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SHORT COMMUNICATION

Performance Evaluation of the Elecsys Growth Hormone and Insulin-Like Growth Factor 1 Assay

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SUMMARY

Background: **Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) are important biomarkers for diagnosis and follow-up of growth-related disorders. Performance of Elecsys hGH and Elecsys IGF-1 (Roche Diagnostics) was evaluated.**
Methods: **The performance of the Elecsys hGH and Elecsys IGF-1 assays on a Roche Cobas e 801 modular analyzer were evaluated. Repeatability, within-laboratory imprecision, and linearity were assessed. Elecsys hGH was compared to hGH [I-125] IRMA (Izotop), and Elecsys IGF-1 was compared to IRMA IGF-1 (Immunotech).**
Results: **The coefficient of variation of within-laboratory imprecision for Elecsys hGH and IGF-1 was less than 1.7%. The linearity values for Elecsys hGH and IGF-1 were confirmed over a range of 0.05 - 24.70 ng/mL and 40.41 - 687.53 ng/mL, respectively. The mean difference between Elecsys hGH and IRMA, and Elecsys IGF-1 and IRMA was 0.284 ng/mL and 14.21 ng/mL, respectively.**
Conclusions: **The analytical performance of Elecsys hGH and Elecsys IGF-1 was clinically acceptable.**
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KEY WORDS

evaluation, growth hormone, insulin-like growth factor 1, performance

INTRODUCTION

Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) play important roles in regulating growth and metabolism. GH and IGF-1 measurements are widely used in diagnosis of GH secretion disorders, evaluation of short stature, management of disorders that cause malnutrition or catabolism, and monitoring of GH and IGF-I replacement therapy [1].
Both GH and IGF-1 have been measured through various methods such as bioassays, immunoradiometric assays (IRMAs), enzyme immunoassays, mass spectrometry assays, and chemiluminescence assays [2,3]. However, there have been limited reports of comparisons between assay methods.
Here, we evaluated the analytical performance of Elecsys hGH (Roche Diagnostics, Mannheim, Germany) and Elecsys IGF-1 (Roche Diagnostics) and compared

Short Communication accepted December 26, 2019

them with IRMAs.

MATERIALS AND METHODS

This study was approved by the Ewha Womans University Seoul Hospital Institutional Review Board (approval number: SEUMC 2019-09-006).

Assay and analyzer

The Elecsys hGH and IGF-1 assays involve an electrochemiluminescence (ECLIA) sandwich immunoassay. For Elecsys hGH, biotinylated monoclonal hGH-specific antibody and a polyclonal hGH-specific antibody labeled with a ruthenium complex form a sandwich complex [4]. For Elecsys IGF-1, sample is pretreated with diluted hydrochloric acid to cleave IGF-1 from insulin-like growth factor binding-protein (IGFBP) and acid-labile subunit (ALS). A biotinylated monoclonal IGF-1-specific antibody and a monoclonal IGF-1-specific antibody labeled with a ruthenium complex react to form a sandwich complex [5]. In the present study, a Roche cobas e801 modular analyzer was used to evaluate performance.

Precision

Repeatability (within-run precision) and within-laboratory imprecision (intermediate imprecision) were evaluated according to Clinical and Laboratory Standards Institute (CLSI) document EP15-A3 [6]. For GH analysis, two levels of PreciControl Multimarkers (Roche Diagnostics) were used. For IGF-1 evaluation, PreciControl Growth (Roche Diagnostics) was used. Each sample material was tested for five days; one run per day with five duplicates.

Linearity

Linearity was evaluated according to CLSI document EP06-A [7]. By mixing high- and low-concentration patient samples, five equally spaced concentrations were prepared. Each sample was analyzed for three replicates. The results were analyzed using polynomial regression. The allowable bias was defined according to the Royal College of Pathologists of Australasia and the Australasian Clinical Biochemist Association Quality Assurance Program [8]. For GH and IGF-1, 1.0 ng/mL (0.333 mIU/L) or ± 15% and 22.9 ng/mL (3 nmol/L) or ± 15% from the assigned value were used, respectively.

Method comparison

For Elecsys hGH and Elecsys IGF-1 assays, 55 and 60 patient samples were used for comparison with IRMA, respectively. Radioactivity was counted on a Packard Cobra II gamma counter (Perkin-Elmer, Waltham, MA, USA) with hGH [I-125] IRMA KIT (Izotop, Budapest, Hungary) and IRMA IGF-1 (Immunotech, Marseille, France). Passing-Bablok regression analysis and coefficients of correlation (r) were calculated according to CLSI EP09-A3 [9]. The allowable mean differences within 1.0 ng/mL or ± 15% for GH and 22.9 ng/mL or ± 15% for IGF-1 were used as acceptance criteria.

RESULTS

Precision

Repeatability %CVs for GH and IGF-1 were 1.2 - 1.3% and 1.0 - 1.1%, respectively. Within-laboratory imprecision %CVs for GH and IGF-1 were 1.4 - 1.5% and 1.4 - 1.6, respectively (Table 1).

Linearity

The linearity values of Elecsys hGH and IGF-1 were analyzed within the ranges of 0.05 - 24.70 ng/mL and 40.41 - 687.53 ng/mL, respectively. Polynomial evaluation showed that both GH and IGF-1 assays were best fit to a third-order polynomial. The difference between third-order polynomial and linear fits of GH was 0.18 ng/mL for level 1, 0.2% for level 2, -2.0% for level 3, -1.4% for level 4, and 1.3% for level 5. The difference between third-order polynomial and linear fits of IGF-1 was 6.68 ng/mL for level 1, 0.7% for level 2, -2.7% for level 3, -2.1% for level 4, and 1.9% for level 5 (Figure 1).

Method comparison

Passing-Bablok regression analysis showed that Elecsys hGH (y) and Izotop hGH [I-125] IRMA (x) yielded a linear equation of $y = 1.068x + 0.088$ ($r = 0.998$). The mean difference between the two methods was 0.284 ng/mL (95% confidence interval, 0.1429 to 0.4242 ng/mL). The Passing-Bablok linear equation between Elecsys IGF-1 (y) and Immunotech IRMA IGF-1 (x) was $y = 0.9854x + 14.99$ ($r = 0.990$). The mean difference between the two methods was 14.21 ng/mL (95% confidence interval, 6.873 to 21.544 ng/mL) (Figure 2).

DISCUSSION

GH is primarily secreted by the pituitary gland and is present in circulation as a mixture of various isoforms. The 22 kDa GH molecule represents more than 90% of the total GH. The second most abundant GH isoform is the 20 kDa GH molecule, representing 10% of the total circulating GH [10]. GH binds to cell-surface growth hormone receptors and stimulates IGF-1 production. In circulation, 99% of IGF-I is bound to IGFBP or ALS. Increased serum GH and IGF-1 produce feedback loops that lead to inhibition of GH releasing hormone and release of somatostatin [11].

The Growth Hormone Research Society, IGF Society, and the International Federation of Clinical Chemistry (IFCC) made a consensus statement on standardization of GH and IGF-1 assays in 2011 [1]. The statement covered standard materials to antibody specificity and interference with regard to GH and IGF-1 assays. In the present study, we evaluated the Elecsys hGH and Elec-

Table 1. Precision profile of Elecsys hGH and Elecsys IGF-1 assay.

Analyte	Level	Mean concentration (ng/mL)	Repeatability		Within-laboratory imprecision	
			SD (ng/mL)	%CV	SD (ng/mL)	%CV
GH	1	0.96	0.01	1.3	0.01	1.4
	2	10.12	0.13	1.2	0.16	1.5
IGF-1	1	58.23	0.60	1.0	0.92	1.6
	2	347.16	3.70	1.1	4.80	1.4

Abbreviations: CV - coefficient of variation, SD - standard deviation.

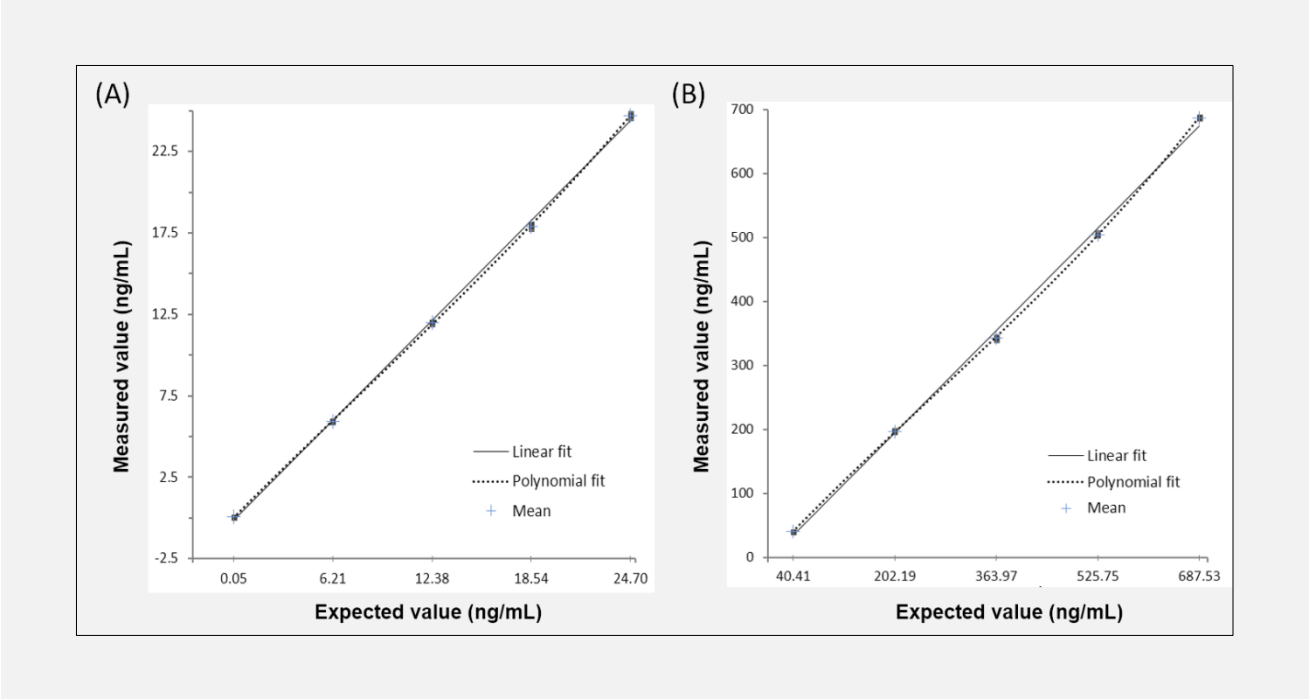

Figure 1. Linearity of (A) Elecsys hGH and (B) Elecsys IGF-1.

The solid black line represents the linear fit and the dashed line depicts 3rd order polynomial fit.

sys IGF-1 assays on a newly developed Roche Cobas e 801 modular analyzer.

Precision analysis of Elecsys hGH and IGF-1 showed a within-laboratory imprecision %CV less than 1.6% and 1.7%, respectively. These results were similar to the manufacturer's performance data [4,5]. Additionally, the within-laboratory imprecision of the Elecsys IGF-1 assay was lower than 7.3%, which was a desirable specification for imprecision criteria derived from intra- and inter-individual biologic variation [12]. Desirable imprecision criteria for GH assay based on biological variation was not available [12].

Elecsys hGH and IGF-1 linearity was confirmed within the ranges of 0.05 - 24.70 ng/mL and 40.41 - 687.53 ng/mL, respectively. The manufacturer proclaimed linearity intervals of Elecsys hGH and IGF-1 was 0.03 - 50.0 ng/mL and 7 - 1,600 ng/mL, respectively. Although polynomial regression showed that the best fit for Elecsys hGH and IGF-1 was a third-order polynomial, the difference between the best fit third-order and linear equations was within the allowable total error, 1.0 ng/mL or ± 15% and 22.9 ng/mL or ± 15% for GH and IGF-1, respectively.

Elecsys hGH showed good correlation with Izotop hGH [I-125] IRMA (y = 1.068x + 0.088, r = 0.998). However, the measured concentration of these two assays differed significantly (95% CI of the mean difference 0.1429 to 0.4242 ng/mL). The difference between these

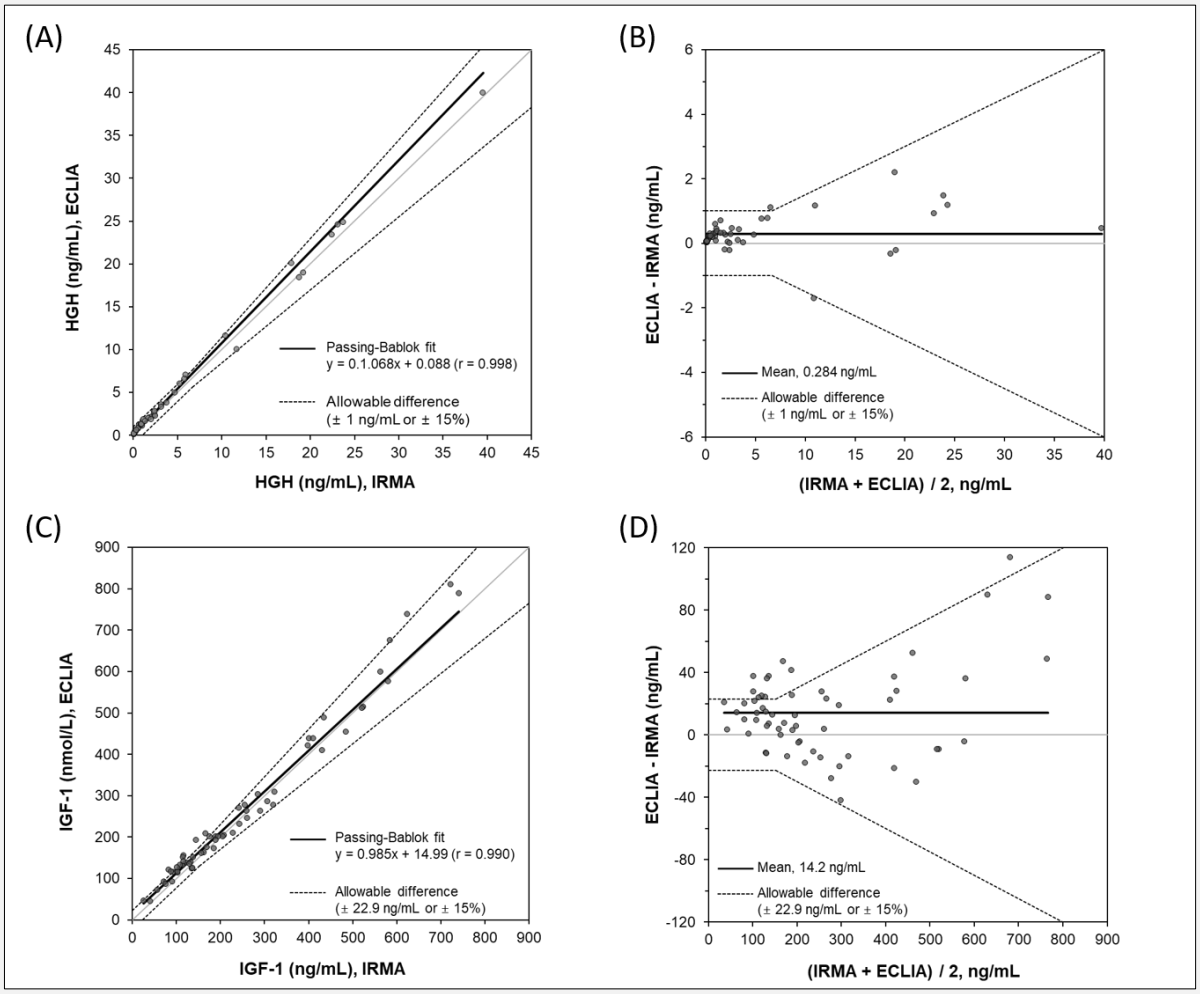

Figure 2. Comparison between the Elecsys hGH, Elecsys IGF-1, and IRMA.

(A) Scatter plot of Elecsys hGH and IRMA. (B) Bland-Altman difference plot of Elecsys hGH and IRMA. (C) Scatter plot of Elecsys IGF-1 and IRMA. (D) Bland-Altman difference plot of Elecsys IGF-1 and IRMA.

two GH assays can be explained by several factors. Elecsys hGH is an ECLIA sandwich assay using monoclonal and polyclonal antibodies. Elecsys hGH targets 22 kDa and 20 kDa GH isoforms and is calibrated against the International Reference Reagent (IRR) 98/574. Izotop hGH [I-125] IRMA uses two monoclonal antibodies only targeting 22 kDa GH and is calibrated against IRR 88/624. Differences greater than 2-fold between the various assays have been reported [13,14]. Such variability is inevitable regarding the heterogeneity of GH, monoclonal or polyclonal antibodies used for detection, and the differences of recognized epitopes. The IFCC recommends using IRR 98/574 for standardization and antibodies specific for 22 kDa GH [1]. Differences in GH levels may also influence medical decision making. Although GH assays with provocative agents use a single cutoff level of 10 ng/mL to diagnose GH deficiency, the diagnostic criterion did not reflect differences between assay principles [15]. Depending on the Passing-Bablok regression analysis, if the GH value of IRMA was 10 ng/mL, ECLIA was 11.09 ng/mL (95% confidence interval, 10.60 to 11.45 ng/mL), which showed a significant difference between the methods. Elecsys IGF-1 also showed good correlation with Immunotech IRMA IGF-1 (r = 0.990). However, there was a significant difference in measured concentration (95% CI of mean difference 6.873 to 21.544 ng/mL). Both assays use monoclonal antibodies and calibrated against IRR 02/254. Since serum IGFBP interfered with the IGF-1 assay, IGFBP should be dissociated before the as-

say. Elecsys IGF-1 uses diluted HCL and Immunotech IGF-1 uses dissociation buffer to pretreat serum. Krebs et al. reported comparative results of five IGF-1 assays and the slopes of regression lines between two assays varied from 0.527 to 1.00, despite all assays being calibrated against the same WHO reference standard [16]. The discrepancies could be attributed to differences in sensitivity to IGFBP [3].
Although there were considerable differences between assays, conversion factors between different GH and IGF-1 assays are discouraged since they do not account for all assay differences [1]. Recently, the Acromegaly Consensus Group published guidelines for using the assay dependent cutoff after the oral glucose tolerance test (OGTT) [17]. Previously, GH nadir value < 1 ng/mL after an OGTT was used to define postoperative cure. However, for newer ultrasensitive GH assays, an OGTT HT cutoff of 0.4 ng/mL is recommended.

CONCLUSION

Elecsys hGH and Elecsys IGF-1 assays yielded clinically acceptable analytical performance. The correlation between Elecsys hGH and Elecsys IGF-1 with IRMA was acceptable. However, since GH and IGF-1 are heterogeneous analytes and can suffer interference due to protein binding, care should be taken when comparing results from different assays.

Declaration of Interest:
The authors have no conflict of interest to declare.

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