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# Rapid detection method and portable device based on the photothermal effect of gold nanoparticles

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ABSTRACT

Gold nanoparticle (GNP)-labeled immunochromatography test strip (ICTS) has been widely used in different fields, but its sensitivity still require further improvement. In this work, a rapid and quantitative test strip detection method based on the photothermal effect of GNPs was established using a temperature sensor. A portable sensor device was fabricated based on the above method, and the main operating parameters were optimized. Three types of analyte models (cells, macromolecules, and small molecules) were chosen to evaluate the application of the sensor device using the commercial ICTS. The detection limit is at least 1 order of magnitude lower than that of the traditional visual detection ICTS. The other strip type of dot immunogold filtration test was adopted to further improve the sensitivity. The sensitivity of the sensor detection method was similar to that of the infrared camera method, and the proposed sensor device has obvious advantage of low-cost, as well as the usage of a small portable instrument, ease of use and rapid test. The portable sensor device based on the photothermal effect of GNPs can be used as a new and promising device for simple, quantitative, rapid, and on-site screening of analyte by ICTS.

## 1. Introduction

Gold nanoparticle (GNP)-labeled immunochromatography test strip (ICTS) have been widely used as a rapid detection method in different fields such as biomedicine, food, and environmental analysis (Du et al., 2017; Sotnikov et al., 2017; Huang et al., 2016; Gong et al., 2018; Fang et al., 2018; Lou et al., 2018). Currently, the signal detection modes of GNP-labeled ICTS mainly included the visual method (Zhang et al., 2015; You et al., 2017; Li et al., 2017) and the simple instrument method with a portable device. The visual method can rapidly obtain qualitative or semiquantitative results by the naked eyes without expensive instruments. However, its sensitivity and repeatability still require further improvement. The simple instrument detection using a portable device such as a color recognition sensor, (Struss et al., 2010; Chen et al., 2014) which can be very helpful for improving the detection precision. However, the sensitivity of this method shows no significant improvement compared with the visual detection method. Herein, a new strategy is proposed to improve the sensitivity of GNP-labeled ICTS based on the photothermal effect of GNPs by a temperature sensor.

GNPs generate heat upon optical stimulation due to surface plasmon resonance (SPR), especially at absorption of 532 nm light (Schlucker, 2014; Langille et al., 2013). Bischof et al. established a creative solution based on the thermal contrast of GNPs to improve the sensitivity of ICTS, which showed a 32-fold improvement compared to visual detection in analytical sensitivity (Qin et al., 2012). And then they presented a single step add-on reader approach based on thermal contrast amplification increasing sensitivity 8-fold in three commercial ICTS (Wang et al., 2016). The effect of the size of GNPs on the analytical performance of lateral flow immunoassays was also investigated (Zhan et al., 2017). However, in previous works, the temperature variation ($\Delta T$) generated by GNPs was recorded using an expensive infrared camera (cost of approximate $6000), which limited the use of this approach in practical applications, especially in field detection.

Herein, a low-cost and more convenient sensor device consisting of a temperature sensor and a laser pen was fabricated to record the $\Delta T$. The main factors affecting the response of the sensor device were investigated, and the performing conditions were optimized. The proposed sensor device has obvious advantage of low-cost (approximate $50), as well as other advantages such as the use of a small portable instrument, ease of use and rapid test. The real feasibility of the proposed sensor device was validated using the commercial ICTS of three

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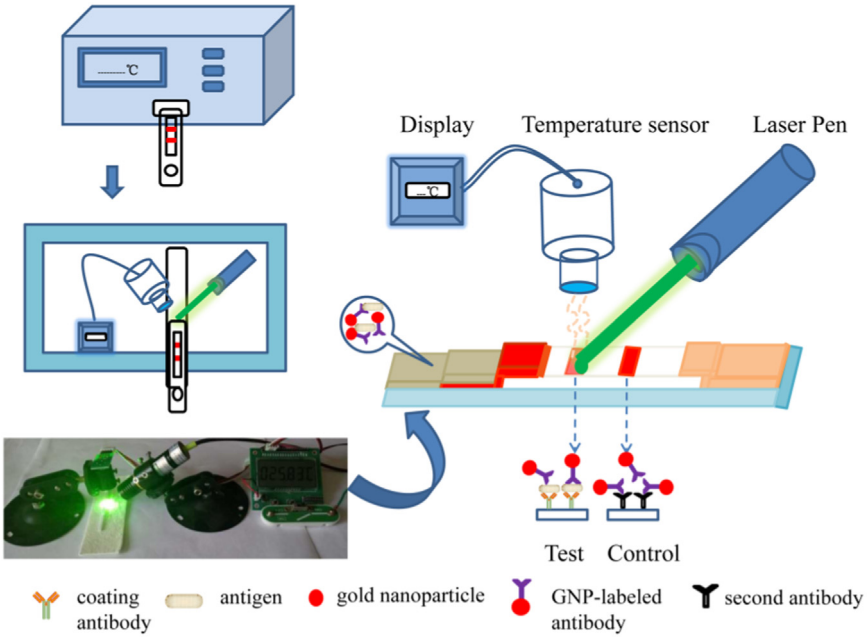


**Scheme 1.** Schematic diagram of photothermal effect based detection.

types of analytes (cells, macromolecules, and small molecules).

The sensor device was mainly fabricated by a temperature sensor, a laser pen and a display as shown in Fig. S2. Scheme 1 shows the schematic diagram of the sandwich ICTS detection based on the photothermal effect of GNPs by the portable sensor device. Coating antibody and second antibody were coated on the nitrocellulose membrane as the test line and control line, respectively. Upon laser irradiation at 532 nm emitted from laser pen, the ΔT of the test line generated by GNPs was recorded with the proposed sensor device. Quantitative results were obtained according to the correlation between the ΔT and the different concentrations of the target analyte (Choi et al., 2016; Werner et al., 2010).

## 2. Material and methods

### 2.1. Reagents and materials

The temperature variation (ΔT) was recorded with a temperature sensor purchased from Shanghai HW Co. Ltd. (Shanghai, China) or an infrared camera (IRS S6) from Suzhou Nuofangke Precision Equipment Co. Ltd. (Suzhou, China). All centrifugations were performed with MOV-112 purchased from Sanyo Electric International Trading Co. Ltd. (Beijing, China). The shaker incubators were obtained from Shanghai East Instrument Equipment Co. Ltd. (Shanghai, China). The laser was purchased from Yiwu Yunmai Electronic Commerce Co. Ltd. (Yiwu, China). Beakers, test tubes, volumetric flasks and other glass equipment were obtained from Jinan Chengsen Trade Co. Ltd. (Jinan, China)

Anti-*Salmonella typhimurium* antibodies were obtained from Beijing Boosen Biosynthesis Biotechnology Co. Ltd. (Beijing, China). *Salmonella typhimurium* (ATCC 14028) was obtained from China Medical Bacterial Preservation Centre (Beijing, China). *Salmonella typhimurium* test strip was purchased from Beijing Zhiyunda Co. Ltd. (Beijing, China). The human chorionic gonadotropin was purchased from Jinan Chengsen Trade Co. Ltd. (Jinan, China). The human chorionic gonadotropin test strip was purchased from Runhe Biological Medicine Technology Co. Ltd. The clenbuterol hydrochloride was purchased from Shenzhen Xiangyun Chemical Co. Ltd. (Shenzhen, China). The clenbuterol hydrochloride test strip was purchased from Jiangsu Wise Science Technology Development Co. Ltd. (Jiangsu, China). Bovine serum albumin (BSA) was purchased from Sigma Aldrich (Steinheim, Germany). Grape juice was purchased from local supermarket (Jinan, China). All of the chemicals were of analytical grade unless otherwise stated.

### 2.2. Construction of the portable sensor device

The sensor device was fabricated with the following parts: a temperature sensor, a laser pen, a power adapter, a display and two on-off. GNPs generate heat at irradiation of 532 nm emitted from laser pen. The temperatures before and after irradiation were recorded using a temperature sensor and read out from the display. The power adapter can convert the voltage from 220 V to 3.3 V. To optimize the irradiation time (in the range of 0–300 s) of the sensor device, the surrounding experimental temperature was approximately 20 °C. The surrounding experimental temperature was optimized at the irradiation time of 50 s, the distance from the laser source to the test line was 2.5 cm and the height of the temperature sensor was 2.5 cm. When optimizing the distance from the laser source to the test line, the irradiation time was 50 s, the surrounding experimental temperature was set at 25 °C , and the height of the temperature sensor was 2.5 cm. The height of the temperature sensor was optimized under the above optimal conditions.

### 2.3. Culture and preparation of Salmonella typhimurium

The culture of *Salmonella typhimurium* was according to our previous work (Zhang et al., 2018). Pure colony cultures of *Salmonella typhimurium* were cultured in Luria broth medium for 12 h at 37 °C as instructed by the American Type Culture Collection. Calculation of the CFU $mL^{-1}$ was determined by conventional plating assay. For real sample analysis, different concentrations of *Salmonella typhimurium* were spiked into 1 mL of grape juice. Then the precipitate was obtained from the mixture by centrifugation at 3000*g* for 5 min, and washed with PBS. The mixture was centrifuged again and the precipitate was resuspended in PBS to the required concentration for detection. Due to safety considerations, all of the bacterial samples were sterilized in an autoclave at 121 °C for 20 min.

### 2.4. Preparation of the standard solution of hCG and clenbuterol hydrochloride

The stock solution of hCG analytes (29.6 IU $mL^{-1}$) were made

according to the sample instruction. A series of work solutions (10, 15, 20, 25, 50, 100, 150, 200, 250, 300 mIU $mL^{-1}$) were prepared by diluting the stock solution with 0.5% BSA for further detection.

The stock solution of clenbuterol hydrochloride analytes (5.29 mg $mL^{-1}$) were also made according to the sample instruction. A series of work solutions (0.5, 1, 2, 3, 4, 5, 6, 7 mg $mL^{-1}$) were prepared by diluting the stock solution with deionized water for further detection.

### 2.5. Preparation of GNP-modified antibody

A total of 40 μL $K_2CO_3$ solution at the concentration of 0.1 mol $L^{-1}$ was added to the GNPs to adjust the pH to 9.0. The antibody was diluted and slowly added to the GNPs with continuous stirring for the total time of approximately 5 min. Then, 0.1 g $mL^{-1}$ of BSA solution was added and stirred for another 30 min. The solution was centrifuged at a rate of 10,000*g* for 30 min. The supernatant was discarded, and the precipitate was suspended in the antibody storage solution.

### 2.6. Preparation of the dot immunogold filtration test strip

Nitrocellulose paper (pore size 8 μm, Whatman AE99) was coated 1 μg/dot with *Salmonella typhimurium* antibody overnight, blocked with 3% BSA for 1 h at 37 °C, washed with modified phosphate-buffered saline tween-20 (PBST) and then dried for storage at 4 °C until use.

### 2.7. Visual detection method of the test strip

For commercial ICTS detection, 80–100 μL (about 3–4 drops) sample solution was slowly added to the sample area of the strip. The result was read in 3–5 min and invalid when exceeding 10 min. For dot immunogold filtration test strip detection, a mixture of 10 μL *Salmonella typhimurium* and GNP-antibodies was added to the strip and the result was observed in 10 min.

### 2.8. Detection method based on the photothermal effect

Standard curves were established according to the ΔT and different concentrations of the target analyte.

$$\Delta T = |\Delta T1 - \Delta T0|$$

Where ΔT was the temperature variation, ΔT0 was the temperature variation of the blank sample (sandwich ICTS) or control sample (competitive ICTS) before and after irradiation, ΔT1 was the temperature variation of the spiked sample before and after irradiation.

## 3. Results and discussion

### 3.1. Characterization of the GNPs

TEM image of the GNPs in Fig. S1a showed that the size of GNPs is about 20 nm. Fig. S1b showed the photothermal effect of different concentration of GNPs to verify the feasibility of the photothermal effect detection method. The result showed that quantitative detection can be achieved by compare the temperature variation of different concentration of GNPs.

### 3.2. Optimization of the effect factors of the sensor device

The main factors affecting the response of the sensor device including the irradiation time, the distance from the laser source to the test line, the surrounding experimental temperature, and the height of the temperature sensor were investigated. The temperature of GNPs increased quickly in the first 50 s and then decreased. To achieve rapid and accurate detection, the irradiation time should be as short as possible. Therefore, 50 s was chosen as the irradiation time (Fig. 1a). The ΔT showed the most significant increase when the ambient temperature was approximately 25 °C (Fig. 1b). Figs. 1c and 1d showed that the optimal irradiation distance from the laser source to the test line and the height of the temperature sensor were 2.5 cm and 2.0 cm, respectively.

### 3.3. Quantitative detection of Salmonella typhimurium

Three representative target analytes, including bacteria cells (Yu et al., 2012), macromolecules (Cui et al., 2011), and small molecules, were chosen to evaluate the application of the sensor device method to the commercial ICTS. First, the feasibility of the sensor device was evaluated using the commercial *Salmonella typhimurium* ICTS. The sensitivities of the visual detection, the infrared camera method and the sensor method were compared. For qualitative analysis, the visual detection limit for *Salmonella typhimurium* was estimated to be $1.13 \times 10^7$ CFU $mL^{-1}$ (Fig. 2a). For the infrared camera method, the linearity in the range of $1.2 \times 10^6 - 3.4 \times 10^7$ CFU $mL^{-1}$ was obtained between the ΔT and the concentration of *Salmonella typhimurium*. The limit of detection was calculated at the signal to noise ratio of 3:1. The ΔT of 10 test strips were measured in the same conditions with an infrared camera, and the standard deviation (SD) of the ΔT was 0.1 °C, which was defined as the value of the noise. Thus, the ΔT corresponding to the detection limit of the *Salmonella typhimurium* should be 0.3 °C, indicating that the detection limit of the infrared camera method was $1.2 \times 10^6$ CFU $mL^{-1}$. These results showed that the analytical sensitivity of the commercial *Salmonella typhimurium* ICTS can be improved 9-fold by using infrared camera than visual detection (Fig. 2c). Subsequently, the ΔT was also measured using the sensor device. The ΔT was linearly related to the concentration of *Salmonella typhimurium* in the range of $1.2 \times 10^6 - 3.4 \times 10^7$ CFU $mL^{-1}$, as shown in Fig. 2d. The detection limit of the photothermal detection obtained by the sensor device was $1.0 \times 10^6$ CFU $mL^{-1}$. The results showed that the sensitivity of the sensor device detection was higher than visual detection, and similar to that of the infrared camera method.

To evaluate the feasibility of the established method for real sample analysis, it was used to detect spiked *Salmonella typhimurium* in grape juice. The visual detection limit for *Salmonella typhimurium* was estimated to be $1.13 \times 10^7$ CFU·$mL^{-1}$ (Fig. 2b). ΔT value was also linearly related to the concentration of *Salmonella typhimurium* in the range of $1.2 \times 10^6 - 3.4 \times 10^7$ CFU $mL^{-1}$. The detection limit of the photothermal detection obtained by the sensor device was $9.7 \times 10^5$ CFU $mL^{-1}$, the recoveries were between 96.2 ± 8.3% and 104.8 ± 5.5% (Fig. 2d). The standard curve coincided with that prepared in PBS, which indicates that the grape juice matrix had almost no effect on the detection. This method can be applied to commercial ICTS, showing the advantages of low-cost and smaller size compared to the infrared camera. Therefore, in our subsequent experiments, the ΔT was only measured by the sensor device.

### 3.4. Quantitative detection of human chorionic gonadotropin

Next, the human chorionic gonadotropin (hCG) analyte was chosen as the representative macromolecule to evaluate the detection performance of the developed sensor device using a commercial ICTS. As shown in Fig. 3a, the visual detection limit achieved by naked eyes was approximately 50 mIU $mL^{-1}$. The ICTS results were also be quantitatively analyzed by determining ΔT using the sensor device. The detection limit of photothermal detection was calculated to be 5.5 mIU $mL^{-1}$ based on the standard curve shown in Fig. 3b. ΔT was linearly related to the hCG concentration in the range of 15–150 mIU $mL^{-1}$. The sensor detection method produced a 9-fold improvement in analytical sensitivity compared to visual detection.

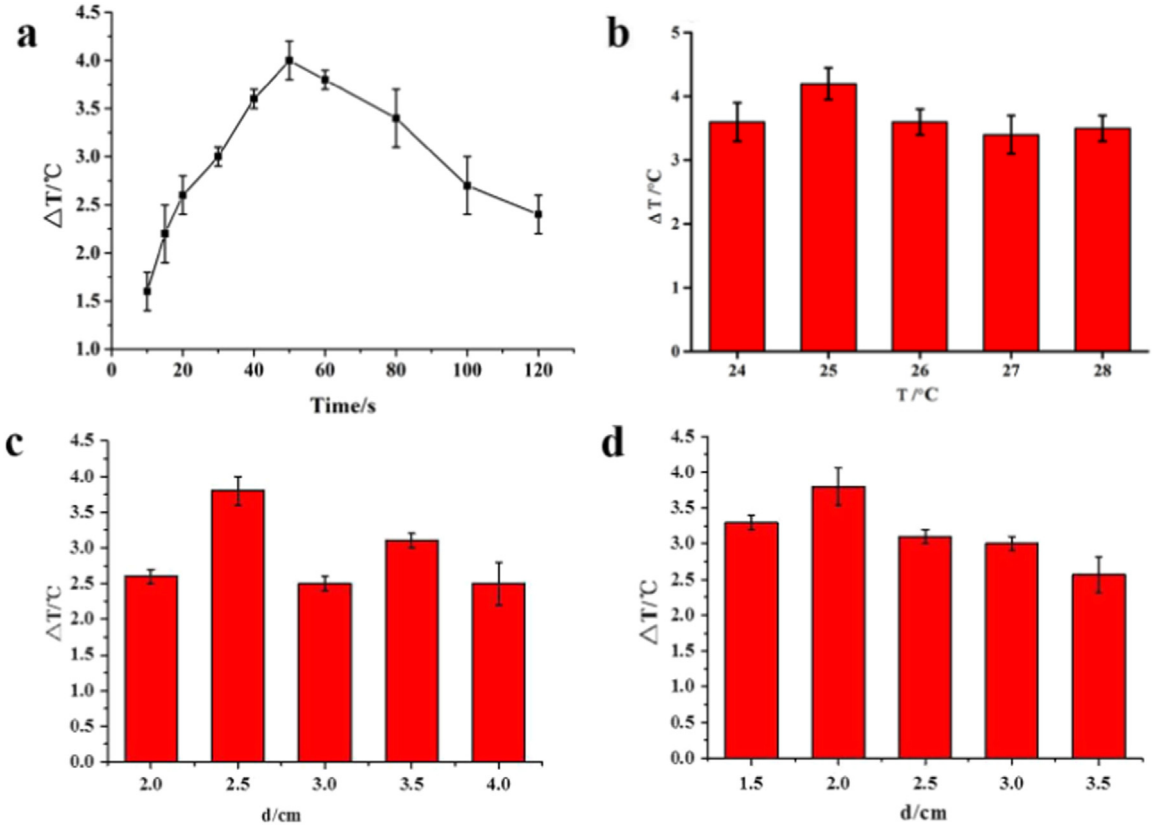


**Fig. 1.** Optimization of effect factors of the sensor device. a: Irradiation time. b: Surrounding temperature. c: Irradiation distance. d: Temperature sensor height.

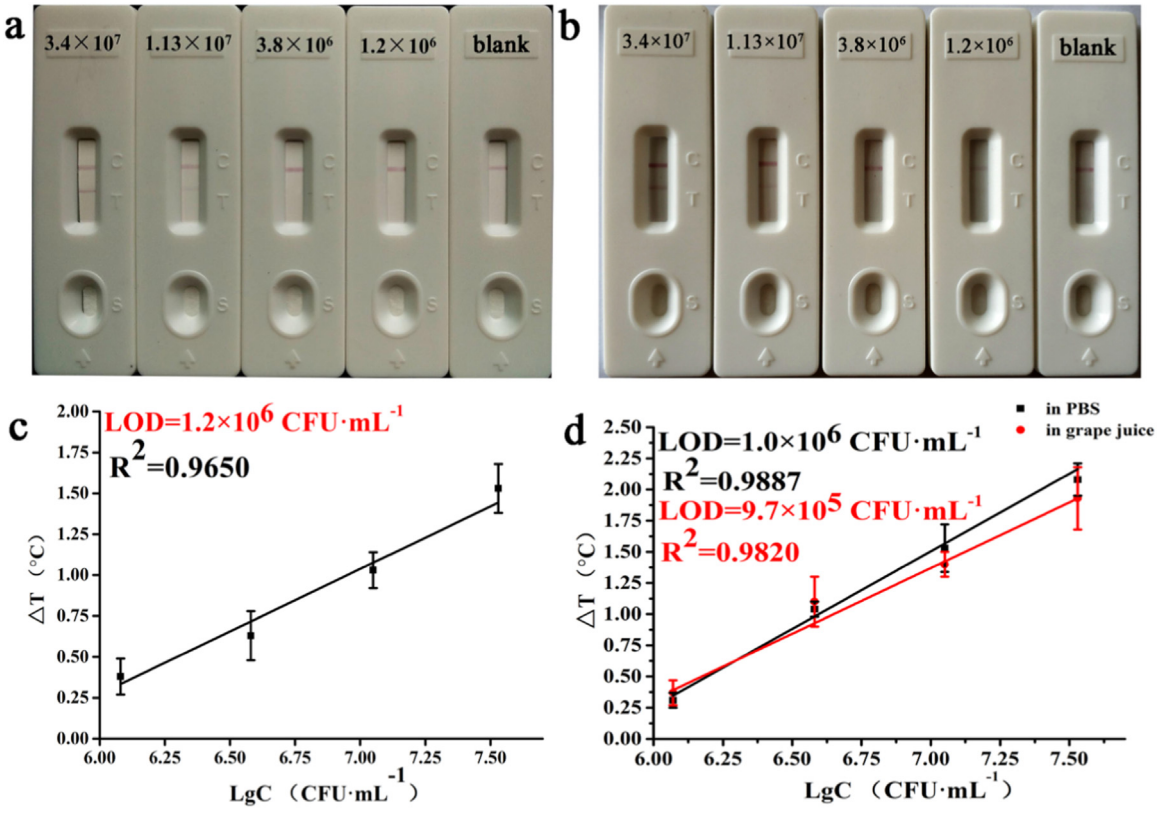


**Fig. 2.** a, b: Photograph of commercial *Salmonella typhimurium* ICTS (from 0 to 3.4 × $10^7$ CFU $mL^{-1}$) in PBS (a) and in grape juice (b). c: Standard curve measured by infrared camera. d: Standard curve measured by sensor device in PBS (the black line) and in grape juice (the red line) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

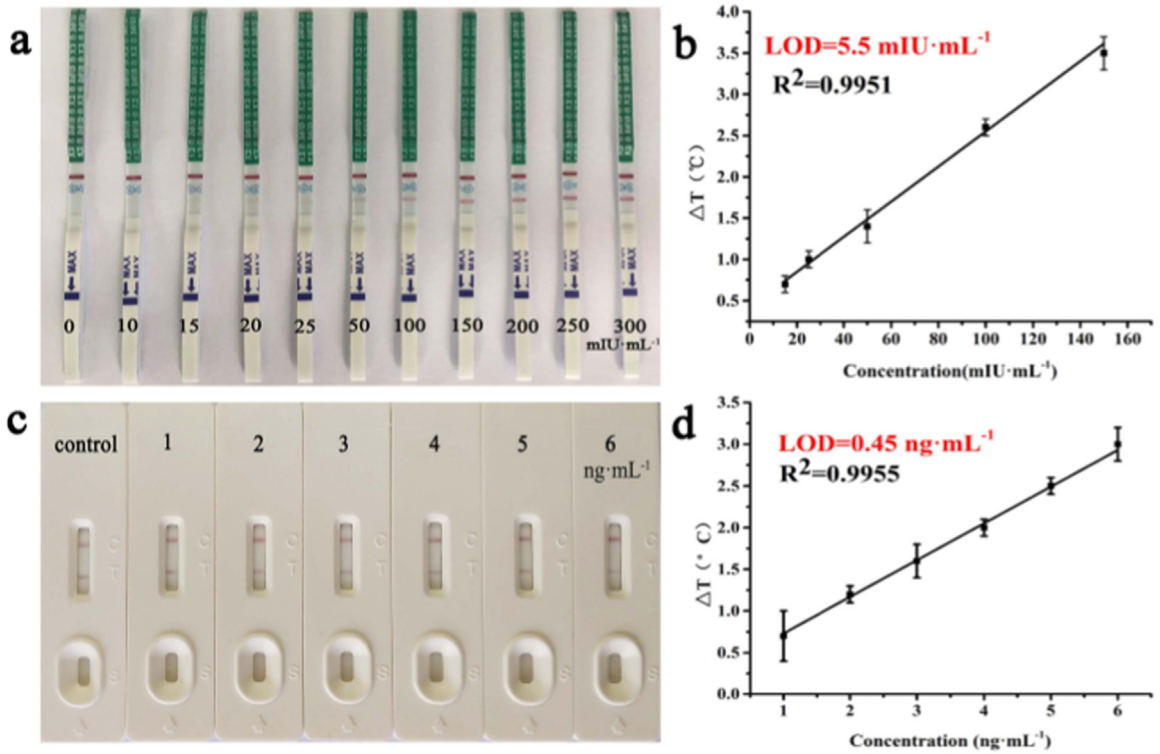


**Fig. 3.** a: Photograph of commercial human chorionic gonadotropin test strips (from 0 to 300 mIU $mL^{-1}$). b: Standard curve of ΔT and human chorionic gonadotropin concentration. c: Photograph of commercial clenbuterol hydrochloride test strips (from 0 to 6 ng $mL^{-1}$). d: Standard curve of ΔT and clenbuterol hydrochloride concentration. ΔT was measured by sensor device.

### 3.5. Quantitative detection of Clenbuterol hydrochloride

Clenbuterol hydrochloride was chosen as the representative of small molecules. Generally, small molecular analytes were detected using competitive immunoassay and give an inverse dependence of the signal on the concentration of the analyte. As shown in Fig. 3c, the visual detection limit achieved by the naked eyes was 6 ng $mL^{-1}$. Fig. 3d showed that the ΔT was linearly related to the concentration of clenbuterol hydrochloride in the range of 1.0–6.0 ng $mL^{-1}$. The detection limit of the photothermal method was 0.45 ng $mL^{-1}$, which was 13-fold improvement in analytical sensitivity than visual detection. The results verified that the sensor device can be applied to the quantitative detection of the commercial test strip and the sensitivity was significantly improved compared to the visual detection.

### 3.6. The dot immunogold filtration detection method of Salmonella typhimurium

Generally, traditional ICTS and dot immunogold filtration test strip are commonly used on-site immunoassays with nitrocellulose membrane as carrier materials. The shape of the detection areas of ICTS and dot immunogold filtration test strip are different, which are line and round dot, respectively. Furthermore, owing to the round dot shape of the detection area of dot immunogold filtration test strip, it is more suitable for the temperature sensor detection than the linear shape of the traditional commercial ICTS. Therefore, the sensitivity of dot immunogold filtration test strip is higher compared to the traditional ICTS (Zhou et al., 2016; Xiao et al., 2017). To further improve the sensitivity of the detection method based on the photothermal effect of GNPs, the dot immunogold filtration test strip was adopted to detect *Salmonella typhimurium*. As shown in Fig. 4a, the visual detection limit achieved by the naked eyes was 5.4 × $10^6$ CFU $mL^{-1}$, which was significantly more sensitive than the traditional ICTS (1.13 × $10^7$ CFU $mL^{-1}$). Fig. 4b showed that ΔT was linearly related to the concentration of *Salmonella*

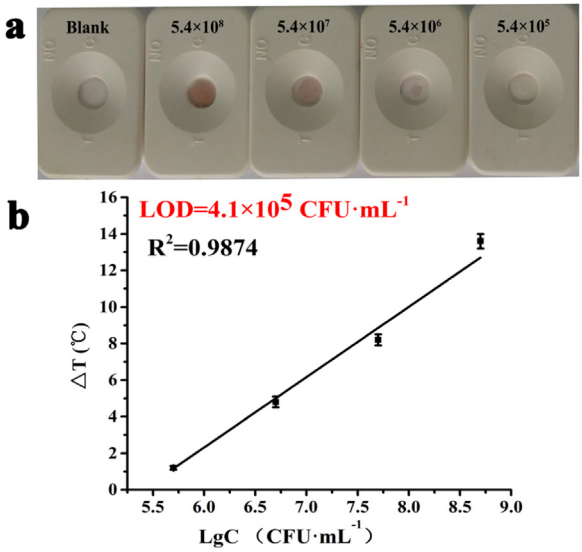


**Fig. 4.** a: The photograph of *Salmonella typhimurium* dot immunogold filtration test strips. b: The standard curve of ΔT and the concentration of the *Salmonella typhimurium*, temperature variation were measured by sensor device.

*typhimurium* in the range of 4.1 × $10^5$ − 5.0 × $10^8$ CFU $mL^{-1}$. The limit of photothermal detection using the sensor device was 4.1 × $10^5$ CFU $mL^{-1}$, which was 13-fold improvement in analytical sensitivity than visual detection for the same type of test strip.

The photothermal effect of GNPs can be applied to ICTS using the proposed sensor device to improve the analytical sensitivity, as shown in Table 1. The results showed that the analytical sensitivity of the commercial *Salmonella typhimurium* ICTS by the sensor device can be improved by 9-fold than visual detection, and the detection limit was 1.0 × $10^6$ CFU $mL^{-1}$, which was similar to the results obtained by the

**Table 1**
The results of different methods for three representative target analytes.

| Analytes | Visual method | Infrared camera method | Sensor device method | Dot immunogold filtration test strip by sensor device method |
|---|---|---|---|---|
| ***Salmonella typhimurium*** | Detection limit: $1.13 \times 10^7$ CFU $mL^{-1}$ | Linear range: $1.2 \times 10^6$–$3.4 \times 10^7$ CFU $mL^{-1}$<br>Cost: $6000 | Linear range: $1.2 \times 10^6$ – $3.4 \times 10^7$ CFU·$mL^{-1}$<br>Detection limit: $1.0 \times 10^6$ CFU $mL^{-1}$<br>Cost: $50 | Visual method: $5.4 \times 10^6$ CFU $mL^{-1}$<br>Linear range: $4.1 \times 10^5$ – $5.0 \times 10^8$ CFU $mL^{-1}$<br>Sensor detection limit: $4.1 \times 10^5$ CFU $mL^{-1}$ |
| **Human chorionic gonadotropin** | Detection limit: 50 mIU $mL^{-1}$ | / | Linear range: 15–150 mIU·$mL^{-1}$<br>Detection limit: 5.5 mIU $mL^{-1}$ | / |
| **Clenbuterol hydrochlorid** | Detection limit: 6 ng $mL^{-1}$ | / | Linear range: 1.0–6.0 ng $mL^{-1}$<br>Detection limit: 0.45 ng $mL^{-1}$ | / |

infrared camera method. The analytical sensitivity of the commercial ICTS for hCG and clenbuterol hydrochloride can be improved by 9-fold and 13-fold than visual detection, respectively. For dot immunogold filtration test strip method, the visual detection limit achieved by the naked eyes was $5.4 \times 10^6$ CFU $mL^{-1}$, which was significantly more sensitive than the traditional ICTS ($1.13 \times 10^7$ CFU $mL^{-1}$). The limit of photothermal detection using the sensor device was $4.1 \times 10^5$ CFU $mL^{-1}$, which was 13-fold greater improvement in analytical sensitivity than visual detection. The whole operation of test strip using the sensor device method or visual method includes sample preparation and detection procedure. The sample preparation for *salmonella typhimurium*, hCG or clenbuterol hydrochloride can be finished in 15 min. The step of photothermal detection and visual method can all be finished in 5 min.

## 4. Conclusion

A rapid and quantitative test strip detection method based on the photothermal effect of GNPs was established using a temperature sensor. A portable sensor device was fabricated based on the above method. Three types of models including cells, macromolecules and small molecules were chosen to evaluate the proposed sensor device. Because of the good photothermal effect of GNPs, the detection limit of ICTS is at least 1 order of magnitude lower than the traditional visual detection. The dot immunogold filtration test strip method, a more sensitive detection method, showed 13-fold improvement in analytical sensitivity than visual results on *Salmonella typhimurium* detection. In summary, the sensor detection method can significantly improve the analytical sensitivity and the proposed sensor device has obvious advantage of low-cost, as well as other advantages such as the use of a small portable instrument, ease of use and rapid test. Furthermore, new type of nanomaterials with excellent photothermal effect can be chosen instead of GNPs to further improve the sensitivity of ICTS combined with the sensor device.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.039.

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