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Evaluation of the highly sensitive Roche thyroglobulin II assay and establishment of a reference limit for thyroglobulin-negative patient samples

Dorien M. Rotteveel-de Groot [a,*], H. Alec Ross [a], Marcel J.R. Janssen [b], Romana T. Netea-Maier [c], Janine D. Oosting [a], Fred C.G.J. Sweep [a], Antonius E. van Herwaarden [a]

[a] Department of Laboratory Medicine, Laboratory of Clinical Chemistry, Radboud University Medical Centre, Nijmegen, The Netherlands
[b] Department of Nuclear Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands
[c] Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Centre, Nijmegen, The Netherlands

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ABSTRACT

Objectives: Thyroglobulin (Tg) measurements are used to monitor for residual thyroid tissue in patients with differentiated thyroid cancer (DTC) after thyroidectomy and radioiodine ablative therapy. In recent years highly sensitive Tg assays have been developed. In this study the analytical performance of the new Roche Elecsys Tg II assay was evaluated and compared with the well documented Access2 Tg assay (Beckman–Coulter).
Design and methods: Analytical performance was examined using various Clinical and Laboratory Standards Institute (CLSI) evaluation protocols. Tg negative patient sera were used to establish an upper reference limit (URL) for the Elecsys Tg II assay.
Results: Non-linearity, drift and carry-over according to CLSI EP10 and EP6 in a measuring range of 0.04–500 ng/mL were non-significant. Total precision according to CLSI EP5 was 10% at a Tg concentration of 0.08 ng/mL. A patient serum comparison performed according to a modified CLSI EP9 protocol showed a significant difference of a factor of approximately 1.4, despite using an identical CRM calibrator. The Elecsys Tg II assay measured Tg with a two-fold higher sensitivity than the Access2 assay. Finally, using human sera without Tg, an URL of 0.05 ng/mL was determined.
Conclusions: In our hands the highly sensitive Elecsys Tg II assay shows a good analytical performance and a higher sensitivity compared to the Access2 Tg assay. An URL of 0.05 ng/mL for the Elecsys Tg II assay was determined which may improve the clinical utility of the assay for the detection of residual DTC or disease recurrence.

1. Introduction

Thyroglobulin (Tg) is a large glycoprotein (about 660 kDa) that is produced exclusively by thyroid follicular cells and therefore is widely used as a tumor marker in differentiated thyroid cancer (DTC) [1]. Depending on the stage of disease, DTC patients are treated by total thyroidectomy and lymph node dissection if necessary, sometimes followed by radioiodine

* Correspondence to: Department of Laboratory Medicine, Radboud University Medical Centre, Geert Grooteplein 10, 6525 GA Nijmegen, The Netherlands.
E-mail address: Dorien.Rotteveel-deGroot@radboudumc.nl (D.M. Rotteveel-de Groot).

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ablation [2]. Tg is measured to monitor patients after this ablative therapy for residual DTC during thyroxine (T4) replacement or, to increase the diagnostic sensitivity of the Tg measurement, after withdrawal of T4 or administration of synthetic TSH [2,3]. Detectable Tg suggests persistence or recurrence of disease. Second generation highly sensitive Tg immunoassays are now available with improved analytical sensitivity and precision at low concentrations [4]. The term *functional sensitivity* or *functional detection limit* is defined as the concentration at which a between-run CV of 20% is observed, and is thus a measure of an assay's precision at low analyte levels (without considering bias) [5,6]. It is a recommended parameter for Tg assay characterization and often serves as the reporting limit [7]. However, immunoassays are usually subject to positive or negative bias near the zero point and assay bias may invalidate decision limits of Tg assays [8]. To circumvent this problem, we previously suggested the use of an upper reference limit (URL) that is based on the one-sided (e.g. 99.9%) confidence interval of measurements in sera that are essentially free of Tg and, as such, takes assay bias into account if present [8]. This appears to be a better approach to determination of a decision limit of a Tg assay. To determine such confidence intervals, we measured Tg levels in patient samples selected from treated DTC patients who did not have clinical or imaging evidence of tumor, and who had undetectable serum Tg levels after stimulation with recombinant TSH.

Immunometric Tg sandwich assays may suffer from interference by Tg autoantibodies causing falsely low or negative Tg measurements [9–11]. These antibodies are detected at diagnosis in approximately 25% of DTC patients and, when present, may complicate Tg monitoring and management of these patients [12]. Guidelines recommend that every Tg test should be accompanied by a Tg autoantibody measurement to validate the Tg result [3]. In addition, quantitative Tg antibody measurements may have potential value as a surrogate post-operative DTC tumor marker in patients with anti-Tg autoantibodies [13]. Therefore, in this study the presence or absence of anti-Tg antibodies was noted in all human samples used in this study.

Roche® have developed a highly sensitive Elecsys Tg II assay and an Elecsys anti-Tg test. In this study we evaluated and compared the Elecsys Tg II assay with the Access2 Tg assay of Beckman Coulter, which is currently used in our laboratory [8]. In addition, anti-Tg autoantibody interferences were examined by using the Roche® Elecsys anti-Tg assay. Finally, a clinical validation using Tg negative patient samples to assess possible assay bias and establish the reporting limit of the Elecsys Tg II assay was performed.

2. Materials and methods

2.1. Materials/patient samples

Patient samples were obtained from a patient population in our academic hospital which serves as a referral institute (level 1) for thyroid carcinoma. Patient samples were selected based on their Tg and anti-Tg values that had been measured previously by luminescence immunoenzymometric assay (LIEMA) (Access2, Beckman Coulter, Brea, CA, USA) and an in-house assay for anti-Tg autoantibodies (see Methods section) [8], respectively. Values in pmol/L were converted to ng/mL by dividing by a factor of 1.5, based on a molecular weight of 660 kDa for Tg. Blood samples were collected in serum gel tubes (Vacutainer blood collection tubes, Becton Dickinson, Franklin Lakes, NJ, USA) and serum was stored in plain tubes at –20 °C until analysis. Samples were anonymized and only used for assay validation and method comparison and therefore approval by the Scientific Ethical Committee was not required. Unless specified otherwise, samples without anti-Tg autoantibodies were selected, as determined with our in-house anti-Tg assay. For most experiments Tg-positive patient samples were used, with Tg concentrations up to 7500 ng/mL (Access2, Beckman Coulter). To determine the URL of the Tg II assay, 12 Tg negative blood samples were selected from DTC patients who underwent complete thyroidectomy followed by radioiodine ablation between 2007 and 2013 (Group A). Blood samples from these patients were collected in 2013 or 2014. At this time these patients did not have clinical or imaging evidence of tumor, and Tg levels after stimulation with recombinant TSH were below the reporting limit of the Access2, and Elecsys Tg II assay (defined as 3 times the within-run SD of duplicate measurements in the lowest concentration range). A second group (Group B) of 10 Tg negative patients was selected from DTC patients who underwent complete thyroidectomy followed by radioiodine ablation between 2004 and 2013, in whom Tg levels before and after stimulation with recombinant TSH were below the reporting limit of the Access2 Tg assay of 0.13 ng/ml [8].

2.2. Methods

Samples were measured on a Modular E170 random access analyzer (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's specifications. The following assays, calibrators and controls were used according to the manufacturer's instructions: Elecsys Tg II, Tg II CalSet, PreciControl Thyro Sensitive, Elecsys anti-Tg, anti-Tg CalSet, and PreciControl Thyro AB (Roche). In addition, in-house prepared serum pools were used as controls. A single lot number of all reagents was used for all measurements unless specified otherwise. Samples were measured on an Access2 random access analyzer (Beckman Coulter) according to the manufacturer's specifications, using the Access2 Thyg reagent pack, Access2 substrate, Access2 washbuffer II, Access2 Thyg sample diluent and Thyg calibrator set (Beckman Coulter). In-house prepared serum pools of patient samples were used as controls. The presence of anti-Tg autoantibodies was assessed by an in-house