Assessing The Reliability of Radioimmunoassay for The Quantification of Testosterone, Progesterone, Luteinizing and Follicle Stimulating Hormones

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ABSTRACT

It is a standard approach to evaluate analytical processes in a laboratory routinely. Especially if it involves the study of key hormones like testosterone, progesterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH), which are required for the diagnosis of various health conditions. The aim of this study was to evaluate the sensitivity, cross-reactivity, precision, parallelism, and recovery of radioimmunoassay (RIA) of these hormone parameters to establish their reliability and suitability for clinical applications. The performance parameters were evaluated by using RIA to assay several control and clinical samples as well as their dilutions and multiple combinations of mixtures. The assays showed high precision, sensitivity, selectivity, and accuracy. The result reaffirms the reliability and robustness of RIA as an analytical tool for quantification of FSH, LH, testosterone, and progesterone. Future studies on other hormone RIAs and across other laboratories are required to further validate the result.

Keywords: Radioimmunoassay, Precision, Sensitivity, Selectivity, Accuracy, Bias, FSH, LH, Testosterone, Progesterone.

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INTRODUCTION

Hormones regulate a diverse set of physiological processes in the human body, including those related to reproduction. FSH drives the follicular development in females. LH activates ovulation and stimulates the formation of the corpus luteum, a transient endocrine gland essential for progesterone production (1). In males, FSH and LH play important roles in spermatogenesis and Testosterone synthesis, respectively (2). Testosterone, the principal male reproductive hormone, promotes the development and Maintenance of the male reproductive system and secondary sexual characteristics while also exerting anabolic effects on skeletal muscle. Progesterone prepares the endometrium for implantation and supports the maintenance of pregnancy (3). Physicians can gain valuable insights into the functional status of the hypothalamic-pituitary-gonadal axis and other endocrine glands by understanding the available hormone concentration. In summary, hormone analysis is an essential component of modern healthcare, enabling clinicians to make informed diagnostic and therapeutic decisions in the management of a wide range of endocrine and reproductive disorders.

Precise and accurate measurement of these hormones are therefore very important in the diagnosis and management of different endocrinological and reproductive disorders. Concerned physician needs to have a certain level of confidence on the methods used in the quantification of hormonal levels. Radioimmunoassay (RIA) is one of the most utilized methods of in-vitro hormone analysis. It has been exceptionally influential in the field of endocrinology and Nuclear Medicine. RIA was established in the late 1950s by Rosalyn Yalow and Solomon Berson (4). It has revolutionized the measurement of trace levels of bioorganic molecules, especially hormones. The principle is based on the exceptional selectivity of antigen-antibody interactions and the high sensitivity of

gamma scintillation detectors (5). However, the reliability of RIA measurements should be thoroughly evaluated to ensure consistency across different laboratories as there are potential sources of systematic and random errors. Each assay depends on numerous variables which may influence the final result (5).

The aim of this study was to ensure that the RIA performed at the in vitro laboratory of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Suhrawardy, is precise and accurate, as a newly established nuclear medicine institute. The key performance parameters chosen for this study are precision, sensitivity, cross-reactivity, parallelism (effect of dilution), and recovery of analyte added to a sample matrix. Inadequate performance in any of these parameters may suggest errors or biases in the assays, which would eventually lead to poor assay results (6). For instance, a high cross-reactivity will result in elevated concentrations of the analytes, potentially leading to untreated disease or unnecessary treatment depending on the actual result.

MATERIALS AND METHODS

Materials and Instruments

RIA kits for FSH, LH, testosterone, and progesterone were purchased from Beijing North Institute of Biotechnology (BNIBT), China. Micropipettes of sizes 1000 µL, 200 µL, and 100 µL from ESCO were used for measuring volumes. After washing, primarily the Shinjin Medics Messiah gamma counter was used to count the gamma photons of the test tubes. Additionally, each tube was counted for a second time using a Perkin-Elmer gamma counter.

Samples Collection and Preparation

To evaluate the performance of the assays at around normal range, 4 serum samples were collected from healthy volunteers. To evaluate the performance of the assays outside the normal range, 8 more serum samples were selected from day-to-day patients. The serums were stored at -26° C until analysis.

Evaluation

For the evaluation of precision, each of the 3 of the selected serums was analysed multiple times (n=5) in the same assay run. The mean and coefficient of variation (CV) for each sample were calculated. For the evaluation of sensitivity, the zero standards (S0) of each parameter were run 10 times. Sensitivity was quantified with lowest detectable dose (LDD), which was defined as (6),

$$LDD = S_o + 3\sigma \quad \ldots \ldots \ldots \quad (2.1)$$

Where S_o = concentration of 0 standard

σ = standard deviaition.

Effect of dilution was determined by measuring the concentration of the most concentrated standards (S_6 or S_7) at 2 times and 4 times dilution, after which the calculated concertations were plotted against the dilution factor to fit against a straight line parallel to the x axis. Recovery was determined by spiking a selected standard with 2 different analyte standards, and then calculating with the following equation (6),

$$\%\ Recovery = \frac{Observed\ Concentration}{concentration\ added\ analyte\ concetration} \times 100\% \quad \ldots \ldots \ldots \quad (2.2)$$

Specificity was determined by measuring the cross-reactivity of an assay with an analyte with similar structure. Since LH and FSH have similar molecular structure, they were checked for cross-reactivity against each other. Similarly, Testosterone and Progesterone has similar structure and checked for cross-reactivity. Generally cross-reactivity is quantified (6),

$$Crossreactivity at 50\%\ bound = \frac{concentration\ of\ standard}{concentration\ of\ competior} 100\% \quad \ldots \ldots \quad (2.3)$$

But generally, the assays are highly specific. As a result, the competitor binding never practically reaches 50%.

Data Analysis

The raw counts per minute (CPM) were collected and transformed into percent bound (B/Bmax). The standards for each parameter were fitted against a 4 points polynomial curve using MyCurveFit add-in for Microsoft Excel. All the unknown concentrations were calculated using the fitted equations.

RESULT AND DISCUSSION

Precision

Table 1 indicates the CV for each hormone assays across three different concentration ranges. In clinical

application a CV of less than 10% is generally acceptable (7). Precision of an assay depends on many things, including but not limited to reaction constants, the speed and completeness of reactions, separation of bound from free antigens, and potential interferences. As long as the reaction time (incubation time) is sufficiently long, the effects of the environmental factors can be minimized.

Table 1: coefficient variation of the selected samples (n= 5) for each hormone parameters.

Analyte	Sample	Shinjin Medics Gamma Counter		Perkin-Elmer Gamma Counter	
		Mean	CV%	Mean	CV%
FSH	X1	20.48 mIU/mL	3.63	20.27 mIU/mL	3.42
	X2	12.82 mIU/mL	6.98	13.07 mIU/mL	6.17
	X3	24.59 mIU/mL	6.51	24.24 mIU/mL	8.04
LH	X4	0.49 mIU/mL	5.60	1.30 mIU/mL	4.43
	X5	0.69 mIU/mL	3.98	1.57 mIU/mL	5.17
	X6	44.25 mIU/mL	5.18	43.15 mIU/mL	5.53
Testosterone	X7	6.83 ng/mL	2.95	6.95 ng/mL	3.67
	X8	8.48 ng/mL	3.84	8.44 ng/mL	3.98
	X9	9.03 ng/mL	2.21	9.12 ng/mL	2.60
Progesterone	X10	1.85 ng/mL	3.03	1.99 ng/mL	3.54
	X11	2.35 ng/mL	2.48	2.45 ng/mL	2.91
	X12	0.94 ng/mL	1.36	0.97 ng/mL	2.11

Even though the CV values were acceptable in this particular study. It only showed that the assays are precise in a single assay run. To prove the precision for inter assay runs, it needs to be repeated across multiple runs.

Sensitivity

For sensitivity study, LDD was calculated as concentration of zero standard raised by 3 times the standard deviation for each hormone parameters with n=10. Both precision and the slope of standard curve can affect LDD. Lower CV and steeper slope can improve sensitivity (8). Testosterone assay was more sensitive because the standard curve was steeper as it was produced for standards in the range of 0-20 ng/mL.

Table 2: Estimated lowest detectable dose for each hormone

Hormone	Lowest Detectable Dose	
	Shinjin	Perkin-Elmer
FSH	0.62 mIU/mL	0.59 mIU/mL
LH	0.85 mIU/mL	0.29 mIU/mL
Testosterone	0.04 ng/mL	0.05 ng/mL
Progesterone	0.10 ng/mL	0.12 ng/mL

The results of the sensitivity study were promising, but it only showed how sensitive the assays were near 0 concentration. Another parameter can be studied to determine sensitivity is the resolution of the assay at every standard.

Cross reactivity

Cross-reactivities between FSH and LH and testosterone and progesterone were checked. Generally, cross-reactivity is quantified as the ratio of the concentration of the analyte standard to the concentration

of the competitor standard (9). Due to the exceptional specificity of RIA, even at the highest available concentration of the competitor standard, 50% binding could not be achieved. Table 3 shows the cross-reactivity percentage of each selected competitor at the highest available concentration against the analyte of our interest.

Table 3: Cross reactivity of selected competitors against the analyte

Analyte	Competitor	Concentration of the competitor	Cross reactivity (%)
FSH	LH	200 mIU/mL	0.68
LH	FSH	100 mIU/mL	0
Testosterone	Progesterone	100 ng/mL	0.4
Progesterone	Testosterone	20 ng/mL	0

Even though there is no visible cross-reactivity of LH in FSH and progesterone in testosterone, FSH in LH and testosterone in progesterone were giving a faint signal. This contrast can be explained by the fact that in our study, FSH standard had the highest concentration, and testosterone assay had the highest sensitivity. Further tests with even greater concentrations of the competitor are required to rule out possible interference by cross-reactivity. There is also the possibility that other cross-reacting species might have even more undeniable affinity towards the assay.

Effect of dilution (Parallelism)

Parallelism shows the effect of dilution. Apparently, the concentrations calculated back from the dilution data gave almost the same results as the undiluted concentration (Figure 1). There are minute slopes visible on the straight lines. The most probable explanation for this outcome might just be the effects of random errors. Particularly because the deviation is almost the same for each instance of dilution and does not increase proportionally with each measurement. Other causes for non-parallelism could be antigen-antibody heterogeneity and incorrect choice of diluents.

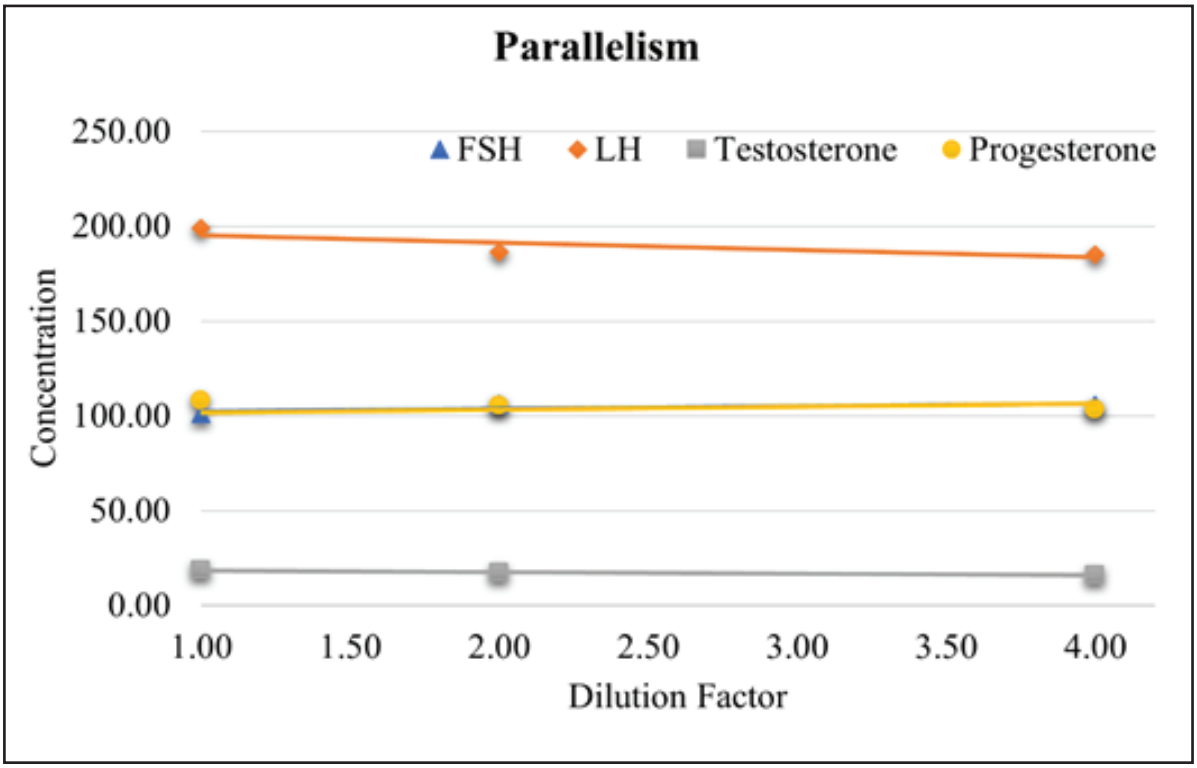

Figure 1: Parallelism study or Effect of dilution

The study found that concentration and diluents did not significantly impact assay results. And while parallelism implies accuracy, it alone cannot guarantee accuracy. More rigorous tests can be done to certify accuracy.

Recovery from spiked sample

Recovery study is one of the most popular methods for testing assay accuracy. Table 4 displays how accurately the assays could detect definite amounts of added analytes.

Table 4: Analyte recovered from spiked base medium

Analyte	Sample	Concentration				Recovered %
		Base Medium	Added analyte	Expected Concentration	Observed Concentration	
Testosterone	X13	2.50 ng/mL	0.50 ng/mL	3.00 ng/mL	3.12 ng/mL	104.13
	X14	8.00 ng/mL	2.50 ng/mL	10.50 ng/mL	11.70 ng/mL	111.42
Progesterone	X15	3.00 ng/mL	1.00 ng/mL	4.00 ng/mL	4.97 ng/mL	124.21
	X16	10.00 ng/mL	2.50 ng/mL	12.50 ng/mL	12.33 ng/mL	98.68
FSH	X17	15.00 mIU/mL	5.00 mIU/mL	20.00 mIU/mL	19.24 mIU/mL	96.19
	X18	40.00 mIU/mL	5.00 mIU/mL	45.00 mIU/mL	41.04 mIU/mL	91.21
LH	X19	45.00 mIU/mL	3.50 mIU/mL	48.50 mIU/mL	39.67 mIU/mL	81.80
	X20	3.50 mIU/mL	1.20 mIU/mL	4.70 mIU/mL	5.16 mIU/mL	109.73

Although 100% recovery is ideal, 100 ± 10% is also tolerable. The X14 sample was barely outside that range. But X15 and X19 are considerably outside the acceptable range. It is unlikely the error arose from some sort of bias in the assay, in view of the fact that the other samples for those assays performed adequately to recover spiked analytes. To eliminate doubts, more of the similar tests should be repeated with a greater number of samples.

CONCLUSION

The objective of the study was to evaluate the performances of RIA of the four hormone parameters at the newly established nuclear medicine institute, INMAS, Suhrawardy. The study has reaffirmed the reliability and robustness of RIA as a remarkable tool for measuring hormone levels. The five qualities of the RIA that were assessed have shown that the assays are adequately precise, accurate, sensitive, and selective. There were some unavoidable limitations, such as the small number of sample sizes and the lack of inter assay assessment of each quality.

For clinical purposes, accurate measurement of FSH, LH, testosterone, and progesterone is crucial. Consequently, a proper evaluation of the methods used for quantification of the analytes is necessary to ensure their validity. Even so, further studies can be done to stren then the confidence in RIA for other analytes. A larger study with a greater number of samples is warranted as well. Potential future studies may incorporate data from multiple centres as well as RIA kits from multiple manufacturers to do some comparison study.

REFERENCES

1. Shrestha, K., Rodler, D., Sinowatz, F. and Meidan, R., 2019. Corpus luteum formation. In The Ovary (pp. 255-267). Academic Press.
2. Ramaswamy, S. and Weinbauer, G.F., 2014. Endocrine control of spermatogenesis: Role of FSH and LH/testosterone. Spermatogenesis, 4(2), p.e996025.
3. Halasz M, Szekeres-Bartho J. The role of progesterone in implantation and trophoblast invasion. Journal of Reproductive Immunology. 2013 Mar 1;97(1):43–50.
4. Yalow RS. Development and Proliferation of Radioimmunoassay Technology. J Chem Educ. 1999 Jun 1;76(6):767.
5. Pappas AA, Glassman AB. Ria: Principles and Application. In: Radiobioassays. CRC Press; 1983.
6. Pimputkar, M.R. and Feldkamp, C.S., 1985. Radioimmunoassay kit evaluation and selection. Journal of nuclear medicine technology, 13(2), pp.88-94
7. Hospes W, Boskma RJ, Brouwers JRBJ. Comparison of an HPLC method with a RIA, EMIT and FIA method for the assay of serum gentamicin with extensive statistical evaluation. Pharmaceutisch Weekblad Scientific Edition. 1982 Apr 1;4(2):32–7.
8. Luisa BG. Immunoassay: A Practical Guide. Elsevier; 2012. 176 p.
9. Sotnikov DV, Zherdev AV, Zvereva EA, Eremin SA, Dzantiev BB. Changing Cross-Reactivity for Different Immunoassays Using the Same Antibodies: Theoretical Description and Experimental Confirmation. Applied Sciences. 2021 Jan;11(14):6581.