



Competitive light-initiated chemiluminescent assay: using 5- α -dihydrotestosterone-BSA as competitive antigen for quantitation of total testosterone in human sera

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Abstract

This paper described a homogeneous method, light-initiated chemiluminescent assay (LICA), for quantitation of total testosterone in human sera. The assay was bead based and built on a competitive-binding reaction format, in which 5- α -dihydrotestosterone (5- α -DHT) competed with the testosterone in serum samples in binding with biotinylated anti-testosterone antibody. The more testosterone in the serum sample, the less 5- α -DHT that bonded with biotinylated anti-testosterone antibodies. 5- α -DHT was coupled with emission beads (doped with thioxene derivatives and Eu(III) as a chemiluminescence emitter) via bovine serum albumin as a linker. Once streptavidin-coated sensitizer beads (modified with phthalocyanine as a photosensitizer) were added, the streptavidin/biotin reaction between 5- α -DHT-bound anti-testosterone antibody and sensitizer beads could bring emission and sensitizer beads together, which allowed energy transfer from sensitizer bead to emission bead. As such, an exciting light (680 nm) impinging on the sensitizer beads led to light emission at 520–620 nm by emission beads. The strength of the emitted light was inversely proportional to the testosterone in serum sample. The detection range of this assay was from 13.3 to 1200 ng/dL. The coefficient variation for intra- and inter-assay was lower than 15%. The recovery of this method ranged from 95.5 to 105.9% for different samples. Moreover, the LICA assay was highly specific with low cross-reactivity and interference. The concentration of testosterone from 58 serum samples analyzed by the LICA method significantly correlated ($y = 0.97x + 1.87$, $R^2 = 0.970$, $p < 0.001$) with those obtained with the SIEMENS Centaur Xp System.

Keywords Testosterone · 5- α -Dihydrotestosterone · Competitive immunoassay · Light-initiated chemiluminescent assay (LICA) · Quantitative assay

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Introduction

Testosterone is the predominant androgenic steroid. In males, testosterone is secreted by adult Leydig cells and controlled principally by luteinizing hormone. Testosterone plays a key role in the development of the testis and prostate in males. A low serum level of total testosterone may indicate hypopituitarism, hypogonadism, renal failure, hepatic cirrhosis, or Klinefelter syndrome in males. Increased serum total testosterone levels in males can be induced by adrenal and testicular tumors, congenital adrenal hyperplasia, or abnormalities of the hypothalamic-pituitary-testicular axis [1–4]. In females, testosterone is generated in the ovaries, adrenal gland, and peripheral fatty tissues, and its serum concentration is normally about tenfold lower than that in males. High serum levels of total testosterone in women may be indicative of polycystic ovary syndrome, ovarian and adrenal tumors, congenital

adrenal hyperplasia, and other disorders of the hypothalamic-pituitary-ovarian axis [5, 6].

Numerous chromatographic methods have been developed to measure testosterone, including high-performance liquid chromatography [7], liquid chromatography tandem-mass spectrometry analysis [8], and gas chromatography coupled with mass spectrometric detection [9]. Though with high sensitivity and accuracy, these methods have certain disadvantages, such as high cost for analyzer instrument, complex operating procedure, and tedious sample pre-treatment. Immunoassays, including radioimmunoassay [10], plasmonic sensor assay [11], microchipselectrophoresis with chemiluminescence (CL) detection [12], and microfluidic indirect competitive immunoassay [13], though efficient, labor-saving, and cost-effective compared with chromatographic assays mentioned above [14, 15], still require several washing steps as well as solid-phase immobilization of the antibody or antigen.

In light-initiated chemiluminescent assay (LICA) system [16, 17], the surface of emission beads and sensitizer beads was coated with thioxene derivatives, Eu(III) and phthalocyanine, respectively. The specific binding of reactants (e.g., antibody) to analytes (e.g., antigen) can be utilized to link emission beads with sensitizer beads (distance less than 200 nm). Under light irradiation (680 nm), singlet oxygen ($^1\text{O}_2$), which was transformed from oxygen near the surface of sensitizer beads, migrated to the emission beads. Emission beads were excited and generated an emission peak with a wavelength of 520–620 nm (Fig. 1). The assay is sensitive, efficient, and easy-to-operate and requires no washing steps [18, 19]; all these virtues make it a promising surrogate for conventional methods.

5- α -dihydrotestosterone (5- α -DHT) is an analogue of testosterone. It contains two more hydrogen atoms in C-4 and C-5 compared with testosterone (Fig. 2). Both structures have a heterocyclic stem nucleus with a

perhydrocyclopentanophenanthrene ring. Testosterone is converted to 5- α -DHT by 5- α -reductase in target tissues that possess specific DHT-binding proteins. This conversion process appears to be an active metabolic process [20]. In this study, 5- α -DHT was linked to bovine serum albumin (BSA) via 3-(O-(carboxymethyl)oxime) as a linker at position 3. The 5- α -DHT-BSA was used as the competitive antigen for determining serum testosterone by means of the equilibrium reaction model [21] instead of sequential saturation model [22]. This competitive LICA system for total testosterone in human sera exhibited exceptional performance, which can meet the needs for clinical diagnosis and laboratory analysis.

5- α -dihydrotestosterone (5- α -DHT)-BSA was used as the competitive antigen and coated with emission beads (E-beads).

Materials and methods

Apparatus and reagents

Serum samples were obtained from Tianjin Central Hospital of Gynecology Obstetrics and stored at -20°C . Uncoupled emission beads (E-beads), streptavidin-coated sensitizer beads (SA-S-beads), and the 96-well assay plates were from Beyond Biotech (Shanghai, China). The monoclonal anti-human testosterone antibody was purchased from Bioventix (Farnham, Surrey, UK). The Sulfo-NHS-LC-Biotin was from Pierce (Rockford, IL). Testosterone-BSA and (5- α -DHT)-BSA were obtained from Fitzgerald (MA, USA). The LICA tests were carried out using a chemiluminescence analyzer instrument (Beyond Biotech, Shanghai, China), equipped with a 680-nm excitation light and 520–620-nm emission filter.

Fig. 1 Schematic diagram of the competitive LICA

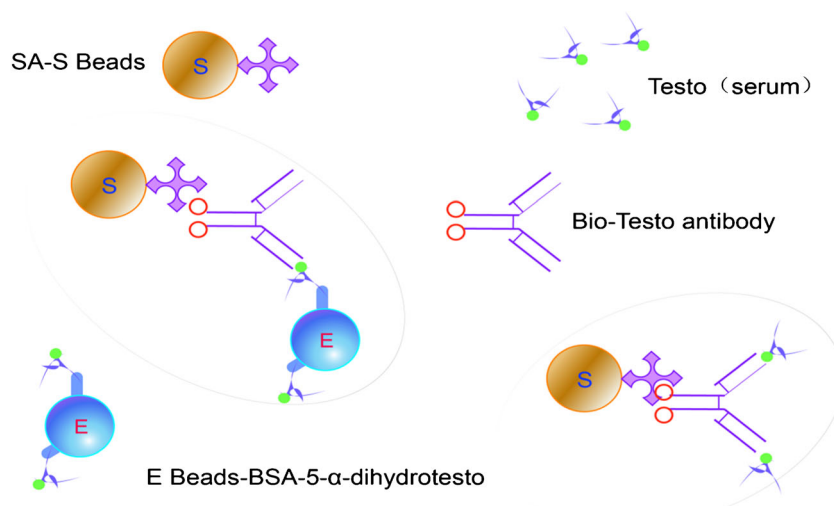
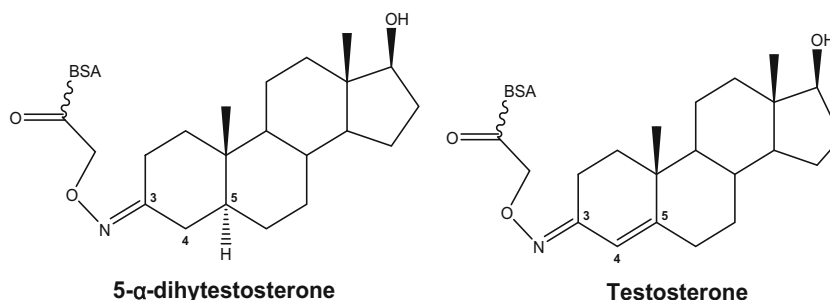


Fig. 2 The chemical structures of testosterone and 5- α -dihydrotestosterone linked to BSA at C-3, respectively



LICA protocol

Coating of antigens to beads

Testosterone-BSA and (5- α -DHT)-BSA were coated on emission beads (E-beads) as previously described [19]. The uncoupled emission beads (8 mg) were suspended in 0.05 M carbonate buffer (pH 9.6) by sonication. Then, 0.1 mg testosterone-BSA and 5- α -DHT-BSA were dissolved in 0.05 M carbonate buffer (pH 9.6), respectively. The E-beads and antigen solutions were mixed and stirred overnight at 37 °C. Then, 10 μ L NaBH₄ (8 mg/mL) was added to reduce the E-beads and 40 μ L glycine (75 mg/mL) was added to block the E-beads. After washing twice, the product was resuspended in 25 mM HEPES, containing 1% BSA and 0.1% ProClin and stored at 4 °C.

Biotinylated antibodies

Anti-testosterone antibodies were dialyzed into 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C. The Sulfo-NHS-LC-Biotin was dissolved in dimethylformamide (DMF) and added into the dialysate at molar ratio of 20:1. After incubation overnight at 4 °C, the mixture was dialyzed in 0.1 M phosphate buffer (pH 7.4) for 8 h. The biotinylated antibodies were verified with the reagent HRP-streptavidin (Thermo Fisher Scientific, MA, USA) by western blotting and stored at 4 °C.

Optimizing the competitive immunoassay

For assay optimization, competitive antigen and the concentration of biotinylated antibody and antigen as well as the buffer composition were optimized. Serum samples, in which testosterone concentrations had been determined by SIEMENS Centaur XP System, were used for assay optimization. The discrimination ability in-between serum samples with different testosterone levels was assessed to decide the optimum assay conditions.

Calibration

Testosterone calibrators were diluted in assay buffer at concentrations of 0, 30, 120, 420, 800, and 1200 ng/dL, designated as S₀, S₁, S₂, S₃, S₄, and S₅, respectively. All calibrators were traced back to testosterone reference standards (ID: 1819, National for Food and Drug Control, Beijing, China) and serum samples with known total testosterone concentrations (measured by the SIEMENS Centaur XP System) [19].

The assay procedure

The buffer used in this assay was 25 mM HEPES, containing 50 mM NaCl, 1% BSA, 0.05% TritonX-100, and 0.05% ProClin-300 and was adjusted to pH 7.4. The testosterone-releasing agent was 0.1 M citrate buffer containing 3% BSA (pH 4.0). The assay was performed in a two-step procedure. Briefly, 20 μ L of testosterone-releasing agent was firstly added into the 96-well plates. Secondly, serum samples or calibrators (20 μ L) were added. Thirdly, 25 μ L of biotinylated antibodies (1:32,000 dilution, 3.12 ng/mL) and 25 μ L E-beads (diluted in 1:1000, 0.1 μ g/mL) were successively supplemented into the wells. The plates were incubated at 37 °C for 17 min by gentle shaking in the chemiluminescence analyzer instrument. Finally, 150 μ L of LICA Common Regent containing the SA-S-beads was added and agitated for another 15 min. The LICA signals were read out through the chemiluminescence analyzer.

Cross-reactivity and interference

To study the specificity of LICA, structural analogs and other reproductive hormones [18, 23] at indicated concentrations were added into serum samples with different concentrations of testosterone (low, 33.0 ng/dL; middle, 76.4 ng/dL; high, 260.9 ng/dL) to assess the cross-reactivity. The results are expressed as follows:

$$1 - C_{\text{pre}} / C_{\text{after}}$$

where C_{pre} and C_{after} meant the concentration value of samples measured before and after the addition of analogs.

Interference tests were performed by adding interfering substances into serum samples. The following interfering substances were added at the stated concentrations: (1) bilirubin (unconjugated) dissolved in dimethyl sulfoxide, (2) hemoglobin from washed hemolyzed erythrocytes, (3) triglyceride in the form of intra-lipid 20% fat emulsion, (4) sodium ascorbate dissolved in 0.01 M PBS, (5) biotin dissolved in 0.01 M PBS [24, 25].

Comparison with the SIEMENS Centaur XP system

The testosterone levels of 58 serum samples from clinical individuals were measured by both methods. A correlation analysis was conducted between the concentrations of total testosterone determined by the two methods.

Data analysis

The data were analyzed using Sigmaplot and GraphPadPrism 5 software.

A four-parameter logistics curve was employed to analyze the standard curve and optimize the assay. The mathematical model was expressed as follows:

$$y = (A-D)/[1 + (x/C)^B] + D$$

In this equation, A , B , C , and D meant maximum effect, slope, ED_{50} , and minimum effect values, respectively [26, 27]. r^2 was used to determine the degree of fitting of the competitive curve.

Results

Assay optimization

Selection of the competitive antigen and reaction model

At the very beginning, we used testosterone-BSA as the competitive antigen for testosterone determination by means of equilibrium reaction model, in which competitive antigen and serum samples were added and incubated simultaneously with biotinylated antibodies. Once the reaction reached equilibrium, SA-S-beads were then added. It turned out that serum samples with diverse testosterone levels were not discriminable and the correlation of serum samples with low concentration of testosterone analyzed by this method and compared with SIEMENS Centaur Xp System was low ($y = 1.16x - 11.35$, $R^2 = 0.870$) (Fig. 3a, b). However, if using the sequential saturation model instead, in which serum samples were preferentially incubated with biotinylated antibodies for 17 min before a further addition of competitive antigen, the discrimination among serum samples with different

testosterone levels and the correlation of serum samples with low concentration of testosterone was obviously improved ($y = 0.95x - 1.68$, $R^2 = 0.970$) (Fig. 3c, d). When 5- α -dihydrotestosterone(5- α -DHT)-BSA was used as the competitive antigen instead of testosterone-BSA and the equilibrium reaction model was applied, the correlation and the discriminatory ability among different serum samples was also satisfactory ($y = 1.08x + 1.13$, $R^2 = 0.982$) (Fig. 3e, f), which is comparable to the second condition, i.e., using testosterone-BSA as competitive antigen by means of sequential saturation model.

Optimization of the working concentrations of the competitive antigen and biotinylated antibodies

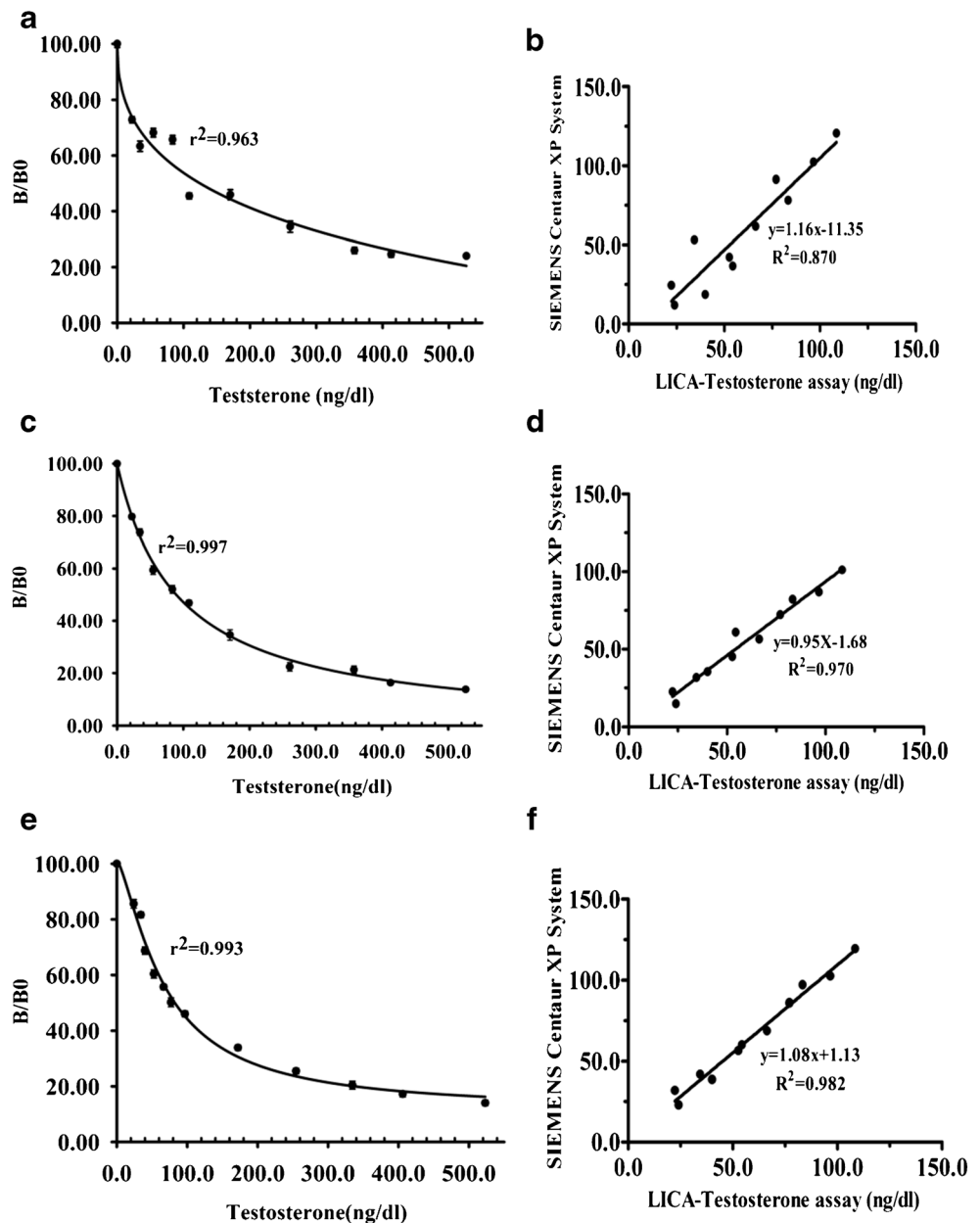
The concentrations of 5- α -DHT-BSA-coupled E-beads and biotinylated antibodies mainly affected the assay's sensitivity and detection range. The optimal working concentration of biotinylated antibodies was studied through plotting calibration curves with six testosterone calibrators. Results showed that the value of ED_{75} , ED_{50} , and ED_{25} was lower (Fig. 4b) when E-beads were 1000-fold diluted (0.1 μ g/mL), indicating that the sensitivity of this assay was higher with E-beads diluted in 1:100 (Fig. 4a). In addition, though the detection range was relatively narrow when using 3.12 ng/mL biotinylated antibodies (diluted in 1/32,000) (Fig. 4c), high sensitivity can be achieved. The functional sensitivity, the lower limit of quantification when $CV\% = 20\%$, obtained by using 49.92 ng/mL, 12.48 ng/mL, and 3.12 ng/mL of biotinylated antibodies was 52.3 ng/dL, 25.1 ng/dL, and 15.4 ng/dL (Fig. 4d), showing that the sensitivity of this assay was higher with biotinylated antibodies diluted in 1/32,000 (3.12 ng/dL).

Optimization of the buffer system

Optimization of the buffer solution Buffer components and the pH are important factors that affect the strength of the binding between antigen and antibody [18]. In this study, 25 mM HEPES buffer (50 mM NaCl, 1% BSA, pH 7.4) provided a more suitable environment for the antibody-antigen specific interaction (see Electronic Supplementary Material (ESM) Fig. S1 a, b, c). The assay performance under three different conditions was analyzed. According to the variation of CL max values and CL_{max}/ED_{50} (see ESM Fig. S1d), the following assays were carried out by using 25 mM HEPES buffer (pH 7.4) containing 50 mM NaCl, 1% BSA, 0.05% Triton X-100, and 0.05% ProClin-300.

Addition of testosterone-releasing agent Most serum testosterone is tightly bound to sex hormone-binding globulin or testosterone-estrogen-binding globulin; it also exists

Fig. 3 Labeled testosterone-BSA and labeled 5- α -DHT-BSA are compared in different competitive assays. **a, b** The assay performance using testosterone-BSA as competitive antigen in equilibrium saturation methods. **c, d** The assay performance using testosterone-BSA as competitive antigen in sequential saturation methods. **e, f** The assay performance in equilibrium techniques using 5- α -DH-BSA as competitive antigen

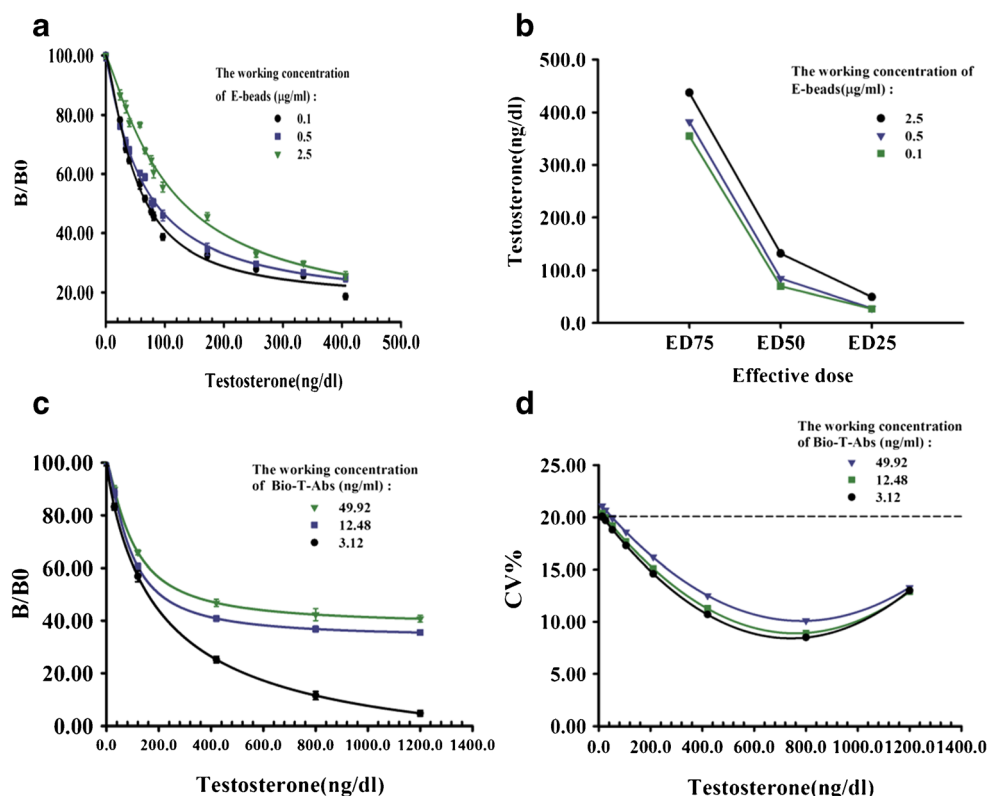


loosely bound to albumin and corticosteroid binding globulin; only less than 2.5% of total serum testosterone is in free state [20, 28]. Hence, it is necessary to convert bound testosterone into free state, which can facilitate testosterone determination, ensuring the accuracy of the assay. Here, 0.1 M citrate buffer (3% BSA, w/v%, pH 4.0) was selected because of the acidic property, under which circumstance the binding of testosterone with other proteins could be destroyed, thus producing free testosterone. The ED_{50} values before and after supplement with the releasing agent were 138.4 and 223.3 ng/dL, respectively, which demonstrated the necessity for an addition of a releasing agent to improve sensitivity of the assay (see ESM Fig. S2).

Calibration curve

The calibration curve was obtained under optimum reaction conditions (Fig. 5). A four-parameter logistic mathematical equation was generated, $y = (100.01 + 5.96) / [1 + (x/176.4)^{0.98}] - 5.96$, $r^2 = 0.999$, from the calibration curve of concentration (x) vs. B/B_0 ratio (y), where B meant the chemiluminescence (CL) signal when serum samples of different concentrations were added and B_0 meant the chemiluminescence signal when buffer solution was added instead of samples. According to the calibration curve, the maximum detection concentration of testosterone by LICA was 1200 ng/dL. The analytical sensitivity of the assay was 13.3 ng/dL, which was calculated from

Fig. 4 The working concentration of the E-beads conjugated with competitive antigens and biotinylated antibodies was optimized. **a** The competitive curves were obtained with 2.5, 0.5, and 0.1 $\mu\text{g/mL}$ E-beads. **b** The value of ED_{75} , ED_{50} , and ED_{25} of competitive curves under different working concentration of the E-beads. **c** Competition curves generated using different concentrations of biotinylated antibodies. **d** The value of CV% obtained by series dilution of the calibrators S_5 (1200 ng/dL)



mean of 20 zero calibrators-3SD. These results demonstrated that the LICA assay was feasible and efficient.

Assay performance

Precision The precision was evaluated by performing duplicate tests of three pooled sera with testosterone concentrations ranging from 15 to 600 ng/dL . In the intra-assay study, 12 duplicates of each pooled sera were randomly placed on a plate and

measured in a run. The coefficients of variation (CV) in the intra-assay were low, from 3.1 to 8.6% (Table 1). For the inter-assay assessment, the three pooled sera were analyzed once a day over a consecutive 12-day period. The inter-assay CV was slightly high, varying between 5.9 and 14.5%.

Accuracy

Spiking recovery was evaluated by supplementing a range of testosterone with different concentrations (0–1000 ng/dL) into 3 serum samples with different testosterone levels ranging from 26.2 to 47.6 ng/dL . The results were expressed as the ratio between the measured and theoretical values. As we can see in Table 2, the recovery rates varied from 95.5 to 105.9%, all within the acceptable range of 80–120%.

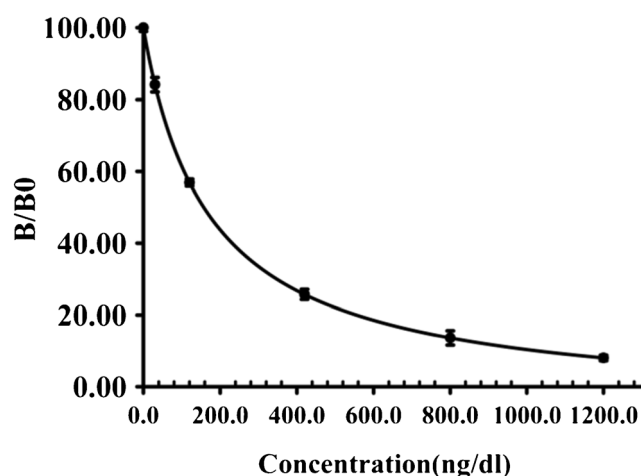


Table 1 Precision test of competitive-binding LICA

| | Inter-assay ($n = 12$, detection of 12 days) | | | Intra-assay ($n = 12$) | | |
|--------|--|-------|------|--------------------------|-------|-------|
| | Mean (ng/dL) | SD | CV% | Mean (ng/dL) | SD | CV% |
| Low | 53.1 | 4.57 | 8.60 | 52.9 | 7.65 | 14.50 |
| Middle | 135.9 | 8.60 | 8.20 | 130.4 | 13.43 | 10.30 |
| High | 546.4 | 17.00 | 3.10 | 557.6 | 32.70 | 5.90 |

CV coefficients of variation

Table 2 Recovery of testosterone added into human serum samples

| Samples | Added (ng/dL) | Measured (ng/dL) | Expected (ng/dL) | Recovery (%) |
|---------|---------------|------------------|------------------|--------------|
| 1 | 0 | 26.2 | – | |
| | 10 | 37.0 | 36.2 | 102.1 |
| | 100 | 127.5 | 233.2 | 101.0 |
| | 500 | 502.6 | 526.2 | 95.5 |
| | 1000 | 986.3 | 1026.2 | 96.1 |
| 2 | 0 | 33.8 | – | |
| | 10 | 46.4 | 43.8 | 105.9 |
| | 100 | 133.2 | 132.8 | 99.6 |
| | 500 | 525.3 | 533.8 | 98.4 |
| | 1000 | 1012.8 | 1033.8 | 98.0 |
| 3 | 0 | 47.6 | – | |
| | 10 | 58.8 | 68.8 | 102.1 |
| | 100 | 145.5 | 333.2 | 98.6 |
| | 500 | 560.3 | 537.3 | 104.3 |
| | 1000 | 1078.6 | 1047.6 | 103.0 |

Specificity The cross-reactivity was assessed by supplementing structural analogs and other reproductive hormones into serum samples with known concentrations (low, middle, high) of testosterone. As was showed in Table 3, with the ratio of cross-reactivity ranging between 11.2 and 15.6%, the steroids including 5- α -DHT, progesterone, and estradiol could compete with testosterone in binding with anti-testosterone antibodies when the testosterone level was low in serum samples. However, when the testosterone was at middle and high concentrations in serum, this cross-reactivity was from 1.7 to 9.5%, which could be ignored.

Interference Anti-interference was assessed by calculating the recovery rate. A recovery rate $\geq 80\%$ was considered acceptable. The concentrations of samples before and after the

addition of interfering substances were analyzed by this assay. The recovery was from 85.0 to 108.7% (Table 4), indicating that bilirubin (20 mg/dL), hemoglobin (500 mg/dL), triglyceride (500 mg/dL), biotin (10 ng/dL), and 6 mg/dL of ascorbic acid (6 mg/dL) at high levels exerted little influence on LICA performance.

Comparison with the SIEMENS Centaur Xp system

Fifty-eight clinical samples were tested by both LICA and the SIEMENS Centaur Xp System. There was a significant correlation between the assay results ($y = 0.97x + 1.87$, $R^2 = 0.970$, $p < 0.001$) (Fig. 6).

Discussion

In this paper, we established a competitive chemiluminescence assay for rapid quantitative detection of testosterone in human sera. Serum samples and the releasing agent were added to a 96-well plate. The competitive antigen-coupled emission beads and biotinylated anti-testosterone antibodies were also added to the wells. The mixture was incubated for 17 min in the analyzer instrument. After the incubation of streptavidin-coated sensitizer beads, the formed complex was excited at 680 nm and a LICA emission peak was detected at 520–620 nm.

The reaction model and competitive antigen were two key points for this competitive LICA. Though the sequential saturation model, or named two-step analysis, has high sensitivity, in which it is possible to relax the requirement of the binding capacity to antibody and dosage of the competitive antigen because of the prior addition of analyte under test, this reaction model is time-consuming and grueling, and the variation is high because of manual operation compared with the equilibrium reaction model. Therefore, we gave priority to the equilibrium reaction mode. In the equilibrium reaction model,

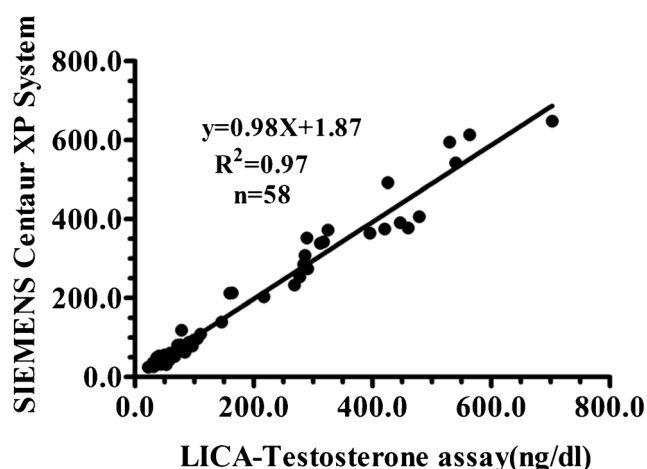
Table 3 Specificity test with structural analogue to testosterone

| Steroid | High | Cross-reactivity (%) | Middle | Cross-reactivity (%) | Low | Cross-reactivity (%) |
|--------------------------|-------|----------------------|--------|----------------------|------|----------------------|
| 5- α -DHT (ng/mL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 100 | 265.3 | 1.7 | 80.2 | 5.0 | 38.2 | 15.6 |
| Progesterone (ng/mL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 100 | 270.3 | 3.6 | 82.1 | 6.9 | 36.7 | 11.2 |
| Estradiol (ng/mL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 100 | 252.7 | 3.1 | 83.7 | 9.5 | 37.6 | 13.9 |

Table 4 Interference test of the present assay

| Interfering substance | High | Recovery (%) | Middle | Recovery (%) | Low | Recovery (%) |
|-----------------------|-------|--------------|--------|--------------|------|--------------|
| Bilirubin (mg/dL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 20 | 254.6 | 102.5 | 74.1 | 103.1 | 34.2 | 96.5 |
| Hemoglobin (mg/dL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 500 | 254.1 | 102.7 | 72.3 | 105.7 | 31.5 | 104.5 |
| Triglyceride (mg/dL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 500 | 265.3 | 98.3 | 74.9 | 102.0 | 34.5 | 95.7 |
| Biotin (ng/mL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 10 | 271.6 | 96.1 | 80.9 | 94.4 | 38.8 | 85.0 |
| Ascorbic acid (mg/dL) | | | | | | |
| 0 | 260.9 | | 76.4 | | 33.0 | |
| 6 | 257.6 | 101.3 | 70.3 | 108.7 | 32.3 | 102.2 |

when testosterone-BSA was used as the competitive antigen, this assay was not able to discriminate serum samples with different testosterone concentrations. However, when changed into sequential saturation reaction model, the assay worked. If 5- α -DHT-BSA was used as the competitive antigen, the serum samples with different testosterone concentrations were discriminable by applying the equilibrium reaction model. From this point of view, we inferred that testosterone-BSA was not suitable for the equilibrium reaction model because of its strong binding capacity with anti-testosterone antibody, while 5- α -DHT-BSA was fit for the equilibrium reaction model due to its less strong binding ability with the same antibody. Hence, the LICA was performed by the equilibrium reaction model and the competitive antigen used in this study was 5- α -DHT-BSA.

**Fig. 6** Correlation between testosterone values of 58 samples measuring in competitive LICA and SIEMENS Centaur Xp System

LICA is a simple, rapid, and high-throughput assay for testosterone quantification. Without washing steps, the assay can be handled in an automatic model [29–32]. In the LICA reaction system, the emission beads and streptavidin-sensitizer beads are general purpose reagents, and competitive antigen and biotinylated anti-testosterone antibodies can be produced in large quantity using a standard production protocol.

The assay performance of LICA-testosterone was comparable to the existing clinical detection methods—SIEMENS Centaur Xp System, in which the detection limit was 11.30 ng/dL with a detection range from 11.30 to 1400.0 ng/dL; the recovery of accuracy was between 83.0 and 95.6%. The detection limit of the LICA for serum testosterone is 13.3 ng/dL. This high sensitivity of the assay can be attributed to the amplified signal resulting from the truth that each donor bead was able to generate 60,000 singlet oxygen molecules under the exciting light (680 nm) [33]. Furthermore, the assay has a high specificity for detecting testosterone, with the recovery rate of interference from 95.5 to 105.9% and the cross-reactivity ratio with structural analogs from 1.7 to 15.6%. The correlation between LICA and SIEMENS Centaur Xp System was significant ($R^2 = 0.970$), confirming the validity of this assay for measurement of serum testosterone. Above all, LICA is a homogeneous luminescent immunoassay, characterized by sensitive, specific, quick, ease of handling, and economical.

Conclusion

In conclusion, the competitive LICA provided a highly sensitive, precise, and specific method for the measurement of total serum testosterone. The LICA for testosterone is a promising assay for clinical application.

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Compliance with ethical standards

The study was approved by the ethics committee of Tianjin Medical University (TMUHMEC2017008). The sera involved in our research were from healthy individuals. Informed consent was obtained from all human participants.

Competing interests The authors declare that they have no conflicts of interest.

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