Assessment of the Precision and Functional Sensitivity of Two Thyroglobulin Assays: Comparison of the Second-Generation Roche Electrochemiluminescent Immunoassay and BRAHAMS Radioimmunoassay

Aerin Kwon1, Eun Hee Lee1, Young Kyung Lee2, and Hee Jung Kang2
1Green Cross Laboratories, Yongin; 2Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea

Background: Thyroglobulin (Tg) is the primary biochemical marker used to monitor patients with differentiated thyroid cancer (DTC) for residual or recurrent disease after total thyroidectomy, as only normal or well-differentiated malignant thyroid cells produce Tg. Here, we evaluated the precision and functional sensitivity (FS) of a recently developed highly sensitive Tg (hsTg) electrochemiluminescent immunoassay (ECLIA) and compared it to that of the radioimmunoassay (RIA) method using pooled human serum with low levels of Tg.

Methods: For the ECLIA method, the Elecsys Tg II kit (Roche Diagnostics, Germany) was used with an E170 analyzer (Roche Diagnostics). For the RIA method, the Tg-plus-RIA kit (BRAHAMS, Germany) was used with a Cobra Quantum gamma counter (Packard Instrument Company, USA). The precision and limit of detection (LOD) were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. FS was determined using a modification of the CLSI guideline.

Results: The total precision of the hsTg ECLIA and RIA methods was 9.6% and 48.2%, respectively. The manufacturer-reported LOD was verified by the hsTg ECLIA (0.04 ng/mL), but not by the RIA method (>0.08 ng/mL). The hsTg ECLIA showed better FS (0.04 ng/mL at a coefficient of variation [CV] of 10%) than the RIA method (0.37 ng/mL at a CV of 20%).

Conclusions: Thus, the hsTg ECLIA performed better than the RIA method in terms of FS, which is extremely important for the early detection of residual or recurrent disease in DTC patients after total thyroidectomy. The excellent performance of the hsTg ECLIA could allow for clinical Tg measurement without thyroid-stimulating hormone stimulation, in contrast to the insufficient performance of the RIA method.

(J Lab Med Qual Assur 2016;38:243-248)

Key Words: Thyroglobulin, Second-generation, Functional sensitivity, Radioimmunoassay, Comparison

INTRODUCTION

Thyroid cancer is the most common cancer in Korea. Most cases of thyroid cancer are differentiated thyroid cancer (DTC), comprising mostly papillary cancers and some follicular cancers, which have excellent prognosis (1). Serum thyroglobulin (Tg) is a large glycoprotein that functions as a substrate for thyroid hormone production...
As only normal or well-differentiated malignant thyroid cells produce Tg, it is used as the primary tumour marker for monitoring DTC patients for residual or recurrent disease after total thyroidectomy (2,3).

The standard assessment during follow-up after total thyroidectomy is neck ultrasound and Tg measurement following thyroid-stimulating hormone (TSH) stimulation (4,5). As the current guideline is based on study results obtained from Tg assays with a functional sensitivity (FS) of approximately 1 ng/mL, patients must get expensive injections of recombinant human TSH (rhTSH) or withdraw levothyroxine to achieve sufficient clinical sensitivity for detection (6).

Recently, a highly sensitive second-generation Tg (hsTg) test with a FS ≤0.1 ng/mL was introduced, which eliminated the need for rhTSH stimulation in most DTC patients (7). Therefore, to investigate if the currently available Tg assays meet the requirements for the Tg measurement without TSH stimulation, we evaluated the precision, limit of detection (LOD), and FS of the hsTg electrochemiluminescent immunoassay (ECLIA) in comparison to the standard radioimmunoassay (RIA) method using low-level pooled human serum.

MATERIALS AND METHODS

For the ECLIA method, the Elecsys Tg II kit (Roche Diagnostics, Mannheim, Germany) was used with an E170 analyzer (Roche Diagnostics). For the RIA method, the Tg-plus-RIA kit (BRAHAMS, Hennigsdorf, Germany) was used with a Cobra Quantum gamma counter (Packard Instrument Co., Meriden, CT, USA).

Evaluation of precision and LOD verification were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (8,9). To evaluate precision, low-level pooled human serum, with an approximate concentration of 0.5 ng/mL when measured by the Elecsys Tg II kit, was assayed in duplicate twice daily for 20 days. To compare the first- and second-generation ECLIA, two levels of commercially available quality control (QC) material (Roche Diagnostics) were also assayed twice daily in duplicate for 20 days. The LOD was evaluated using two blank samples and two low-level samples with concentrations close to the manufacturer’s reported LOD. Each sample was assayed once daily with four replicates for three days. FS was evaluated by using a modification of the CLSI guideline (9). First, eight dilutions (expected concentrations: 2.00, 1.00, 0.75, 0.50, 0.25, 0.10, 0.06, and <0.04 ng/mL) were prepared by combining pooled serum samples from patients with approximate concentrations to achieve concentrations between 2.00 and <0.04 ng/mL when measured by the Elecsys Tg II kit. Each sample was assayed in duplicate once daily for 10 days. Estimates of mean and within-run precision were calculated for each dilution and were tabulated and plotted as the precision profile. Based on the shape of the precision profile, a power function model was used to fit the datasets by regression analysis. The FS was estimated as the concentration at the intersection of the power function model fit line with an accuracy goal of a 10% CV (or 20% if 10% was not possible).

Statistical analysis was performed using EP evaluator ver. 10.0 (Data Innovations, South Burlington, VT, USA).

RESULTS

As shown in Table 1, the second-generation ECLIA showed excellent precision with low-level QC material and low-level pooled human serum. In contrast, the RIA method showed poor precision with low-level pooled human serum, which was similar to that of the first-generation ECLIA (data not shown).

The manufacturer’s reported LOD was verified with the second-generation ECLIA, and the experimental value was

|                     | Second-generation ECLIA (ng/mL) | RIA (ng/mL) |
|---------------------|---------------------------------|-------------|
| Low-level QC material | 3.1 (1.12±0.03)                | NT          |
| High-level QC material | 4.1 (19.67±0.80)              | NT          |
| Pooled serum        | 9.6 (0.43±0.04)                | 48.2 (0.18±0.09) |

Values are presented as CV% (mean±SD). Abbreviations: ECLIA, electrochemiluminescent immunoassay; RIA, radioimmunoassay; NT, not tested.
were not directly comparable, as they were reported at different CV% (0.1 ng/mL at a CV of 30% for the second-generation ECLIA and 0.2 ng/mL at a CV of 20% for the RIA). From the calculated precision profile, a cut-off value corresponding to a CV of 10% for ECLIA and 20% for RIA was used as the FS. The CV% was uniformly low, and was all less than 10% for the ECLIA method. However,

Table 2. Detection capability of the second-generation ECLIA and RIA

| Expected value (ng/mL) | Second-generation ECLIA (ng/mL) | RIA (ng/mL) |
|------------------------|---------------------------------|-------------|
| 2.00                   | 3.46 (1.98±0.07)                | 13.52 (1.13±0.15) |
| 1.00                   | 2.80 (0.96±0.03)                | 10.75 (0.46±0.05) |
| 0.75                   | 3.37 (0.71±0.02)                | 14.72 (0.35±0.05) |
| 0.50                   | 3.67 (0.47±0.02)                | 19.49 (0.26±0.05) |
| 0.25                   | 3.89 (0.22±0.01)                | 27.22 (0.17±0.05) |
| 0.10                   | 8.15 (0.08±0.01)                | 38.42 (0.12±0.05) |
| 0.06                   | 6.51 (0.04±0.00)                | 56.11 (0.09±0.05) |
| <0.04                  | NA (all <0.04)                 | 61.33 (0.09±0.05) |

Values are presented as CV% (mean±SD).

Abbreviations: ECLIA, electrochemiluminescent immunoassay; RIA, radioimmunoassay.

A CV of 10% could not be applied to the RIA method due to its low precision. The fitted CV% of the measured functional sensitivity was 15.2 (95% confidence interval, 10.3–20.0).

Table 3. Precision profile of the second-generation ECLIA and RIA method with low-level pooled human serum

| Expected value (ng/mL) | Second-generation ECLIA (ng/mL) | RIA (ng/mL) |
|------------------------|---------------------------------|-------------|
| 2.00                   | 3.46 (1.98±0.07)                | 13.52 (1.13±0.15) |
| 1.00                   | 2.80 (0.96±0.03)                | 10.75 (0.46±0.05) |
| 0.75                   | 3.37 (0.71±0.02)                | 14.72 (0.35±0.05) |
| 0.50                   | 3.67 (0.47±0.02)                | 19.49 (0.26±0.05) |
| 0.25                   | 3.89 (0.22±0.01)                | 27.22 (0.17±0.05) |
| 0.10                   | 8.15 (0.08±0.01)                | 38.42 (0.12±0.05) |
| 0.06                   | 6.51 (0.04±0.00)                | 56.11 (0.09±0.05) |
| <0.04                  | NA (all <0.04)                 | 61.33 (0.09±0.05) |

Values are presented as CV% (mean±SD).

Abbreviations: ECLIA, electrochemiluminescent immunoassay; RIA, radioimmunoassay; NA, not applicable.

Fig. 1. Functional sensitivity of the (A) second-generation ECLIA and (B) RIA for the detection of thyroglobulin in human serum. From the precision profile data (Table 3), a curve was drawn to estimate the relationship between the mean measured concentration and CV%. A cut-off value, corresponding to a CV of 20% for RIA, was used as the functional sensitivity (the value at which the upper 95% CI of the fitted curve has a CV of 20%). The functional sensitivity of the second-generation ECLIA could not be estimated because all specimens had a CV% less than the target CV of 10%. Therefore, the limit of detection was used as the functional sensitivity. Abbreviations: ECLIA, electrochemiluminescent immunoassay; RIA, radioimmunoassay; CV, coefficient of variation; CI, confidence interval.
for the RIA method, a gradual increase in CV% was observed as the mean concentration of the pooled serum decreased (Table 3, Fig. 1).

**DISCUSSION**

The second-generation ECLIA showed excellent precision when used with low-level pooled human serum (a CV% of 9.6 at a mean concentration of 0.43 ng/mL). In contrast, the RIA showed precision similar to that of the first-generation ECLIA method when evaluated with low-level pooled serum.

In this study, the FS of the second-generation ECLIA and RIA methods was 0.04 ng/mL at a CV of 10% and 0.37 ng/mL at a CV of 20% (95% confidence interval, 10.3% to 20.0%), respectively. Thus, the FS of the second-generation ECLIA was at least 10-fold better than that of the RIA. Both the manufacturer’s reported LOD and the FS as determined by the RIA were two-fold higher than those of the second-generation ECLIA, and neither parameter was acceptable. In contrast, the reported LOD was verified with the second-generation ECLIA (0.04 ng/mL), and the measured FS was much better than the reported FS (0.04 ng/mL).

FS is defined as “the concentration of the analyte at which the precision of a measurement procedure, under the stated experimental conditions, meets a stated performance requirement” [9]. In other words, it is a “measure of the imprecision of an assay at a low analyte concentration and involves variation that would be observed in many repeated measures of a single biological sample under unchanging conditions that is usually defined at the concentration resulting in a CV of 20%” [10]. Currently, FS is widely used as a measure of the clinical utility of Tg assays, and each successive generation shows significantly improved FS [10].

After the recent introduction of the hsTg test, many reports have suggested that rhTSH stimulation is no longer necessary in low-risk DTC patients with undetectable Tg concentrations, as the hsTg test is sufficient and has a high negative predictive value (NPV) [10]. In a meta-analysis of nine studies including 3,178 DTC patients, it was suggested that undetectable hsTg concentrations (for example, below the FS) without rhTSH stimulation, have a very high NPV (98%-100%) and an adequate sensitivity for the detection of recurrent disease (88%-98%) [11]. Spencer et al. [7] not only insisted that the enhanced FS of hsTg obviates the need for rhTSH stimulation but also indicated that an increasing trend in basal hsTg levels (non-rhTSH-stimulated hsTg) is a more sensitive tool for detecting disease recurrence than a conventional rhTSH-stimulated Tg test. Giovanella et al. [12] also suggested that a Tg assay with a FS of 0.2-0.3 ng/mL is accurate enough to forgo rhTSH stimulation in low-risk DTC patients. Undetectable hsTg concentrations suggest a low risk of DTC recurrence. Even when hsTg becomes detectable during follow-up, evaluation of the hsTg-slope over a three to six month period accurately identifies recurrence during both short- and long-term follow-up, with an NPV of 100% [12]. Therefore, Tg concentrations that are undetectable using an insensitive first-generation assay, such as the RIA method, should be interpreted with caution, as Tg levels might be detectable when using the hsTg test. In this study, we showed that the FS of the RIA method was approximately 10-fold lower than that of the ECLIA method; therefore, when using the RIA method, clinicians could miss early detection of residual or recurrent disease, which would prevent timely management. However, despite the growing number of studies, each laboratory and clinic must establish its own guidelines for interpretation and cut-offs for the hsTg test for various clinical decisions [10].

This study has several limitations. First, although both the second-generation ECLIA and RIA reagents are calibrated against the international certified reference material 457, these two reagents yielded different Tg values due to the inherent differences in the assay methods, instruments, calculations, etc. Therefore, the LOD and FS values of those two reagents should be compared with caution. Second, we determined the FS using a modification of the CLSI guideline, which does not reflect the true LOD or FS.

Thus, the second-generation ECLIA method exhibited better performance than the RIA method in terms of
FS, which is extremely important for the early detection of residual or recurrent disease in patients with DTC after total thyroidectomy. The excellent performance of the second-generation ECLIA should allow clinical Tg measurement without TSH stimulation when monitoring DTC patients after total thyroidectomy: in contrast to the RIA method, which had insufficient performance and requires TSH stimulation.

REFERENCES

1. National Cancer Information Center. Cancer prevalence statistics. http://www.cancer.go.kr/mbs/cancer/subview.jsp?id=cancer_040402000000 (Accessed May 12, 2015).
2. Grogan RH, Mitmaker EJ, Clark OH. The evolution of biomarkers in thyroid cancer-from mass screening to a personalized biosignature. Cancers (Basel) 2010;2:885-912.
3. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016;26:1-133.
4. Busaidy NL and Cabanillas ME. Differentiated thyroid cancer: management of patients with radioiodine nonresponsive disease. J Thyroid Res 2012;2012:618985.
5. Pacini F, Castagna MG, Brill L, Pentheroudakis G; ESMO Guidelines Working Group. Thyroid cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v214-9.
6. Giovanella L, Feldt-Rasmussen U, Verburg FA, Grebe SK, Plebani M, Clark PM. Thyroglobulin measurement by highly sensitive assays: focus on laboratory challenges. Clin Chem Lab Med 2015;53:1301-14.
7. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies. Curr Opin Endocrinol Diabetes Obes 2014;21:394-404.
8. Clinical and Laboratory Standards Institute. Evaluation of precision of quantitative measurement procedures: approved guideline. 3rd ed. Wayne (PA): Clinical and Laboratory Standards Institute, 2014.
9. Clinical and Laboratory Standards Institute. Evaluation of detection capability for clinical laboratory measurement procedures: approved guideline. 2nd ed. Wayne (PA): Clinical and Laboratory Standards Institute, 2012.
10. Giovanella L, Clark PM, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, et al. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. Eur J Endocrinol 2014;171:R33-46.
11. Giovanella L, Treglia G, Sadeghi R, Trimboli P, Ceriani L, Verburg FA. Unstimulated highly sensitive thyroglobulin in follow-up of differentiated thyroid cancer patients: a meta-analysis. J Clin Endocrinol Metab 2014;99:440-7.
12. Giovanella L, Maffioli M, Ceriani L, De Palma D, Spriano G. Unstimulated high sensitive thyroglobulin measurement predicts outcome of differentiated thyroid carcinoma. Clin Chem Lab Med 2009;47:1001-4.
Thyroglobulin 검사법의 정밀도 및 기능성 민감도 평가: 2세대 로슈 전기화학발광 면역측정법과 브람스 방사선면역측정법의 비교

권애린1 • 이은희1 • 이영경2 • 강희정2
1녹십자료재단, 2한림대학교 의과대학 한림대학교성심병원 진단검사의학과

배경: Thyroglobulin (Tg)은 정상 또는 분화갑상선암세포에서만 분비되므로 분화갑상선암 (differentiated thyroid cancer, DTC) 환자에서 갑상선전절제술 후 잔존질환 및 재발의 확인과 추적 관찰에 가장 중요한 생화학표지자이다. 저자들은 최근 개발된 전기화학발광 면역측정법(electrochemiluminescent immunoassay, ECLIA)을 이용한 고민감도 Tg (highly sensitive Tg, hsTg) 시약의 정밀도와 기능성 민감도를 저농도 혼주혈청을 이용하여 방사선면역측정법 (radioimmunoassay, RIA)과 비교평가하였다.

방법: ECLIA법을 이용한 Elecsys Tg II 시약(Roche Diagnostics, Germany)과 RIA법을 이용한 Tg-plus-RIA 시약(BRAHAMS, Germany)의 정밀도, 검출한계 및 기능성민감도를 Clinical and Laboratory Standards Institute (CLSI) 지침에 따라(기능성 민감도는 CLSI 지침을 수정하여 평가) 각각 E170 장비(Roche Diagnostics, Germany)와 Cobra Quantum 감마카운터기(Packard Instrument Company, USA)를 사용하여 평가하였다.

결과: ECLIA법을 이용한 hsTg 시약과 RIA법을 이용한 시약의 정밀도는 각각 9.6%과 48.2%였다. 제조사가 제시한 검출한계는 ECLIA법을 이용한 hsTg 시약에서는 검증되었지만(0.04 ng/mL), RIA법을 이용한 시약에서는 제조사가 제시한 것보다 월등히 높았다(0.08 ng/mL 이상). 기능성 민감도 역시 ECLIA법을 이용한 hsTg가(10%의 정밀도에서 0.04 ng/mL) RIA법을 이용한 시약보다(20%의 정밀도에서 0.37 ng/mL) 월등히 우수하였다.

결론: ECLIA법을 이용한 hsTg 시약이 RIA법을 이용한 시약보다 기능성 민감도가 우수하였는데, 이는 갑상선전절제술을 시행한 DTC 환자에서 잔존질환 및 재발의 조기검출에 매우 중요한 요소이다. ECLIA법을 이용한 hsTg 시약의 우수한 성능은 RIA법을 이용한 시약의 낮은 성능에 비해 갑상선자 극초초음파의 투여 없이도 갑상선전절제술 후 DTC 환자의 추적관찰에 임상적으로 유용하게 쓰일 수 있을 것으로 판단된다.

(J Lab Med Qual Assur 2016;38:243-248)

교신저자: 강희정
주14068 경기도 안양시 동안구 관평로170번길 22, 한림대학교 의과대학 한림대학교성심병원 진단검사의학과
Tel: 031)380-3929, Fax: 031)380-3934, E-mail: kangheejeung@hallym.or.kr