

Clinical Value of a Rapid Fully Automated Thyroid Autoantibody Assay in the Diagnosis and Management of Graves Disease

HANITA O¹, AZURA NR¹, FAIZAL MMZ²

¹Department of Diagnostic Laboratory Services, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaakob Latif, 56000 Bandar Tun Razak, Cheras, Kuala Lumpur, Malaysia

²Department of Biomedical Science, Faculty of Allied Science, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

ABSTRAK

Penyebab utama hipertiroidisme adalah Penyakit Grave (GD). Ia merupakan penyakit autoimun dimana berlaku pengikatan antibodi terhadap reseptor hormon perangsang tiroid (TRAb). Tujuan kajian ini adalah untuk memvalidasi pengujian TRAb dari segi kejituhan, sensitiviti, spesifisiti dan nilai *cut-off*. Ujian kejituhan (CV) dilakukan secara dalam masa dan sela masa menggunakan dua tahap kawalan kualiti pada julat kepekatan rendah 3.78-7.02 IU/L dan julat kepekatan tinggi 13.5-21.2 IU/L. Untuk menentukan sensitiviti, spesifisiti dan nilai *cut-off*, 124 sampel serum dari 46 GD, tujuh tiroiditis Hashimoto (HD), 11 goiter nodular *non-autoimmune* (NAG), dua kanser tiroid and 58 normal sebagai kawalan telah diambil secara retrospektif. Kejituhan dalam masa adalah 2.4% pada kepekatan 3.90 IU/L(julat: 3.78-7.02 IU/l) and 0.8% pada kepekatan 20.80 IU/L (julat:13.5-21.2 IU/l). Kejituuan keseluruhan adalah 3.8% pada kepekatan 3.80 IU/L (julat:13.5-21.2 IU/l) and 1.0% pada 20.8 IU/L (julat:13.5-21.2 IU/l). Analisa *Receiver-operating characteristic* (ROC) menunjukkan sensitiviti terbaik (94%) dan spesifisiti terbaik (98%) adalah pada nilai *cut-off* 1.69 IU/L. *Positive predictive value (PPV)* dan *negative predictive value (NPV)* berada pada 95% dan 94%. Pada nilai 1.69 IU/l ini, didapati sensitiviti untuk 29 sampel pesakit yang baru didiagnosa dengan GD berada pada 94%. Dari kajian ini didapati esai TRAb yang mengambil masa 27 minit ini mempunyai kejituuan yang baik, sensitiviti yang tinggi untuk mengesan penyakit GD dan spesifisiti yang bagus untuk membezakan GD dari penyakit tiroid yang lain dan membantu dalam rawatan pesakit tiroid.

Kata kunci: penyakit Graves, reseptor hormon perangsang thyroid, kejituuan, sensitiviti, spesifisiti

Address for correspondence and reprint requests: Dr. Hanita Othman, Department of Diagnostic Laboratory Services, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaakob Latif, Bandar Tun Razak, Cheras,56000, Kuala Lumpur . Tel: 603-91455449 Fax: 603-91456579. Email: drhanita@ppukm.ukm.edu.my

ABSTRACT

The most common cause of hyperthyroidism is Graves disease (GD) which is characterised by the presence of autoantibodies which binds to the TSH receptor (TRAb). Recently, a rapid, fully automated electrochemiluminescent immunoassay Elecsys® Anti-TSHR for detection of autoantibodies to TSH receptor was made available for routine clinical use. The objective of this study is to evaluate this assay and to determine the sensitivity, specificity and cut-off value. Interassay and total imprecision (CV) were determined at 3.78-7.02 IU/L and 13.5-21.2 IU/L respectively. A total of 124 samples which comprised of 46 GD, seven Hashimoto thyroiditis (HD), 11 non autoimmune nodular goitre (NAG), 2 thyroid cancers (Ca) and 58 normal controls were retrospectively analysed to determine the sensitivity, specificity and cut-off value. Inter-assay CV's were 2.4% at a concentration of 3.90 IU/L (range: 3.78-7.02 IU/l) and 0.8% at 20.80 IU/L (range:13.5-21.2 IU/l). Total imprecision was 3.8% at a concentration of 3.80 IU/L (range:13.5-21.2 IU/l) and 1.0% at 20.8 IU/L (range:13.5-21.2 IU/l). The ROC analysis of patients with GD, other thyroid disorders and normal controls revealed that the highest sensitivity (94%) and specificity (98%) were seen at cut-off value of 1.69 IU/L. Positive predictive value (PPV) and negative predictive value (NPV) was 95% and 94% respectively. At this derived cut-off value of 1.69 IU/L, we found that the sensitivity of TRAb positivity within the group of 29 newly diagnosed GD patients was 94%. Our results demonstrate that this fully automated assay with testing time of 27 minutes has high sensitivity in detecting GD and high specificity for discriminating other thyroid disease and represent major improvement in the diagnosis and management of patients with thyroid diseases.

Key words: Graves disease, TSH Receptor, reproducibility, sensitivity, specificity

INTRODUCTION

Graves disease (GD) is a thyroid-specific autoimmune disease characterised by the presence of autoantibodies which binds to the TSH receptor (TRAb) and leads to overactivity of the thyroid gland which clinically manifest as hyperthyroidism. Besides that, the inflammation of the eye muscles by attacking autoantibodies give rise to Graves orbitopathy (GO).

The detection of TRAb is valuable in confirming the diagnosis of GD in mild hyperthyroidism, euthyroid Graves ophthalmopathy without

goiter, differentiating GD from other toxic nodular goiter or factitious thyrotoxicosis (Weetman et al. 2000) and recent studies have shown that TRAb detection is potentially useful in predicting outcome in patient with GD and GO respectively. (Smith et al. 2007) The measurement of TRAb has been shown to be valuable in determining the causes of post partum thyrotoxicosis either due to post-partum thyroiditis or Graves disease. Furthermore this marker is useful in predicting the likelihood of either foetal or neonatal thyrotoxicosis in women undergoing

treatment or those who had thyroid ablation (Saravanan & Dayan 2001).

In tandem with advance in technology, great improvement has been achieved in TRAb detection methodologies. At present, the bioassay and competitive TSH inhibition binding assays have been established. (Sanders et al. 2002; Scott et al. 2005; Smith et al. 2007). Among them, the competitive assays are the only validated routine test for TRAb analysis. These assays are based on the ability of TRAb to inhibit TSH receptor (TSHR) binding of labelled bovine TSH because purified bovine TSH has higher affinity towards TSH receptor than human TSH and it is a more suitable ligand for ^{125}I or biotin labelling (Morgenthaler 1999). An alternative to bovine TSH have been available, for example, labelled mouse thyroid-stimulating monoclonal antibodies with similar performance (Sanders et al. 2002). More recently, Smith et al. (2005) developed an enzyme-linked immunosorbent assay (ELISA) using a labelled human thyroid stimulating monoclonal autoantibody M22 (Smith et al. 2005). In this assay, TRAb are detected based on their ability to inhibit the binding of labelled human monoclonal thyroid stimulating antibody (M22) to porcine TSH-coated ELISA plates.

Three comparison studies have been published (Kamijo 2003; Kamijo 2007; Liu et al. 2008) and two studies have found that the M22-biotin based ELISA have excellent sensitivity and specificity (Kamijo 2003; Kamijo 2007) while the remaining study showed relatively poor precision ($\text{CV} > 20$) (Liu et al. 2008).

As with other manual assays, the drawback of this ELISA assay is

on their sensitivity, specificity and reproducibility. Furthermore, the typically long incubation times and intense manpower needed to perform this assay, make it unpopular in busy service laboratories.

Recently, a rapid (27 minutes), fully automated electrochemiluminescent immunoassay Elecsys®Anti-TSHR for detection of autoantibodies to TSH receptor was made available for routine clinical use. The methodology of this assay incorporated the used of solubilized porcine TSH receptor immunocomplexed with a biotynylated mouse monoclonal antibody to the porcine TSH receptor and M22 human monoclonal antibody as a ruthenium-labelled assay ligand.

The aim of this study was to evaluate the Elecsys®Anti-TSHR assay system in terms of reproducibility, sensitivity, specificity and to determine the cut-off value of this assay and the feasibility of this assay to be offered as a routine laboratory test for UKM Medical Centre (UKMMC).

MATERIALS AND METHODS

The study was conducted in the Chemical Pathology Laboratory, Department of Diagnostic Laboratory Services, UKMMC.

Sample collection

The samples were acquired from the Endocrinology and Chemical Pathology laboratories and the diagnosis of the patients were based on the patients' record from the hospital or laboratory information system.

Inclusion criteria: Samples from patients with thyroid disorders which

include GD, Hashimoto thyroiditis (HD), non autoimmune nodular goitre (NAG), thyroid cancer (Ca).

Reproducibility study (Coefficient variation, CV):

The intra-assay imprecision CV was determined by 21 replicates in a single run on Cobas e411 electrochemiluminescent immunoassay analyzer using a manufacturer control material of two levels PreciControl Thryo 1 (PCThyro 1 range: 3.78-7.02 IU/L) and PreciControl Thryo 2 (PCThyro 2 range: 13.5-21.2 IU/L).

Total imprecision CV study was performed according to CLSI/NCCLS guideline EP5 A2 using aliquots of control materials PC Thryo 1 (range: 3.78-7.02 IU/L) and PC Thryo 2 (range: 13.5-21.2 IU/L) which were analysed in two determinations per run and two runs per day for five days.

Sensitivity, specificity and cut-off value:

The determination of optimal decision threshold level for positivity by receiver-operating characteristic (ROC) analysis was performed using serum from apparently healthy subjects, patients with GD and patients with other thyroid diseases which were acquired retrospectively.

Duration of assay performance

To validate the claim by the manufacturer that the total duration of this assay is 27 minutes, we examined the time from the samples was loaded in the analyzer and the time the result was made available by the analyzer.

Test principle

The Elecsys® Anti-TSHR assay is a third generation assay using a preformed immunocomplex based on native porcine TSHR solubalised from a thyroid cell membrane preparation and biotinylated anti-porcine TSHR mouse monoclonal capture antibody and a ruthenium labeled human TSHR stimulating monoclonal detection antibody (M22). The capture antibody binds to the C-terminal moiety of the porcine TSHR (Bolton et al. 1999) which does not interfere with the binding of TRAb or M22 to the TSHR. After addition of streptavidin-coated microparticles and ruthenium labeled M22, serum TRAb bound to the TSH receptor are detected by the ability to inhibit the binding of labeled M22. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. Anti-TSHR is calibrated against the *National Institute for Biological Standards and Control* (NIBSC) 90/672 standard. The test measuring range was 0.3-40 IU/l. The within run CV for PCThyro 1 and PCThyro 2 were 3.1 and 1.4 respectively and the total CV for PCThyro 1 and PCThyro 2 were reported at 5.5 and 2.4 respectively. The cut-off value was 1.75 IU/l. However, each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Statistical analysis

All clinical and laboratory data were stored and analyzed using the Statistical Package for Social Science (SPSS) software version 17.0.

A receiver operator characteristic (ROC) curve was constructed to determine the sensitivity, specificity and cut-off value.

In all statistical analyses, $p < 0.05$ (95% confidence interval) were considered significant.

RESULTS

Reproducibility study (Coefficient variation CV):

The mean value for PreciControl Thyro 1 and 2 were 3.90 IU/l (range: 3.78-7.02 IU/l and 20.80 IU/l (range 13.50-21.20 IU/L) respectively. The intra-assay CVs were 2.4% and 0.8% for level PreciControl Thyro 1 and 2 respectively. The total imprecision were 3.8% and 1.0% for PreciControl Thyro 1 (mean: 3.8 IU/l, range: 3.78-7.02 IU/l) and PreciControl Thyro 2 (mean: 20.80 IU/l, range 13.50-21.20 IU/L). Results of the reproducibility study are summarized in Table 1.

Sensitivity, specificity and cut-off value:

A total of 124 samples which comprised of 46 GD (29 newly diagnosed GD and not on antithyroid therapy), 7 HD, 11 NAG, 2 Ca and 58 normal controls were retrospectively analysed to determine the sensitivity, specificity and cut-off value. The patients' general characteristics are shown in Table 2. The samples were divided into two cohorts; sensitivity cohort comprises of the GD patients and the specificity cohort are made up by 20 patients with other thyroid diseases and 58 normal

control subjects. The mean age of the study population was 40.3 ± 16.1 years (range: 14-69 years). There was significant difference between the age of the patients in the sensitivity cohort and those in the specificity cohort, (35.3 ± 15.7 years vs 42.7 ± 16.4 years, ($p < 0.05$)). Female preponderance was seen in both sensitivity and specificity cohorts. The majority of the study population was Malay followed by Chinese and Indian in both cohorts. (Table 2)

The ROC analysis showed an area under curve (AUC) of 0.99 (95% CI: 0.98-1.0). The optimal pair of sensitivity (94%) and specificity (98%) was found at a TRAb level of 1.69 IU/l (Figure 2). Positive predictive value (PPV) and negative predictive value (NPV) was 95% and 94% respectively

Duration of assay performance

The mean total duration of the assay was 25.70 ± 0.02 minutes (n=124). This finding validated the manufacturer's claim of the total assay duration of 27 minutes.

DISCUSSION

The diagnosis of Graves hyperthyroidism is based on the clinical presentation and biochemical changes of hyperthyroidism. Traditionally, serum TSH and free T4 are measured to confirm the diagnosis of hyperthyroidism.

The measurement of serum TRAb is not routinely done because the diagnosis of GD can nearly always be made correctly based on the clinical findings. Furthermore, the inability

Table 1: Reproducibility study (Coefficient variation, CV)

| Material | Range(IU/l) | Mean (IU/l) | CV |
|--|--------------|-------------|------|
| Inter assay CV, n =21 replicates in a single run | | | |
| PreciControl Thyro 1 | 3.78-7.02 | 3.90 | 2.4% |
| PreciControl Thryo 2 | 3.50-21.20 | 20.80 | 0.8% |
| Total CV, n = 40 replicates in 10 runs | | | |
| PreciControl Thryo 1 | 3.78-7.02 | 3.80 | 3.8% |
| PreciControl Thryo 2 | 13.50-21.20 | 20.80 | 1.0% |

Table 2: General characteristic of the study population

| Patients Characteristic | (n=124) | Sensitivity Cohort (Graves Disease) (n = 46:29 newly diagnosed) | Specificity Cohort (Normal Control Non Graves Disease) (n = 78) |
|-------------------------|----------------------------------|---|--|
| Age (Mean±sd) | 40.3±16.1 (range 13-72 years) | 35.3±15.7 (14-61 years) | 42.7±16.4 (range: 13-72 years) |
| Gender | | | |
| Male | 48 (38.7%) | 17 (36.9%) | 33 (42.3%) |
| Female | 76 (61.3%) | 29 (63.1%) | 45 (57.7%) |
| Race | | | |
| Malay | 72 (58.1%) | 20 (43.5%) | 37 (47.4%) |
| Chinese | 46 (37.1%) | 24 (52.2%) | 35 (44.9%) |
| Indian | 6 (4.8%) | 2 (4.3%) | 4 (5.1%) |
| Others | | | 2 (2.6%) |
| Normal Control | 58 | | |
| Non Graves Disease | 20 | | |
| Graves Disease | 46 | | |

of the routine laboratory to perform this test partly due to the facts that different methodology gives different sensitivity and specificity and the test method can be cumbersome. With great improvement in TRAb detection methodologies, this test can be done routinely in most laboratories.

We evaluated the third generation, fully automated electrochemiluminescent immunoassay Elecsys[®]Anti-TSHR for detection of autoantibodies to TSH receptor.

From this study, the intra-assays and total imprecision CVs were found to be less than 5%. This finding showed that Elecsys[®]Anti-TSHR assay meets the requirement CVs of less than 20%. (Hermsen et al. 2009)

With regards to the cut-off value, the optimal pair of sensitivity (94%) and specificity (98%) was found at TRAb level of 1.69 IU/L. The calculated Positive predictive value (PPV) and Negative predictive value (NPV) were 96% and 97% respectively. We further

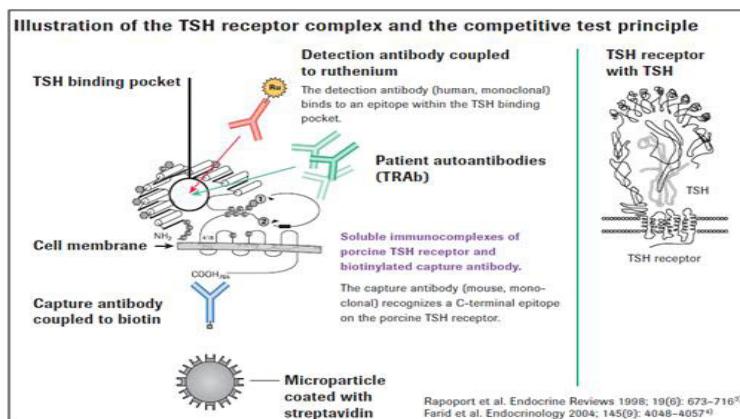


Figure 1: Illustration of the TSH receptor complex and the competitive test principle

studied the 29 newly diagnosed GD patients; at the derived cut-off value of 1.69IU/L, we found that the sensitivity of TRAb assay was 94%.

Our finding was different from the manufacturer expected value and other studies (Hermsen et al. 2009; Schott et al. 2009) which reported the cut-off value as 1.75 IU/l. When we used the calculated cut-off for TRAb positivity of 1.75 IU/l, we found the sensitivity and specificity of 87% (95% CI: 0.86- 0.93) and 99% (95% CI: 0.97- 0.99).

This discrepancy in the cut-off value could be due to the difference in the studied population and small sample numbers. In this study we included the GD which already received antithyroid drug therapy including GD patient on long-term follow up, while in the study by Hermsen et al. (2009) only the untreated GD patients were included as sensitivity cohort. We included this group of patient to simulate the requirements in clinical routine when patients are included with long-lasting history of GD and potential negative TRAbs. Schott et al. (2009), performed two sets of ROC analysis, the first

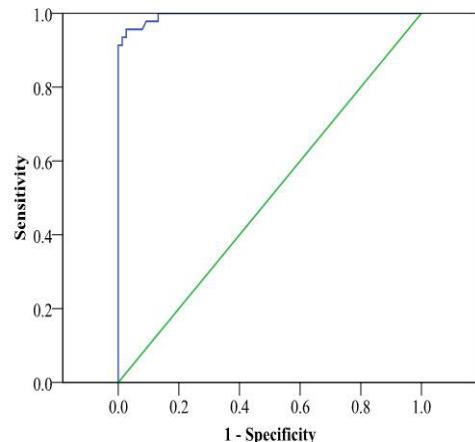


Figure 2: The ROC curve performed on 46 GD (sensitivity cohort), 20 patients with other thyroid disease and 58 apparently normal control (specificity cohort) The optimal cut off value was calculated at 1.69 IU/l (sensitivity 94%, specificity 98%), AUC 0.99 (95% CI: 0.98-1.0)

analysis include only the untreated GD patients as sensitivity cohort whilst the other include all the GD patients; they found that at 1.75IU/l, lower sensitivity and good specificity were found when all GD patients were analysed together; a finding which was similar to ours.

From this study, we concluded that the fully automated

electrochemiluminescent immunoassay Elecsys®Anti-TSHR assay with testing time of 27 minutes has excellent reproducibility with high sensitivity in detecting GD and high specificity for discriminating other thyroid diseases. Moreover, with the rapid turn-around time and less man power needed than the manual assay, it is feasible to be offered as a routine laboratory test and represent a major improvement in the diagnosis and management of patients with thyroid diseases.

Authors' disclosures of Potential Conflicts of Interest: All authors completed and signed the disclosure conflict of interest form before conducting this study.

Role of sponsor: The funding organization play no role in the design of this study, choice of enrolled patients, review and interpretation of data or preparation or approval of this manuscript.

ACKNOWLEDGEMENT.

This study was supported by Roche Diagnostics.

REFERENCES

- Bolton, J., Sanders, J., Oda, Y., Chapman, C., Konno, R., Furmaniak, J., Rees Smith, B. 1999. Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA. *Clin Chem* 45(12): 2285-2289.
- Hermsen, D., Broecker-Preuss, M., Casati, M., Mas, J.C., Eckstein, A., Gassner, D., van Helden, J., Inomata, K., Jarausch, J., Kratzsch, J., Mann, K., Miyazaki, N., Navarro Moreno, M.A., Murakami, T., Roth, H.J., Noh, J.Y., Scherbaum, W.A., Schott, M. 2009. Technical evaluation of the first fully automated assay for the detection of TSH receptor autoantibodies. *Clin Chem Acta* 401(1-2):84-89.
- Kamijo, K. 2003. TSH-receptor antibody measurement in patients with various thyrotoxicosis and Hashimoto thyroiditis: a comparison of two steps assay using porcine TSH-receptor and human recombinant TSH receptor. *Endocr J* 50(1):113-116.
- Kamijo, K. 2007. TSH-receptor antibody determined by first, second and third generation assay. *Endocr J* 54(4): 619-624.
- Liu, C., Hermsen, D., Domberg, J. 2008. Comparison of M22-based ELISA and human-TSH-receptor-based luminescence assay for the measurement of thyrotropin receptor antibodies in patients with thyroid diseases. *Horm Metab Res* 40(7):479-483.
- Morgenthaler,N.G 1999. New assay systems for thyrotropin receptor antibodies. *Curr Opin Endocrinol Diabetes* 6:251-260.
- Sanders, J., Jeffreys, J., Depraetere, H., Richards, T., Evans, M., Kiddie, A., Brereton, K., Groenen, M., Oda, Y., Furmaniak, J., Rees Smith, B. 2002. Thyroid stimulating monoclonal antibodies. *Thyroid* 12(12):1043-1050.
- Saravanan, P., Dayan, C.M. 2001. Thyroid autoantibodies. *Endocrinol Metab Clin North Am* 30(2):315-337.
- Schott, M., Hermsen, D., Martina, B.P., Marco, C.J. 2009. Clinical value of first automated TSH receptor autoantibody assay for the diagnosis of Graves disease: an international multicentre trial. *Clin Endocrinol* 63 enclosure 6:1-8.
- Schott, M., Scherbaum, W.A., Morgenthaler, N.G. 2005. Thyrotropin receptor autoantibodies in Graves disease. *Trends Endocrinol Metab* 16(5):243-248
- Smith, B.R., Bolton, J., Young, S., Collyer, A., Weeden, A., Bradbury, J., Weightman, D., Perros, P., Sanders, J., Furmaniak, J. 2005 A new assay for thyrotropin receptor autoantibodies. *Thyroid* 14(10):830-835.
- Smith, B.R., Sanders, J., Furmaniak, J. 2007. TSH receptor antibodies. *Thyroid* 17(10):923-38
- Weetman, A.P. 2000. Graves' Disease. *N Engl J Med* 343(17): 1236- 48.