Original Article

Performance characteristics of three automated immunoassays for thyroid hormones

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Abstract

Background: Since the introduction of the first radioimmunoassay, several improvements have been made in the design of immunoassays such as method of antibody production, labeling, automation and detection technology. We performed an analytical evaluation of the new electrochemiluminescent immunoassay (ECLIA) for serum TSH, FT4 and T3 in the Elecsys 2010 immunoassay system and compared the results of this method with those of radioimmunoassay (RIA) [immunoradiometric (IRMA) for TSH] and Elisa.

Methods: Fasting serum from 112 hypo, hyper and euthyroid patients were used to evaluate the minimum detectable concentration, intra- and inter-assay precisions for TSH, FT4, T3, linearity for TSH assay and method comparison study.

Results: Within the analytical range tested, intra-assay coefficient of variation was < 2.3% for TSH, 2.3% for FT4 and 7.8% for T3. The inter-assay coefficient of variation was < 2.9% for TSH, 2.5% for FT4 and 12.3% for T3. The measurement of diluted sera indicated a desirable percentage of recovery for TSH. No correlation was found between Elecsys 2010 and Elisa /IRMA for TSH. The comparison of results of the Elecsys ECLIA assay with those of Elisa and RIA for T4 were: T4 (ECLIA) = -0.612+0.999, T4 (Elisa, r= 0.88) and T4 (ECLIA)=0.642+0.942 T4 (RIA, r=0.957), while ECLIA assay with Elisa and RIA for T3 were: T3 (ECLIA)= 0.242+0.908 T3 (Elisa, r=0.8) and T3 (ECLIA) = -0.029+1.01 T3 (RIA, r=0.957).

Conclusion: The results show that Elecsys 2010 is an automated reliable, efficient and technically excellent instrument to use in the measurement of serum TSH, T4 and T3.

Keywords: Luminescent Measurements/methods, Thyroid hormones, Immunoassay

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Thyroid disease is one of the most common endocrine disorders (1). The laboratory diagnosis and monitoring of thyroid diseases such as hypo and hyper thyroidism are based on serum TSH measurement along with serum T4 and T3 (both free and total) (2). The National Academy of Clinical Biochemistry (NACB) has recommended that the functional sensitivity of TSH assay be less or equal to 0.02 mIU/L. This permits patients with nonthyroid illness to be distinguished from those with primary hyperthyroidism. This is particularly important in patients hospitalized with nonthyroid illness where TSH concentration as low as 0.02 mIU/L may be encountered (3).

The analytical sensitivity of TSH assay and its ability to reliably distinguish between euthyroid and hyperthyroid patients especially in subclinicial stages, where T4 and T3 levels are in normal range makes it a very sensitive marker of primary thyroid function abnormalities (4). Several years ago, the most commonly used assay for the measurement of TSH was radioimmunoassay which was considered as the first generation method with functional sensitivity of 1 mIU/L, IRMA was the second generation method with functional sensitivity of 0.1 mIU/L from the 1990s to date, and the third generation method was electrochemiluminescence assay that had been introduced with improved functional sensitivity (5).

Electrochemiluminescence or electrogenerated chemiluminescence (ECL) is a kind of luminescence produced during electrochemical reactions in solution. In electrogenerated chemiluminescence, electrochemically generated intermediates undergo a highly exergonic reaction to produce an electronically excited state that emits light (6). ECL excitation is caused by energetic electron transfer (redox) reactions of electrogenerated species. Such luminescence excitation is a form of chemiluminescence where one/all reactants are produced electrochemically on the electrodes (7). ECL is usually observed during the application of potential (several volts) to electrodes of electrochemical cell that contains solution of luminescent species (polycyclic aromatic hydrocarbons, metal complexes) in aprotic organic solvent (ECL composition). ECL proved to be very useful in analytical applications as a highly sensitive and selective method. It combines the analytical advantages of chemiluminescent analysis (absence of background optical signal) with ease of reaction control by applying electrode potential. Enhanced selectivity of ECL analysis is reached by variation of electrode potential thus controlling species that are oxidized/reduced at the electrode and take part in ECL reaction (8).

It generally uses Ruthenium complexes, esp $[Ru(Bpy)3]^{2+}$ (which releases a photon at ~620 nm) regenerating with TPA (Tripropylamine) in liquid phase or liquid-solid interface. It can be used as monolayer immobilized on an electrode surface (made e.g. of nafion, or special thin films made by Langmuir-Blogett technique or self-assembly technique) or as a coreactant or more commonly as a tag and used in HPLC, Ru tagged antibody based immunoassays, Ru Tagged DNA probes for PCR etc., NADH or H_2O_2 generation based biosensors, oxalate and organic amine detection and many other applications and can be detected from picomolar sensitivity to dynamic range of more than six orders of magnitude. Photon detection is done with photomultiplier tubes (PMT) or silicon photodiode or gold coated fiber-optic sensors. ECL is heavily used commercially for many clinical lab applications (9).

With respect to the increasing competition among laboratories in order to define the best sensitive assay with good reliability, we performed an analytical evaluation of the new electrochemiluminescent immunoassay (ECLIA) for serum TSH, FT4 and T3 in the Elecsys 2010 immunoassay system and compared the results of this method with those of RIA (IRMA for TSH) and Elisa.

Methods

Blood was collected from 112 hypo-hyper- and euthyroid individuals after 12 hours of fasting. Serum specimens were used to evaluate the minimum detectable concentration and intra- and inter-assay precisions for the three analytes (i.e.TSH, FT4, T3), linearity for the TSH assay and method comparision study. The ECLIA method was compared with those of Elisa and IRMA for TSH, Elisa and RIA for serum T3 and FT4 measurements.

Immunoassay on the fully automated Elecsys 2010 analyzer involves the electrochemiluminescent reaction of ruthenium (Ru II). Tris with triproplamine leads to the amplification of the light signal that allows high speed and dynamics of signal generation and measurement with this system.

TSH measurement is based on the sandwich principle whereas T3 and FT4 measurements are based on competition principle. RIA and Elisa assay for measurement of TSH are both based on sandwich principle whereas T3 and FT4 are based on competition principle (2, 10). To determine the minimum detectable concentration of TSH, Elecsys 2010 calibrator of zero concentration was used. Low detection limits was inferred from the means of 10 times a day measurement of the mentioned zero standard.

Imprecision was determined by analyzing the 2 levels of commercial control materials (low and high) and serum pools with low, mid and high concetrations. For intra-assay run imprecision estimation, the analytes were analyzed 20 times a day and 20 different non-consecutive days in one month for the inter-assay imprecision study (11, 12).

For linearity study, three serum samples with high concentrations of TSH were diluted with the universal BM diluent (Cat No. 155922) at 1.2 , 1.5, 1.10 of serum. Each dilution was tested in duplicate, the calculations were made by percentage differences between the expected and the observed values.

To evaluate the recovery for TSH serum of a well known hypothyroid patient, presumably that of containing a high concentration of TSH was selected. TSH measurement was repeated 4 times a day to assure its level. The mean of those determinations was considered the TSH value for the recovery evaluation.

The different amounts of this serum were added to three serum samples of the different concentration levels of TSH. As TSH was under the upper limit of analytical range of measurement, dilution was not found necessary (2). The

evaluation was made by the differences between the expected and observed values (recovery percentage). The results of Elecsys 2010 assay versus Elisa and RIA assays were subjected to Pearson correlation analysis and linear regression.

Results

The minimum detectable concentration was obtained when zero standards were processed for TSH with Elecsys 2010, Elisa and IRMA were 0.005, 0.3 and 0.1 mIU/L, respectively. The result obtained by Elecsys 2010 only coincided with the minimum detectable concentration proposed by manufacturers. The minimum detectable concentration for FT4 obtained by Elecsys 2010 and RIA were 0.3 pmol/L which coincided with the minimum detectable concentration proposed by the manufacturers. However, with Elisa method, the minimum detectable concentration did not meet the manufacturers' claim. The results of precision studies are summarized in table 1.

Table 1. Intraassay and Interassay Imprecision for the Elecsys 2010 TSH,FT_4 and T_3 assay

Imprecision	Sample	Mean			Coefficient of variation (%)		
		TSH	FT_4	T_3	TSH	FT_4	T_3
Intraassay	Precicontrol Low	1.01	14.5	2.2	2.3	1.9	5.2
	Precicontrol high	8.3	38.4	6.1	2.2	2.3	4.3
	Pool serum 1	0.81	15.1	1.6	2.0	2.0	7.8
	Pool serum 2	4.4	7.2	2.1	1.8	1.7	7.5
	Pool serum 3	78.9	5.5	3.2	1.1	1.9	5.9
Interassay	Precicontrol Low	0.95	14.7	2.38	2.9	2.5	5.9
	Precicontrol high	8.1	36.3	6.4	1.8	1.6	4.6
	Pool serum 1	0.78	16.1	1.9	1.5	2.1	12.3
	Pool serum 2	4.2	7.8	2.3	1.7	1.5	5.1
	Pool serum 3	75.6	5.3	2.9	1.4	1.4	5.9

The intra-assay coefficient of variation ranged from 1.1 to 2.3% for TSH, from 1.7 to 2.3% for FT4, and from 4.3 to 7.8% for T3 with Elecsys 2010 method. The inter-assay coefficient of variation varied from 1.4 to 2.9 % for TSH, from 1.4 to 2.5 % for FT4, and from 4.6 to 12.3% for T3. Based on the results, the obtained percentage of recoveries for TSH assay using Elecsys 2010 were 107, 106 and 95.6 for 12.6, 18.9 and 37.8 mIU/L concentrations, respectively (table 2).

Table 2. Recovery analyses of the TSH assay using Elecsys 2010

TSH Values (mIU/L)		
Thearetical values	Measured values	Recovery (%)
12.6	13.3	107
18.9	20.1	106
37.8	36.2	95.6

The results of linearity analyses of the TSH assay by Elecsys 2010 given in table 3 is also illustrated in figure 1. As expected at higher concentrations, typically there was deviation from linearity.

Table 3: Linearity analyses of the TSH assay using Elecsys 2010

Theoretical value	Dilution	Measured values (mIU/L)	Percentage difference
48.75	1/2	53.8	110
	1/5	46.2	94
	1/10	48.1	92
19.5	1/2	22.2	113
	1/5	22.8	116
	1/10	17.5	89
9.75	1/2	11.6	119
	1/5	10.1	87
	1/10	11.5	124

The inspection results from the method comparison study indicates no correlation between Elecsys 2010 and Elisa/IRMA for TSH. However, there were significant correlations between Elecsys 2010 and Elisa T4 (r=0.88, p<0.001), Elecsys 2010 and RIA for T4 (r=0.957, p<0.001), Elecsys 2010 and Elisa for T3 (r=0.8, p<0.001) and Elecsys 2010 and RIA for T3 (r=0.957, p<0.001).

The regression analysis equations obtained from the comparison of the results of Elecsys 2010 assay with Elisa and RIA are as follows: for T4; T4 (ECLIA)=-0.612+0.999 T4 (Elisa) and T4 (ECLIA)= 0.642+0.942 T4 (RIA), for T3; T3 (ECLIA)=0.242+0.908 T3 (Elisa) and T3 (ECLIA)= -0.029+1.01 T3 (RIA).

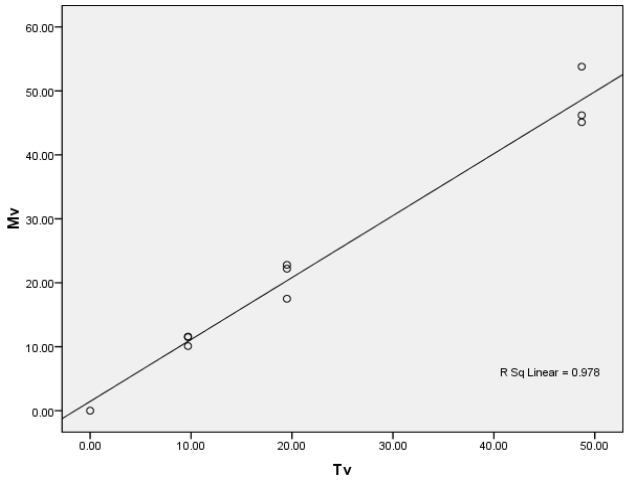

Figure 1. Graphical presentationof linearity plot of TSH assay by Elecsys 2010

Mv = measured value Tv = Theoretical value

Discussion

The minimum detectable concentration for TSH, FT4 and T3 by Elecsys 2010 was lower than the other commercial immunoassay methods. When zero standards were processed, the minimum TSH measurement was 0.005, 0.3 and 0.1 mIU/L by Elecsys 2010, Elisa and IRMA, respectively. The reason for this difference is the use of the 3rd generation TSH by Elecsys 2010 which is capable of measuring TSH concentration as low as 0.005 mIU/L. Another reason for this ability is that, Elecsys 2010 immunoanalyzer is based on a new detection technology that uses an electrochemiluminescent label. This is particularly important in its ability to differentiate the subclinical and clinical hyperthyroidism states.

This analyzer shows no carry–over in the measurement which can be expected in an automated system that changes its tips and curettes with every sample (13, 14). Linearity assays which were verified by diluting samples with Elecsys 2010 buffer indicated a desirable percentage of recovery. Based on percentage recovery, the obtained Elecsys 2010 assay for TSH, T3 and FT4 were more satisfactory than IRMA and Elisa methods.

In dilution studies performed for IRMA and Elecsys unlike Elisa; TSH, T3 and FT4 measurement results were independent of dilution factor .

Certain amount of carry–over has been reported with most immunoassay systems, however, with Elecsys method in which solutions are provided by the company itself , tips and curettes are changed with every sample and no carry – over has been found (13, 14). In this study, we did not evaluate the effect of lipemia, hemolysis and icterus on the hormone measurement, however, Kroll et al. have shown that in the Elecsys 2010 method, these parameters have no effect on TSH measurement (15).

According to the manufacturers, in the guidelines of Elecsys 2010, no hook effect for TSH concentrations up to 100 mU/L is expected. Because of the wide measuring range of the Elecsys method for TSH (0.005-100 mIU/L), the possibility of false low concentration for TSH is unlikely, whereas for IRMA and Elisa methods, the maximum reporting range proposed by manufacturers is 40 mIU/L (2). In pregnant women, because of high HCG concentration, there is a possibility of cross-reactivity in TSH assay ,however, it has been shown that high concentrations of HCG, FSH and LH have no cross-reactivity with the Elecsys TSH assay (16).

Regression analysis results showed no correlation between the Elecsys 2010 and the Elisa and IRMA methods. The results of our study indicate that Elecsys 2010 FT4 does correlate well with those measured by RIA and Elisa. The calibration curve stability of Elecsys 2010 is for at least 2 months and there is no need for daily calibration in contrast to IRMA for TSH and the RIA for FT4 and T3 (10, 2). This method shows a high degree of reproducibility and linearity with no carry-over effect. The low detection limit for TSH by Elecsys 2010 makes it a sensitive method for detecting patients with thyroid disorders.

In conclusion, we found that Elecsys 2010 is an automated reliable, efficient and technically excellent instrument to use in the measurement of serum TSH, FT4

and T3. The electrochemiluminescence technology of Elecsys 2010 shows the advantages in system performance. This method is particularly superior to other laboratory methods for the measurement of serum TSH since its minimum TSH concentration detectibility of 0.005 mIU/L facilitates the diagnosis of subclinical hyperthyroidism from euthyroid state with low serum TSH.

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Conflict of Interest: There is no conflict of interest.

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