

Supporting Information for

A Microfluidic Chemiluminescence Immunoassay for Measurement of Testosterone in Serum and Urine

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Materials

Polydimethylsiloxane (PDMS) base with curing agent (Sylgard 184) was from Dow Corning Inc. Testosterone-bovine serum albumin(T-BSA) was purchased from Fitzgerald (1 mg/ml, USA); Testosterone-mAb (T-mAb) was purchased from Abcam (2 mg/ml, UK); HRP conjugated Goat Anti-Mouse (IgG-HRP) was purchased from Haohan Co.Ltd (2 mg/ml, Beijing, China). Testosterone-standard was purchased from sigma (power, USA).

10 mM phosphate buffer solution (PBS) of pH 7.4 was used as the baseline buffer. The wash buffer was prepared by dissolving Tween-20 in 10 mM PBS of pH 7.4 (PBST). 0.3 g Bovine Serum Albumin (Prospec, Israel) was dissolved in 10 ml PBS of pH 7.4 to prepare 3% BSA as the blocking agent. Testosterone-standard was dissolved in ethyl alcohol.

All urine and serum samples were provided by Peking Union Medical College Hospital.

Fabrication of the microfluidic chip for MIC

The microfluidic chip for MIC consists of two polydimethylsiloxane (PDMS) layers, in which the top layer contains seven parallel microchannels for sample introduction, and the bottom layer is immobilized with detection antigens (Fig. 1b). The top layer replica with embossed channels was fabricated by molding PDMS against the polymethyl methacrylate (PMMA) mould. Degassed PDMS (mixed in a 12:1 ratio of PDMS base with curing agent) was coated over the PDMS replica, baked at 70 °C for 30 min in oven, and released from the replica. The inlets and outlets of PDMS top layer were punched

using a syringe needle with flattened tip. The height and width of each embedded microchannel are 500 μm , and the channel length is 4 mm. The bottom layer is a thin layer of PDMS (mixed in a 12:1 ratio of PDMS base with curing agent) of 2 mm thick. The top PDMS layer was adhered to the bottom PDMS layer by the hydrophobic interaction.

Determination of testosterone in different samples

Although the blood samples are more complicated than urine samples, the concentrations of testosterone in serum was relatively low. To prepare serum samples with certain amount of testosterone, we chose the serum with trace amount of testosterone (0.2 ng/mL), and spiked 9.8 ng/mL or 0.8 ng/mL of testosterone into serum samples. The final concentration of testosterone in serum is 10 ng/mL or 1 ng/mL, which was tested by traditional ELISA and MIC (Table 1). The relative error (RE) for serum samples between ELISA and MIC was less than $\pm 10\%$. For urine samples, the original concentration of testosterone was much higher than that in serum, so that we used ELISA and MIC to assay the diluted urine directly, and compared the measured value of testosterone by ELISA and MIC. The RE for urine samples was slightly higher than that for serum samples, probably due to the intrinsic variation between ELISA and MIC, and the lack of proteins in urine which kept the stability of hapten such as testosterone. However, the RE for urine samples was still within $\pm 10\%$.

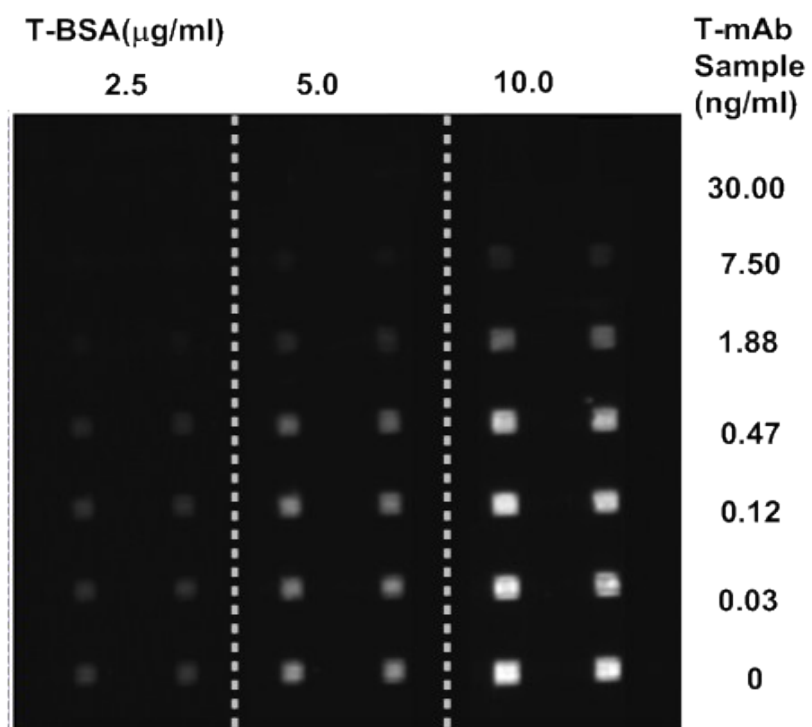


Fig. S1 Optimization of T-BSA concentrations from 2.5 to 10 $\mu\text{g/ml}$. The concentration of T-mAb is 1.67 $\mu\text{g/ml}$. Sample is the standard testosterone with different concentrations from 0.03 to 30 ng/ml.

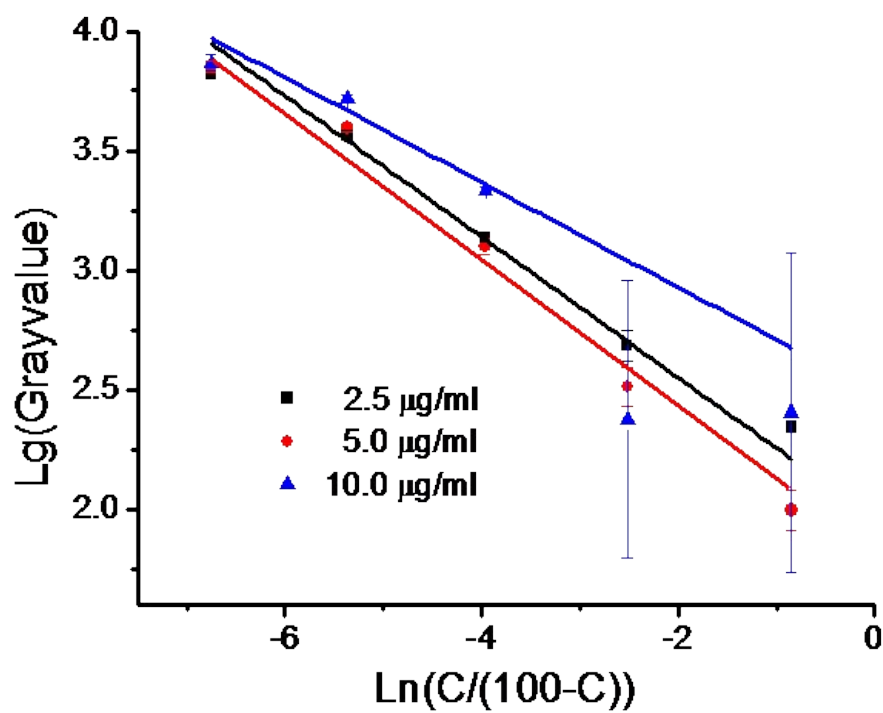


Fig. S2 Optimization of T-BSA concentrations: T-BSA was serially diluted from 10 to 2.5 $\mu\text{g/ml}$. $R^2_{2.5} = 0.9473$; $R^2_{5.0} = 0.9637$; $R^2_{10.0} = 0.8458$

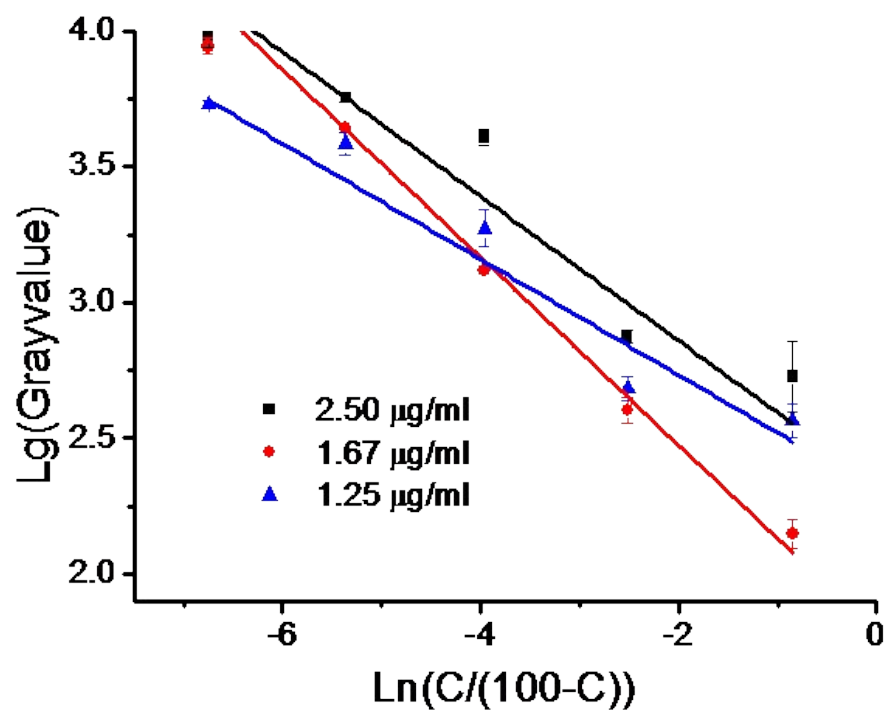


Fig. S3 Optimization of T-mAb concentrations: two-fold serial dilution of Testosterone-mAb were prepared, from 1:800 to 1:1600 (according to 2.5 to 1.25 µg/ml).

$R^2_{2.50}=0.9386$; $R^2_{1.67}=0.9894$; $R^2_{1.25}=0.9734$.

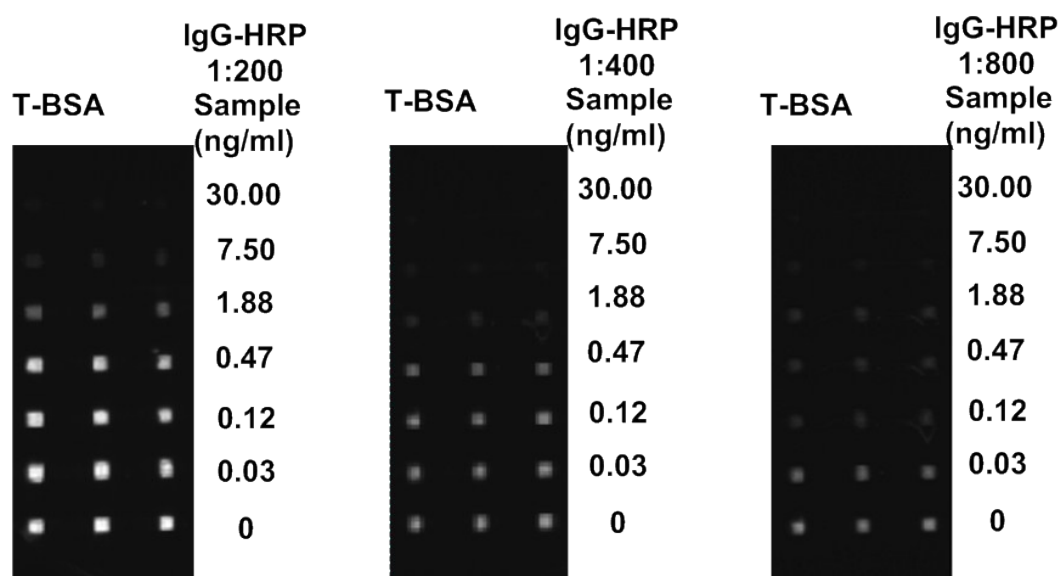


Fig. S4 Optimization of second antibody concentrations: second antibody was serially diluted from 1:200 to 1:800 (10.0 to 2.5 µg/ml). (The concentration of T-BSA was 5 µg/ml, and the dilution of T-mAb is 1:1200 (1.67 µg/ml)).

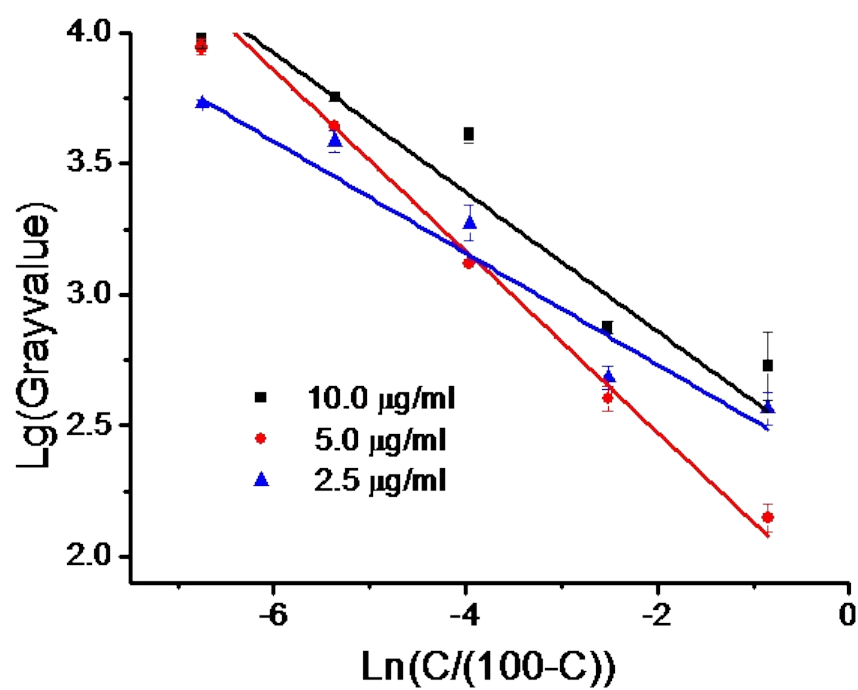


Fig. S5 Optimization of second antibody concentrations: second antibody was serially diluted from 2.5 to 10.0 $\mu\text{g/ml}$. $R^2_{2.5}=0.8181$; $R^2_{5.0}=0.9631$; $R^2_{10.0}=0.9473$.

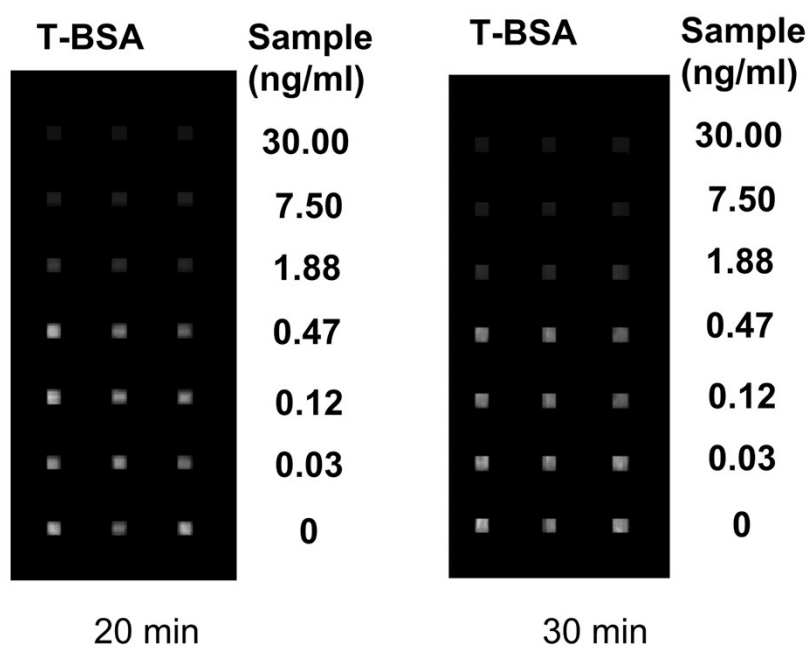


Fig. S6. Optimization of the incubating time for hapten and antibody binding: 20 min and 30min (The concentration of T-BSA was 5 $\mu\text{g/ml}$, second antibody was 5 $\mu\text{g/ml}$) and the dilution of T-mAb is 1:1200 (1.67 $\mu\text{g/ml}$)).

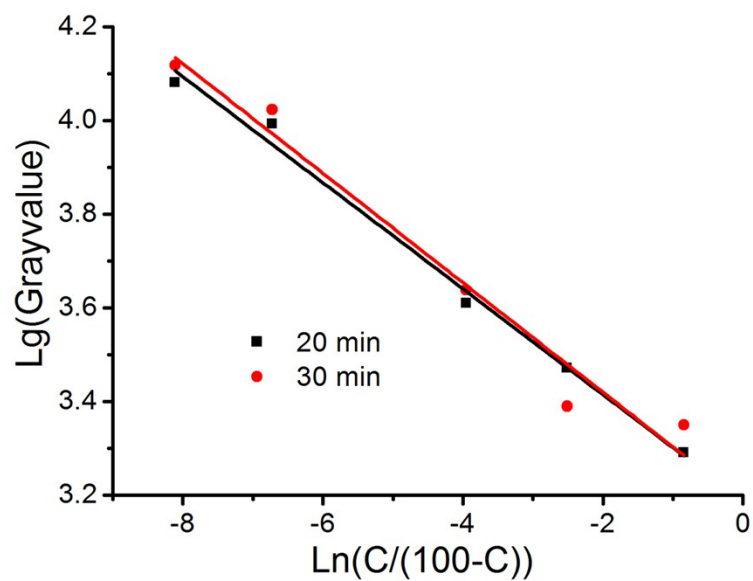


Fig. S7. Optimization of the incubating time for hapten and antibody binding: 20 min and 30 min. 20 min: $R^2=0.9953$; 30 min: $R^2=0.9795$.

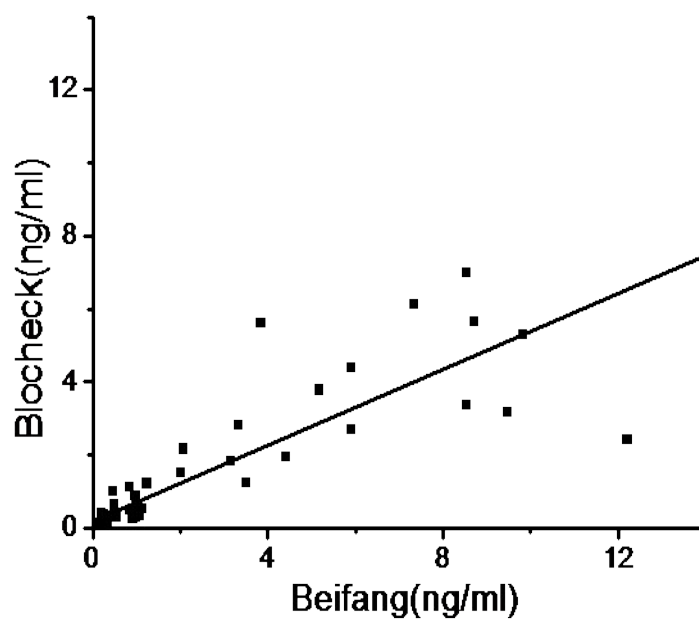


Fig. S8. Testosterone measurement in 49 human serum samples. Each serum sample is measured by ICEIA, Biocheck's and Beifang's ELISA kit, to compare the performance of these two commercial kits