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ORIGINAL ARTICLE

Measurement Differences Between Two Immunoassay Systems for LH and FSH: A Comparison of Roche Cobas e601 vs. Abbott Architect i2000sr

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SUMMARY

Background: **Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) regulate the growth and reproductive activity of gonadal tissue and determine the concentration of LH is essential for the prediction of ovulation. Collectively, FSH and LH are important measurements to ascertain the causes of infertility as well as diagnosing disorders such as polycystic ovary syndrome and pituitary and gonadal dysfunction. This study compares the correlation between LH and FSH measurements during examination with two different systems, Architect i2000sr (Abbott Laboratories; Lake Bluff, IL, USA) and Cobas e601 (Roche; Geneva, Switzerland), and assesses the differences between these systems.**

Methods: **Serum analysis was performed for 95 patients using both the Cobas e601 and Architect i2000sr systems. The method used to compare the systems was Passing-Bablok regression analysis with a Bland-Altman agreement plot. Inter-rater agreement was analyzed using a concordance correlation coefficient.**

Results: **Architect i2000sr and Cobas e601 have strong correlations in their LH and FSH results. However, the Bland-Altman plot shows that LH and FSH measurements in Cobas e601 are about 1.31 times and 1.26 times higher than those in Architect i2000sr, respectively. Passing-Bablok regression analysis also shows significant proportional deviation between them.**

Conclusions: **The difference between the test results for LH and FSH in Cobas e601 and Architect i2000sr indicate that the results from one system cannot be directly used to evaluate the other system.**

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LH, FSH, comparison, Architect i2000sr, Cobas e601, immunoassay

INTRODUCTION

The incidence of infertility has increased year-over-year in recent years due to environmental, psychological, and other factors [1-3]. The diagnosis of infertility is devastating for those planning lives in which having and raising children is a central desire; the psychological impact of the news is similar to that occurring with a cancer di-

agnosis [4]. Fortunately, treatments for infertility (such as embryo transfer) have made considerable progress in recent years [5].

Monitoring the daily concentrations of LH is important for predicting ovulation. Since increasing concentrations of LH precede ovulation, patients who wish to choose the best time for artificial insemination must monitor LH concentration to predict the ovulation period. When the follicles and the egg cells in follicles mature, the LH peak promotes follicular rupture, releasing egg cells. Because injection of spermatozoa requires precise timing with this process of follicular rupture, fertilization *in vitro* requires more frequent sampling of the LH [6]. Van Rooij et al. note that FSH levels can affect the embryo implantation rate [7], and high FSH levels in mature women can stimulate follicular development and promote testicular gametes. Another study found that with a FSH/LH concentration ratio ≥ 2, the number of eggs, MII eggs, and high-quality embryos was significantly reduced, significantly reducing the clinical pregnancy rate with ovulation induced by assisted reproductive technology (ART) [8-10]. In men, LH mainly stimulates Leydig cells to produce testosterone, regulating testicular seminiferous tubules in support of cell spermatogenesis through the production of testosterone and FSH [11], which also plays an important role in pregnancy. Gonadotropin deficiency is usually an early sign of pituitary dysfunction. Concentrations of LH and FSH are low in people suffering from this disease. Therefore, the high concentration of LH and FSH can diagnose gonadal failure, which is one of the main causes of infertility. In addition, high concentrations of LH and FSH are also observed in women with primary amenorrhea, during menopause, or with polycystic ovary syndrome or other diseases[6].

Measurement of LH and FSH using Architect i2000sr and Cobas e601 is widely used in the clinical setting as part of diagnosing and treating various disorders. Many large hospitals and medical centers have a number of different automated immunoassay systems on site, each of which may give different ranges for the concentrations of items that are present. In addition, a patient whose illness is diagnosed at a different hospital than he or she will be treated and may also undergo similar tests on several different immunoassay systems. At this point, it is necessary to take into account the bias introduced by use of different detection systems. This bias may lead to significant differences in the results yielded, potentially creating statistically incongruent results that lead to apparent clinical contradictions.

Currently, a fairly large body of literature is being generated to compare the results from different immunoassay systems. However, few reports exist on the difference between LH and FSH in different detection systems. In order to avoid unacceptable errors when navigating between test results yielded by two different systems, this study analyzed the LH and FSH results as determined by the Cobas e601 and Architect i2000sr systems to explore the correlation between them.

MATERIALS AND METHODS

Samples

Ninety-five fresh peripheral blood samples were obtained from August 5 to December 25, 2016 through a process of random selection; the test results were based on data yielded by Cobas e601. The intent of random selection was to cover the entire measurement range of LH and FSH concentrations. After fasting, venous blood samples were collected into a coagulation activation tube with a sterile serum separator. Samples were centrifuged at 2500 rpm for 10 minutes and tested after 30 minutes. Lipid, severe hemolysis, jaundice, and samples that were insufficient or were collected with inappropriate test tubes were excluded from the study.

Methods

Cobas and Architect were both calibrated using the calibrators found in their respective assembly kits to ensure the instruments were in good condition. Prior to sample analysis, we conducted an accuracy study based on CLSI EP05-A2. The serum samples of 2 concentration levels were analyzed every day, two times a day, and the measured intervals were more than 2 hours a day for a period of 20 days. Repeatability and intermediate precision were computed. The samples were first tested on Cobas and then repeated on Architect within 2 hours on the same day. Samples that exceed the linear range concentration are automatically diluted using the system, and all methods were analyzed according to the manufacturer's instructions, with particular attention to interference and pre-analysis factors.

The linear range of FSH determination in Architect and Cobas E601 is 0.05 - 150 mIU/mL and 0.1 - 200 mIU/mL, respectively. LH is 0.09 - 250 mIU/mL and 0.1 - 200 mIU/mL, respectively.

Statistical analysis

Data analysis was performed using MedCalc Version 11.4.2.0 (Mariakerke, Belgium); data processing was performed using Excel 2003. The non-parametric Wilcoxon rank-sum [12] test was used to calculate the significant differences in the paired approach, with Passing-Bablok for regression analysis [13]. The Bland-Altman agreement plot was used to evaluate the difference in measurement results between the two systems [14], with the differences (ratios) between the two techniques then plotted against the averages of the two techniques in this graphical method. The concordance correlation coefficient (CCC) ρ_c was used to evaluate the degree to which pairs of observations fell on the 45° line through the origin [15,16]. It contains a measurement of precision ρ (ρ is the Pearson's correlation coefficient, which measures how far each observation deviates from the best-fit line, and is a measure of precision) and accuracy C_b (C_b is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy): $\rho_c = \rho \cdot C_b$. All reported p-values in the study were two-tailed, and there

Table 1. Performance characteristics of Architect i200sr and Cobas e601 for LH and FSH.

	Descriptive data of evaluated parameters			Precision data of devices				
	Instruments	Measurement range	Analytical sensitivity	Median	Repeatability		Intermediate precision	
					SD	CV, %	SD	CV, %
LH (mIU/mL)	Cobas e601	0.10 - 200	0.10	16.60	0.20	1.20	0.32	1.91
				58.04	0.56	0.96	1.06	1.82
	Architect i2000sr	0.09 - 250	0.09	12.10	0.24	1.98	0.29	2.37
				46.82	0.98	2.09	1.28	2.74
FSH (mIU/mL)	Cobas e601	0.10 - 200	0.10	10.28	0.22	2.13	0.29	2.84
				42.87	0.93	2.18	0.99	2.31
	Architect i2000sr	0.05 - 150	0.05	8.19	0.21	2.55	0.26	3.12
				31.52	0.76	2.41	0.80	2.55

LH - luteinizing hormone, FSH - follicle stimulating hormone, SD - standard deviation, CV - coefficient of variance.

Table 2. Passing-Bablok regression parameters for Architect i2000sr and Cobas e601.

Cobas vs. Architect			Passing-Bablok regression analysis			
	Measurement range	Sample size, n	Intercept A	95% CI	Slope B	95% CI
LH	0.1 to 200 (mIU/mL)	95	0.2517	0.0856 to 0.4967	1.2783	1.2568 to 1.3047
FSH	0.1 to 200 (mIU/mL)	95	0.0387	-0.1774 to 0.1808	1.2268	1.1929 to 1.2621

LH - luteinizing hormone, FSH - follicle stimulating hormone, 95% CI - 95% confidence interval.

Table 3. Correlation analysis and Wilcoxon test of evaluated parameters between Architect i2000sr and Cobas e601.

Cobas vs. Architect		Concordance correlation coefficient analysis				Wilcoxon test	
	Measurement range	CCC(ρc)	95% CI	ρ	C_b	Large sample test statistic Z	Two-tailed probability
LH (mIU/mL)	0.1 to 200 (n = 95)	0.9110	0.8841 to 0.9319	0.9907	0.9196	8.418611	p < 0.0001
FSH (mIU/mL)	0.1 to 200 (n = 95)	0.9480	0.9324 to 0.9601	0.9933	0.9544	8.463039	p < 0.0001

LH - luteinizing hormone, FSH - follicle stimulating hormone, CCC(ρc) - concordance correlation coefficient, ρ - Pearson's rho C_b - bias correction factor, ρc = ρ·Cb, 95% CI - 95% confidence interval.

was a statistically significant difference when $p < 0.05$.

RESULTS

The accuracy studies of Architect and Cobas for LH and FSH measurements are included in Table 1, indicating that both of them have good performance.

LH

Concordance correlation coefficient analysis shows that CCC is 0.911 and C_b is 0.9196 (Table 3), indicating excellent correlation between the Cobas and Architect values. However, Passing-Bablok regression showed that the intercept of the Cobas data for the *y*-axis was 0.2517 and the slope was 1.2783 (Table 2), indicating a significant proportional difference in the Cobas and Architect values and a negligible constant difference. The Wil-

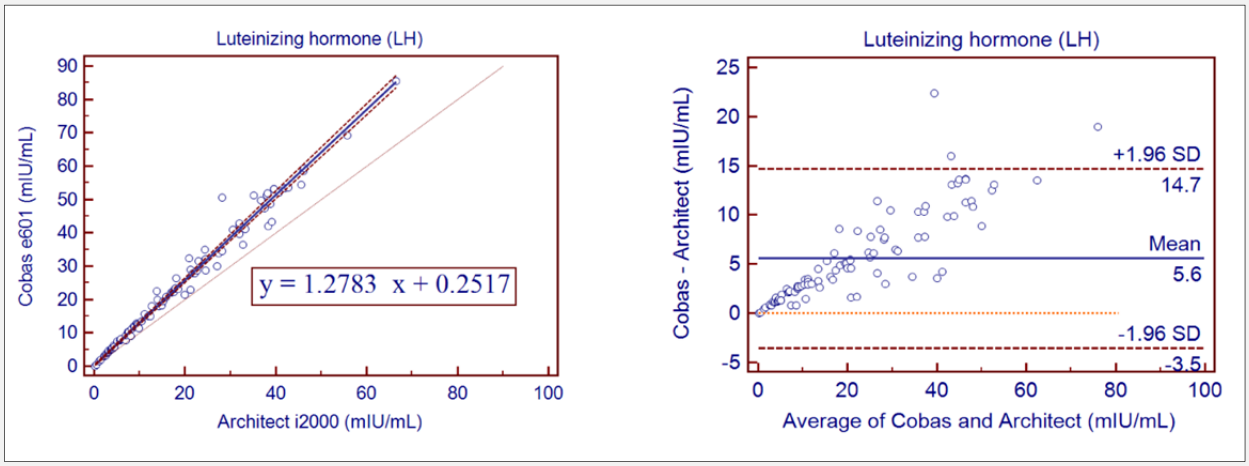

Figure 1. Passing-Bablok regression lines and Bland-Altman agreement plots of LH for Architect i2000sr and Cobas e601.

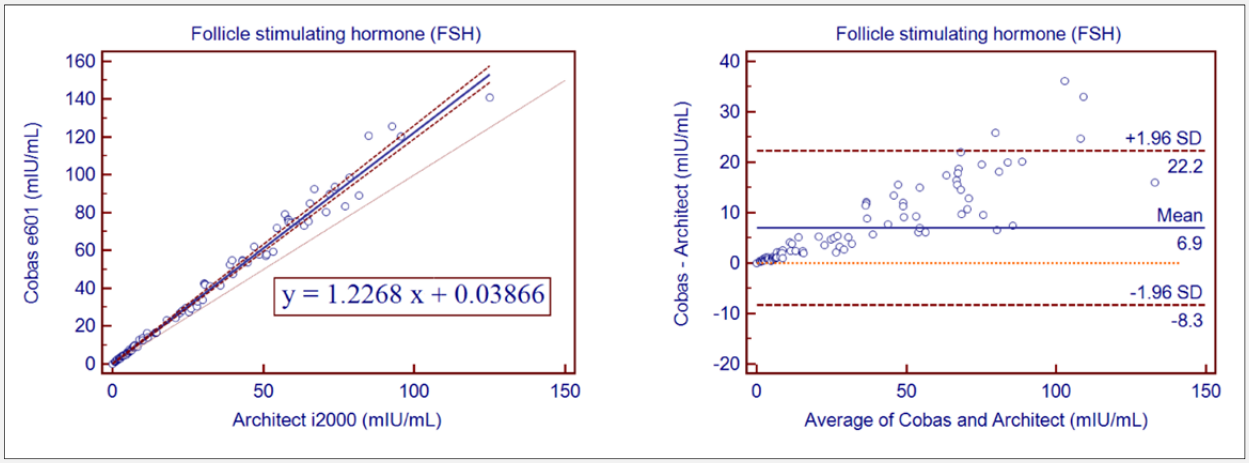

Figure 2. Passing-Bablok regression lines and Bland-Altman agreement plots of FSH for Architect i2000sr and Cobas e601.

coxon rank-sum test ($p < 0.001$, Table 3) showed a statistically significant difference between the Cobas and Architect data. The average deviation of the two according to the Bland-Altman agreement plot is 5.6 mIU/mL (Figure 1), with the difference mostly above the 0-line value (difference = 0), revealing that the Cobas test yields values higher (about 1.31 times higher; Figure 3) than those for Architect.

FSH

The trend for FSH is similar to that of LH. Concordance correlation coefficient analysis showed that CCC is 0.948 and C_b is 0.9544, indicating a strong correlation between Cobas and Architect. Passing-Bablok regression shows that the *y*-intercept is 0.0387 and the slope is 1.2268, indicating significant proportional deviation between the Cobas and Architect values, with a negligible constant difference. The Wilcoxon rank-sum test

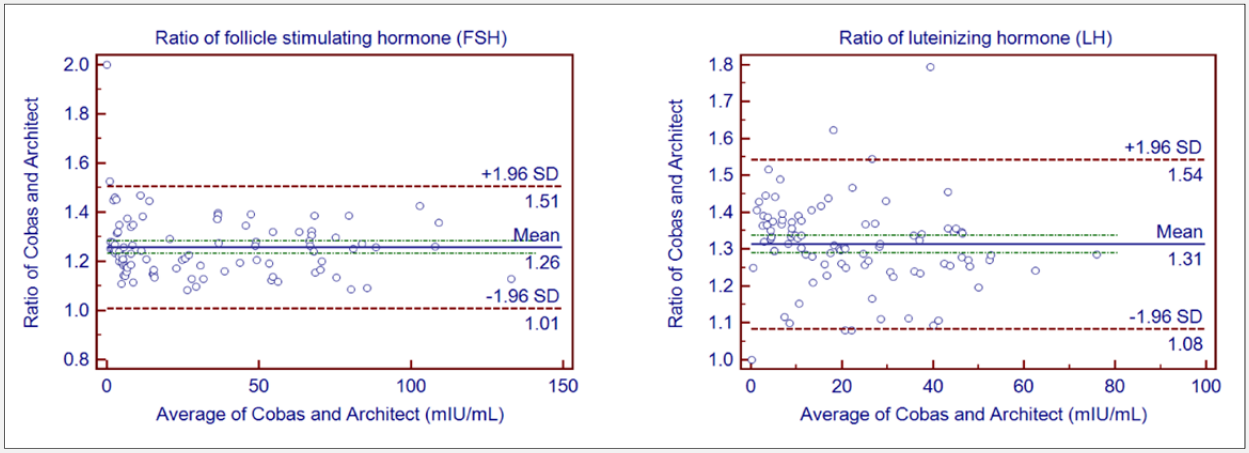

Figure 3. Bland-Altman plots of the ratios of LH and FSH for Architect i2000sr and Cobas e601.

($p < 0.001$) showed a statistically significant difference between Cobas and Architect data. The average deviation of the two with the Bland-Altman test was 6.9 mIU/mL (Figure 2). 95.79% (91/95) of the values reside within these limits, with most of the difference being above the balance line, revealing that Cobas yielded higher values (1.26 times higher) than did Architect (Figure 3).

DISCUSSION

With the rapid development of medical technology and equipment, many hospital laboratories are equipped with multiple large-scale automatic immune analysis systems. With the increasing awareness of quality management, people are becoming more concerned about and interested in the accuracy of test results. LH and FSH are widely used in the test requests of most doctors, especially during tests for infertility, pituitary gonadal dysfunction, and polycystic ovary syndrome, or in patients trying to choose the best time for pregnancy. As these data are the key factors that influence clinical decision making, making the results obtained from these analyses more reliable is important.

The literature that compares different assay systems is considerable in its scope. A recent study from Masika shows that low concentrations of TT3 in the Cobas 6000 system and LC-MS/MS system test results may bring some problems to the clinical use of drugs [17]. Another study from Guan XY et al. [18] showed that HCG's test results on Architect systems were about 25% higher than those for Cobas. Additionally, other studies have shown that the average deviation in 25-hydroxyvitamin D levels between the Architect and Roche systems with LC-MS/MS is 15.1% and -14.1%, respectively [19]. Sarkar R [20] also reported that TSH levels on the Cobas system measured 28.7% higher than on the Architect. These reports, along with our findings, show that the huge difference is ubiquitous with use of different platforms.

In this study, Cobas and Architect both performed well in terms of accuracy, and both systems can in fact be used for clinical testing. However, although the correlation between the two is good, the LH measured in the Cobas is about 1.31 times higher and the FSH 1.26 times higher than in Architect. This has posed challenges in situations involving clinical diagnosis for treatment of various conditions. For example, when LH and FSH are initially measured on Architect, the referral may be assigned to Cobas when patients must receive treatment at a different hospital. The patient or clinician may refer to these two results, and the high result may cause the clinician to over-treat the patient. Conversely, low results may lead to inadequate medication or discontinuation of medication. When a patient wants to monitor the ovulation period, the results of Cobas (followed by a switch to Architect) may cause the best ovulation fertilization period to be missed, as these patients have their FSH and LH levels monitored frequently enough that only one of the two systems can serve to provide consistent baseline values. Additionally, according to the literature, inconsistent results in LH/FSH tests can lead to difficulty in clinical diagnosis of disorders like polycystic ovary syndrome [21], as specific values for these two items are related to diagnosis and treatment.

In this study, according to Passing-Bablok regression and the Bland-Altman test, there are differences between the Cobas and Architect data. Many factors can

explain the difference between the two measurements. Cobas e601 and Architect i2000sr are two different detection methods using two different luminescent matrices. The Cobas e601 system uses an electrochemiluminescence immunoassay (ECLIA); the chemiluminescent reagent is ($[Ru(bpy)_3]^{2+}$). Architect i2000sr is a quantitative assay of a chemiluminescent microparticle immunoassay (CMIA); the chemiluminescent reagent is acridinium ester. Some scholars have suggested that different chemiluminescent reagents may lead to inconsistent results [18]. Second, it has been reported that the substance is not a single substance in the serum of patients; they may contain other substances, such as heterophilic antibodies [22-24]. There may be immune cross-reactivity; the ability of different systems to identify this interference is different, and the results obtained on different platforms are different. But the emergence of such differences due to the cross reaction in LH and FSH remains to be confirmed. Third, the difference in calibration between different detection systems may lead to differences in test results. Abbott Architect FSH can be traced back to the World Health Organization (WHO) FSH 1st International Standard (92/510). Roche Cobas FSH can be traced back to the WHO 2nd reference material internal reference point (IRP 78/549). However, LH in Cobas and Architect are traceable to the WHO LH 2nd International Standard (80/552). Further analysis potentially shows that calibration for the Architect method involves the presence of a phosphate buffer containing a heat-inactivated bovine serum albumin stabilizer. The matrix of the Cobas test calibrator is made from saline containing human serum. Different substrates lead to differences in concentration, which remains to be confirmed by further research.

In the different scenarios posed by this study, which (in general) require results to be compared with previous results, to avoid the differences caused by multiple analysis procedures results should be obtained from an analyzer, and the laboratory should use the same instrument for all measurements whenever possible. If a laboratory has a different system analyzer, it is necessary to compare the test results of each system. Such a comparison method would be based on the CLSI EP09-A2 document [25]. In projects that achieve consistency in values across different tests, the detection system is the main characteristic, and the results from other detection systems are consistent. However, this is only relevant in cases where all the tests are performed at the same laboratory, and a type of detection system that can compare results from different laboratories has not yet been developed with respect to having a unified standard. When test results are derived from different systems between different laboratories, the results between them should not be used to determine whether the concentration is increased or decreased. It is important that clinical and laboratory specialists be aware of the differences between the methods they use or the analysis procedure, as these differences may directly affect the physician's judgments related to the disease.

This study has several limitations. First, although 95 samples did meet the requirements for statistical significance, the sample size is relatively small. Second, the relatively small study population is also a limitation - all samples are from a single tertiary unit, and our findings may not apply to other groups. Third, this comparative study only involved Roche Cobas e601 and Abbott Architect i2000sr. Further analysis is needed to find out whether differences exist between these two systems and other analytical systems.

CONCLUSION

The values of LH and FSH in Cobas e601 are higher than in Architect i2000sr. Overall, LH and FSH values measured in Cobas are about 1.31 times and 1.26 times higher than in Architect, respectively. Clinicians, especially those working in departments of gynecology and andrology, need to be aware of the differences between the two different systems to avoid potential misdiagnosis and unnecessary treatment. Laboratory experts should also pay attention to these problems in their daily work when changing their methods and compare the results from different platforms.

Ethical:
Compliance with ethical standards.

Funding:
No specific funding was obtained for this study.

Ethical Approval:
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study prior to any study procedure.

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Declaration of Interest:

We have no possible conflict of interest to mention. We certify that we have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, honoraria).

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