Original Article

Performance characteristics of the light-initiated chemiluminescent assay for quantitative determination of progesterone

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Background: To study the performance of quantitative determination of progesterone by light-initiated chemiluminescent assay (LICA).

Methods: Clinical samples of serum were used for detection of progesterone by LICA. The precision study was performed according to Clinical and Laboratory Standards Institute (CLSI) EP15-A3, the linear range validation was performed according to CLSI EP06-A, accuracy was evaluated according to CLSI EP9-A3, and the performance of detection capability was confirmed according to CLSI EP17-A2. All data were analyzed using SPSS software. Function regression analysis was performed by OriginPro software.

Results: The LICA-800 system exhibited low coefficients of variation (CVs) and high reproducibility, and the calculated synthetic CV was 2.16%. The access progesterone assay showed excellent linearity in the assay measuring range (0.37–40 ng/mL) using the polynomial regression method in accordance with CLSI EP06-A. Bias assessment was used to verify accuracy, and the percentage deviation met the quality requirements of the laboratory's allowable deviation of 10.00%. In terms of the detection capability of LICA, the calculated limit of blank (LoB) was 0.046 ng/mL, limit of detection (LoD) was 0.057 ng/mL, and the limit of quantitation (LoQ) value was 0.161 ng/mL.

Conclusions: The competitive LICA provided a highly sensitive, accurate and precise method for measuring serum progesterone level.

Keywords: Progesterone; light-initiated chemiluminescent assay (LICA); quantitative assay; analysis performance

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Introduction

Progesterone (P4) is a steroid hormone that plays an important role in reproductive function (1). It is produced by the corpus luteum during early pregnancy, and mainly used clinically to determine ovulation, for progesterone treatment monitoring and early pregnancy evaluation (2,3). In addition, P4 can also prevent and treat osteoporosis in women, prior considers that P4 is essential for development of adolescent peak bone mineral density (4). P4 can also induce neuroprotection (5). Thus, accurate detection of P4 is hugely important in clinical work.

The light-initiated chemiluminescent assay (LICA) is an emerging homogeneous quantitative immunoassay technology. Previous studies have shown good repeatability

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and intermediate imprecision in detecting total testosterone, estradiol, thyroid-stimulating hormone (TSH) etc. by the LICA (6,7).

In this study, we evaluated the performance of LICA for quantitative determination of P4 in human serum. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3119).

Methods

Apparatus and reagents

All clinical samples were serum samples. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Tianjin Beichen Hospital. Informed consent was obtained from all human participants. All the tests were carried out using the LICA 800 (China).

Precision study

The precision study was performed according to the according to CLSI EP15-A3: User Verification of Precision and Estimation of Bias, 3rd Edition (8).

In terms of sample selection, according to the precision confirmation report provided by the reagent manufacturer, the samples that were close to the concentration value of the imprecision parameters confirmed by the manufacturer and consistent with the matrix were selected as verification samples, in which: PC Land PC H are low-value and high-value quality control products provided by the manufacturer; Sample 1, Sample 2 are the clinical serum samples; Bio-M and Bio-H are intermediate and high-value quality control products from the Bio-Rad company.

Linearity study

The linear range validation was performed according to CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (9).

Clinical samples with a linear range of 0.37–40 ng/mL were collected, and high-value samples (HV) and low-value samples (LV) were diluted according to a certain proportion. Five samples were obtained: 1 mL LV for sample 1; 0.75 mL LV + 0.25 mL HV for sample 2; 0.50 mL LV + 0.50 mL HV for sample 3; 0.25 mL LV + 0.75 mL HV for sample 4; and 1 mL HV for sample 5.

Accuracy study

Accuracy was evaluated according to CLSI EP9-A3: Measurement Procedure Comparison and Bias Estimation using Patient Samples; Approved Guideline 3rd Edition Scheme (10). Numerical consistency was compared with Beckman. All samples were tested three times before the mean value was taken.

Detectability performance confirmation

The performance of detection capability shall be confirmed according to CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, 2nd Edition (11).

Statistical analysis

The data were analyzed using SPSS software. Function regression analysis was performed by Originpro software. $P<0.05$ was considered statistically significant.

Results

Precision verification

The results are shown in *Table 1*. Paired *t*-test was conducted between the sample concentration and the concentration in the precision confirmation report provided by the manufacturer (T=1.179, P=0.304). There was no significant difference between the concentration value selected and the concentration value provided by the manufacturer, so the sample concentration had been selected appropriately. The PC LV, Sample 2, Bio-M and Bio-HV samples were less than the imprecision of the manufacturer's declaration and passed directly; the PC HV and Sample 1, which were greater than the imprecision during the manufacturer's declaration, passed the verification by calculating the verification value (UVL), and finally they were less than the UVL of the imprecision according to the manufacturer's declaration.

In terms of the precision test data set: the standard deviation (SD) and CV of each sample were calculated after four times of repeated detection. The CV trend was relatively constant among repeated testing of cross-grade samples, and the calculated synthetic CV was 2.16%,

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Table 1 Precision verification data (ng/mL)

Sample	Mean value of detection	Repeatability		Intermediate imprecision		Manufacturer's declared CV (%)	UVL
		SD	CV (%)	SD	CV (%)		
PC LV	4.12	0.156	3.79	0.166	4.01	4.26	–
PC HV	19.77	0.812	4.11	0.812	4.11	3.68	5.59
Sample 1	11.80	0.499	4.23	0.499	4.23	4.02	5.98
Sample 2	0.80	0.029	3.67	0.035	4.36	4.95	–
Bio-M	5.30	0.224	4.23	0.301	5.68	5.91	–
Bio-H	20.68	0.956	4.62	1.056	5.11	5.50	–

Table 2 Summary of curve estimation results

Order	Coefficient symbol	Coefficient value	Coefficient standard error	*t*-test	P value	Standard error of regression coefficient
First order	a	0.210	0.249	2.857	0.009	0.677
	b1	0.976	0.010	94.362	0.000	
Second order	a	0.167	0.266	0.629	0.536	0.565
	b1	1.075	0.031	34.239	0.000	
	b2	–0.002	0.001	–3.259	0.004	
Third order	a	0.067	0.300	0.225	0.824	0.571
	b1	1.124	0.073	15.391	0.000	
	b2	–0.006	0.005	–1.286	0.213	
	b3	5.56E-5	0.000	0.750	0.462	

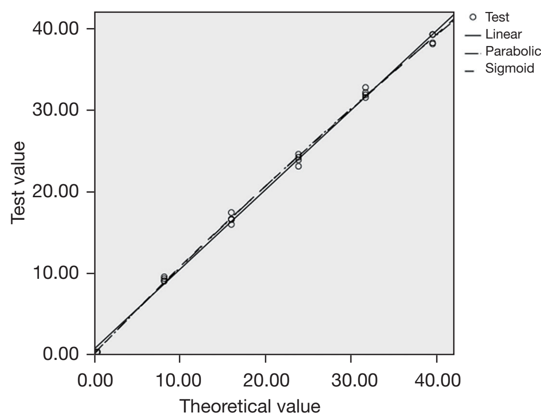

Figure 1 Linear regression fitting curve (X is the theoretical value; Y is the measured value).

which was less than the allowed imprecision (7.50%) of the project established by our laboratory, and so the precision verification was passed.

Linearity test

SPSS software was used to input the theoretical value and test value of the data set. The regression analysis curve estimation is shown in *Table 2*. The optimal fitting equation was $Y = -0.002X^2 + 1.075X + 0.167$ (X is the theoretical value), and the curve is shown in *Figure 1*.

As for the judgment of the nonlinear degree of the data group, the results are shown in *Table 3*. The fitting values of the optimal fitting equation and the first-order

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Table 3 EP6-A2 judgment of nonlinear degree

Sample	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Measured mean value	0.42	9.31	16.70	23.99	32.10	38.73
Second-order fitting value	0.62	8.89	16.92	24.70	32.23	39.53
First-order fitting value	0.62	8.25	15.88	23.52	31.15	38.78
Difference	0.00	–0.64	–1.03	–1.18	–1.09	–0.75
Percentage deviation	0.42	–6.84	–6.17	–4.92	–3.38	–1.93

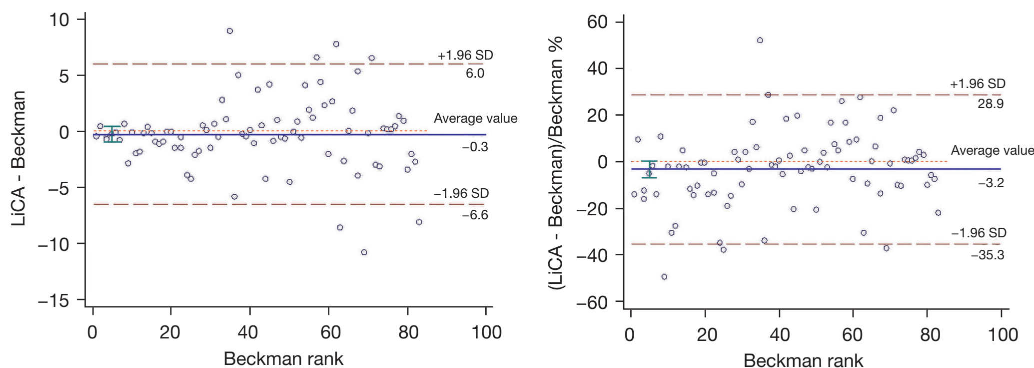

Figure 2 Bland-Altman graph: the vertical axis of (left) is the deviation, and the vertical axis of (right) is the percentage deviation.

equation were calculated and compared with the measured mean value; the percentage deviation was calculated and compared with the allowable deviation of 10.00%. The results are shown in *Table 3*, which are all tests passed.

Accuracy evaluation

Outlier test and homogeneity analysis of each data segment

According to the quality requirements of the quality assessment program of the Royal Society of Pathologists, P4 ≤10 nmol/L (3.15 ng/mL), and the allowable deviation is ±2 nmol/L (0.63 ng/mL). The Beckman test result was <3.15 ng/mL, which was not included in the analysis. A total of 33 cases were not included in the analysis, and 30 cases (90.91%) met the requirements of the allowable deviation.

The remaining data group used the generalized extreme studentized deviate (ESD) method (α=0.01) to eliminate outliers, no outliers, and the 83 valid data groups.

Preliminary estimation of bias

A Bland-Altman diagram was drawn to observe the characteristics of the paired data of the two groups (*Figure 2*). The data group basically showed the characteristics of constant CV. Shapiro-Wilk test showed that the data group was normal, w=0.9706 (P=0.0537). The normality was accepted, the bias was expressed by the mean of percentage deviation, and the bias was –3.24% (–5.03% to –1.45%).

Regression analysis

The best models (OLR, WLS, Deming and Passing-Bablock) were used for regression analysis of the data group. The specific results are shown in *Table 4*. The best fitting curve was WLS: y = 1.005x – 0.379; the Spearman correlation coefficient was 0.940 (*Table 4*).

Calculation of bias at medical decision-making level

This was evaluated by substituting the clinical attention diagnostic points 5.09, 11.52 and 18.62 ng/mL into the above equation with the least proportional bias. The results are shown in *Table 5*. The percentage deviation of each concentration point met the quality requirements of the laboratory's allowable deviation of 10.00%.

Detectability performance confirmation

LoB

We used the diluent provided by the manufacturer for each

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Table 4 Summary of four linear fitting models

Regression model	Regression curve	Slope 95% CI	Intercept 95% CI	Proportional bias (%)
OLR	Y=1.004X −0.354	0.930–1.078	−1.944 to 1.236	−7.0 to 7.8
WLS	Y=1.005X −0.379	1.001–1.009	−0.464 to −0.295	0.1 to 0.9
Deming	Y=1.063X −1.482	0.991–1.135	−2.532 to −0.432	−0.9 to 13.5
Passing-Bablok	Y=1.057X −1.363	1.008–1.111	−2.083 to −0.299	0.8 to 11.1

95% CI, 95% confidence interval.

Table 5 Summary of bias at the medical decision-making level

Determining level (ng/mL)	Estimated value (ng/mL)	Deviation (ng/mL)	Percentage deviation (%)
5.09	4.74	−0.35	−7.20
11.52	11.20	−0.32	−2.83
18.62	18.33	−0.29	−1.55

Table 6 Summary of LoD performance validation data

Sample	Mean value (ng/mL)	SD (ng/mL)
1	0.046	0.0062
2	0.065	0.0070
3	0.125	0.0092
4	0.258	0.0162
5	0.311	0.0188

LoD, limit of detection.

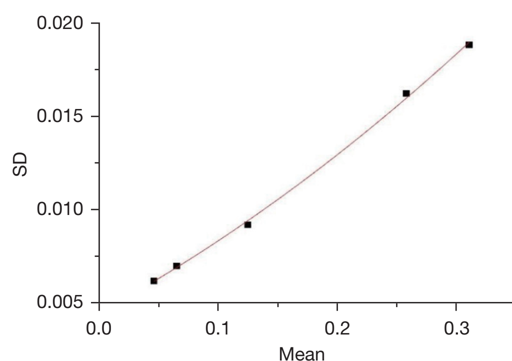

Figure 3 Precision curve (x-axis is each average analyte concentration; y-axis is intralaboratory variation).

sample, and each of the 5 samples was repeatedly tested 12 times, for a total of 60 test results. The Curve Calculator software provided by the manufacturer was used to fit the results of the cubic spline algorithm.

Sort the 60 test results according to size, calculated the percentile (PctB) of the result distribution of blank specimens, and α=0.05, PctB = 1 − α =0.95 were required. The corresponding arrangement position for calculating the percentile was as follows: arrangement position = 0.5 + (B × PctB), where B is the total number of samples, and the 60 test results are arranged at 57.5; LoB should be calculated according to the 57th and 58th digits, namely LoB = X57 + 0.5 (X58 – X57).

In this study, X57=0.16 and X58=0.17, so the calculated LoB was 0.046 ng/mL.

LoD

We used the diluent provided by the manufacturer to dilute the LV clinical samples to obtain serial concentration samples. Each sample was tested for 3 days, and each sample was tested 4 times a day. The mean value and CV of each sample were calculated and shown in *Table 6*.

The homogeneity of variance test was carried out for the above groups of data. The F value was 248.3, P=0.000, and variance was uneven. LoD was calculated using the precision curve scheme with Originpro software. The results are shown in *Figure 3*. The specific curve selection method was consistent with the verification of linear range, and the final precision curve was $Y = 0.0385X^2 + 0.0344X + 0.0045$.

Substituting LoB as X into the above equation, SD_{WL} =0.018, using the regression line variance to calculate the SD_{WL} value under the corresponding measured

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Table 7 Measured concentration (MC), SD_{WL} and trial LoD values and bias

MC	SD_{WL} (ng/mL)	Trial LoD (ng/mL)	Bias (ng/mL)
0.050	0.006	0.056	0.006
0.052	0.006	0.057	0.005
0.054	0.006	0.057	0.003
0.056	0.007	0.057	0.001
0.057	0.007	0.057	0.000
0.058	0.007	0.057	−0.001
0.059	0.007	0.057	−0.002

LoD, limit of detection.

Table 8 Summary of LoQ performance validation data

Sample	Mean value (ng/mL)	CV (%)
1	0.032	16.76
2	0.052	14.21
3	0.075	11.22
4	0.099	9.53
5	0.121	8.26
6	0.142	8.02
7	0.265	6.25
8	0.398	4.21
9	0.523	3.99

LoQ, limit of quantitation.

concentration (MC). then used the SD_{WL} value obtained to calculate the test LoD value. The bias was calculated by subtracting the MC value from the test LoD value. When the bias changes from positive to negative, the step length between the two concentrations can be reduced to determine the test LoD value when the bias is equal to 0. The test LoD value was the final LoD value. The results are shown in *Table 7*; LoD =0.057 ng/mL.

LoQ

Thelow-value clinical samples were diluted with the diluent provided by the manufacturer. Each sample was tested for 20 days, and two analysis batches were made every day, each batch and each sample were tested twice. The CVs and mean values are shown in *Table 8*.

The functional regression analysis was carried out using Originpro software. The allowable imprecision was

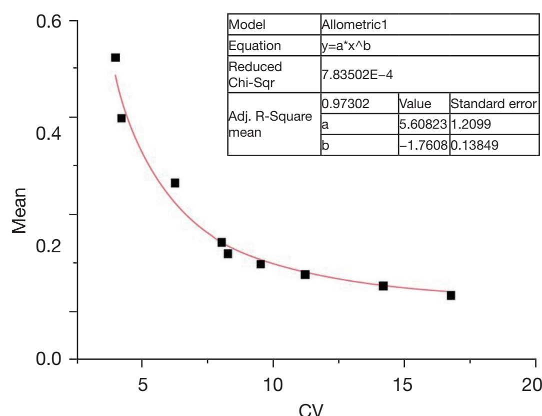

Figure 4 Function curve (x-axis is the coefficient of variation; y-axis is mean value).

derived as 7.50% based on the current technical level. The regression curve is shown in *Figure 4*. The available function was Y = 5.6082X − 1.761; Y=0.161 ng/mL was obtained by substituting the coefficient of admissible variation as X in the equation.

In summary, the LoQ value was 0.161 ng/mL (the allowable error is 7.50%).

Discussion

In this study, we used established LICA equipment for quantitative detection of P4 in human serum. The basic principle of light-induced chemiluminescence is a homogeneous immunoassay. The antigen or antibody on the surface of the luminescent particle forms an immune complex with the antigen or antibody to be tested in the liquid phase, and then the two types of particles are pulled closer by combining the biotin-avidin system with photosensitive particles coated with avidin. Under laser excitation, the energy transfer of ionic oxygen between particles occurs, and the high-energy red light is generated. The photon number is converted into a target molecular concentration by single photon counter and mathematical fitting. In contrast, when there is no target molecule in the sample, the immune complex cannot form between the two particles. The distance between the two particles exceeds the range of ionic oxygen transmission, the ionic oxygen is rapidly quenched, and no high-energy red light signal is generated. The P4 concentration in human serum negatively correlates with the signal value and can be calculated quantitatively according to the calibration curve.

As a quantitative assay, the advantages of the LICA are

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as follows: long-arm biotin-labeled progesterone, in which biotin is a small molecule, coupled with small molecular progesterone, has little effect on biological activities. Second, biotin-labeled progesterone is coupled through a specific chemical bond, giving the labeled progesterone better specific activity, which is helpful for improving the sensitivity of the analysis. In contrast, when labeled with a macromolecular enzyme protein, the immune activity of progesterone is affected. Finally, the LICA is based on a one-step homogeneous immunoreaction, and the homogeneous environment does not requiring washing processes, which avoids the possibility of cross-contamination. Yang *et al.* consider this an important factor responsible for the increased precision because it contributes to a more stable reaction system (12).

Based on the results presented here, we confirmed low CVs and high reproducibility (repeatability CV ≤7.5%) in the detection of P4 on the LICA-800 system, and precision was verified. Furthermore, the access progesterone assay showed excellent linearity in the assay measuring range (0.37–40 ng/mL) using the polynomial regression method in accordance with CLSI EP06-A. Bias assessment was used to verify the accuracy, and the percentage deviation met the quality requirements of the laboratory's allowable deviation of 10.00%, which means that the LICA is accurate for progesterone quantification. In addition, we confirmed the detection capability of LICA: the calculated LoB was 0.046 ng/mL, LoD was 0.057 ng/mL, and the LoQ value was 0.161 ng/mL, which means that LICA is sensitive in detecting P4. Therefore, the LICA exhibited good analytical performance and fulfilled the accuracy requirements.

Chemiluminescence immunoassay is widely used in clinical laboratories. Though with high sensitivity and accuracy, it also has certain disadvantages. For example, the above immunoassay requires several washing steps as well as solid-phase immobilization of antibodies, which is time-consuming and laborious (13). Similarly, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is also an emerging technology for the detection of small molecular hormones (14,15), but its high cost and low degree of automation limit its popularity and application in clinical laboratories. While LICA has shown good repeatability and intermediate imprecision in detecting progesterone, total testosterone, estradiol, TSH etc. (6,7). All these findings confirm the practicability of this assay in detecting small molecular hormones. From this point of view, LICA may be more suitable for the routine detection of small molecular hormones such as progesterone.

Conclusions

The competitive LICA provided a rapid, highly sensitive, accurate and precise method for the measurement of serum progesterone. The LICA is a promising assay for clinical application.

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Footnote

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Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-3119

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Tianjin Beichen Hospital. Informed consent was obtained from all human participants.

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