; Elisei, R. Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumor outcome. Acta Endocrinol. **1988**, *119*(3), 373–380.
22. McLachlan, S.M.; Rapoport, B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies? Thyroid **2004**, *14*(7), 510–520.
23. Sapin, R.; d'Herbomez, M.; Gasser, F.; Meyer, L.; Schlinger, J.L. Increased sensitivity of new assay for anti-thyroglobulin antibody detection in patients with autoimmune thyroid disease. Clin. Biochem. **2003**, *36*(8), 611–616.

Received March 10, 2008
Accepted August 4, 2008
Manuscript 3279

Downloaded At: 09:57 16 January 2011