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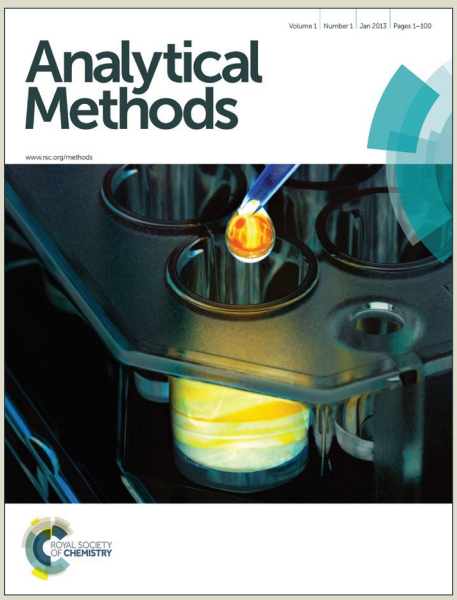

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Colorimetric determination of *Salmonella typhimurium* based on aptamer recognition

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Colorimetric determination of *S. typhimurium*

Abstract A sensitive method for colorimetric determination of *Salmonella typhimurium* (*S. typhimurium*) was developed based on aptamer recognition and gold nanoparticles. First, the biotinylated specific aptamer for *S. typhimurium* was immobilized on the avidin-coated microplate wells. Then, target bacteria, biotinylated aptamer and avidin-catalase were successively introduced into the microplate wells. Finally, the hydrogen peroxide and freshly prepared gold (III) chloride trihydrate were added, and the absorbance of the reaction product was measured with a plate reader. Under the optimized conditions, there was a linear relationship between the absorbance (A550 nm) and the concentration of *S. typhimurium* over the range of 10^1 to 10^6 cfu mL^{-1} (R^2=0.9920), with a detection limit of 10 cfu mL^{-1}. The simple rapid method provided a promising tool for on-site screening of *S. typhimurium* in raw chicken samples.

Keywords Aptamer**;** Colorimetric determination**;** Gold nanoparticles**;** *Salmonella typhimurium* (*S. typhimurium*)

1. Introduction

Salmonella typhimurium (*S. typhimurium*) is one of the most important and common food-borne pathogens in humans. It can be transmitted to humans through some animal-related food including meat, eggs and milk, which can cause severe diseases and even death,[1] such as food-poisoning, gastroenteritis and septicemia. The World Health Organization (WHO) has reported that salmonellosis caused by *Salmonella* sp. is the most frequently reported food-borne disease worldwide.[2] To date, several detection methods for *S. typhimurium* have been achieved through traditional culturing methods, immunological and genetic methods.[3] Although these developed detection methods have been successfully applied in various fields, these methods have some disadvantages, such as detection time, price and performance. For example, the enzyme-linked immunosorbent assays (ELISA) is relatively expensive, time consuming and complex to perform,[4] and the traditional culturing methods are labor-intensive and time consuming. Therefore, development of a simple, specific and sensitive detection method for *S. typhimurium* is of great significance.

Aptamers are single-strand DNA or RNA sequences, which can recognize and bind to their target with high affinity and specificity. Factually, aptamers are stable, inexpensive, simply synthesized and minimally immunogenic. Based on these merits, aptamers have been widely used as a useful recognition element tool for detection of pathogenic bacteria,[5-7] heavy metal ions,[8, 9] toxins [10, 11]and proteins.[12, 13]

Gold nanoparticles (AuNPs) have been successfully employed in chemical and biological detection due to their excellent physical, chemical and unique optical

properties. When AuNPs are well-dispersed in solution, it shows red color; while AuNPs are aggregated, it appears purple or blue depending on the degree of aggregation.[14] Based on this distinct color change, AuNP-based colorimetric assays have been used for the detection of various analytes, including acetamiprid,[15] melamine,[14, 16] cadmium (Ⅱ),[17] mercury (Ⅱ),[18] Ochratoxin A,[19] ramoplanin,[20] and DNA.[21]

In this study, the biotinylated specific aptamer fixed on the microplate wells was applied to recognize and bind to *S. typhimurium* with high affinity and specificity, which helped to caputure the target bacteria on the microplate. And the avidin-catalase can bind to another biotinylated aptamer on the surface of the target bacteria through biotin–avidin interactions. The catalase consumeed hydrogen peroxide (H_2O_2) which resulted in a decreased concentration of H_2O_2. Consequently, this reaction slowed down the kinetics of crystal growth, and aggregated nanoparticles were formed, which leaded the solution to blue color. In the absence of the target *S. typhimurium*, H_2O_2 concentration was high, and the reduction of gold ions via H_2O_2 occurred at a rapid rate, which formed non-aggregated and spherical nanoparticles, and the solution was red.[22] Based on this principle, the colorimetric detection method for *S. typhimurium* was established and the detection could be realized by monitoring the color change of the AuNPs solution with bare eyes.

2. Experimental

2.1 Reagents and apparatus

Anhydrous sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), 30% hydrogen peroxide (30% H_2O_2), sodium chloride (NaCl), gold (Ⅲ) chloride trihydrate ($HAuCl_4$), dimethyl sulfoxide (DMSO), potassium chloride (KCl), tris (hydroxymethyl) aminomethane, disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), dipotassium hydrogen phosphate (K_2HPO_4), agar powder, tryptone, yeast extract, ethanol, Tween-20, and bovine serum albumin (BSA) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). SM (PEG) 24 was purchased from Thermo Scientific (Shanghai, China). MES was purchased from the Aladdin industrial Corporation (Shanghai, China). The catalase from bovine liver and avidin were purchased from Sigma-Aldrich (Saint Louis, MO). *S. typhimurium* ATCC 50761 was obtained from the American Type Culture Collection (ATCC). *S. typhimurium* aptamer[23] 5'-biotin-C6-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G-3' was synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). The ultrapure water (18.2 MΩ cm) used in the experiments was prepared with a Millipore Direct-Q® 3 system (MA, U.S.A.). A Molecular Devices SpectraMax M5 plate reader (M5, Molecular Devices, USA) was used for scanning signals.

2.2 Preparation of avidin-catalase

The process was based on the reported method by Yuan[24] as follows: 4 μL of SM (PEG) 24 (250 mmol L-1 in dry DMSO) and 1 mL of avidin (1 mg mL^{-1}) were mixed, and the reaction was incubated for 30 minutes at room temperature. The excess cross-linker was removed with a dialysis bag. Then, 5 mg of catalase was added, and

the reaction mixture was incubated at room temperature for 30 minutes. Finally, the avidin-catalase conjugation was stored at 4 °C until used.

2.3 Colorimetric determination of *S. typhimurium*

First, 200 μL of avidin (0.01 mg mL^{-1} in carbonate buffer, pH 9.6) was added into each microplate well for overnight at 4 °C. The wells were washed 3 times with wash buffer (0.01 mol L PBS, 0.05% Tween-20). Second, the microplate wells were blocked with blocking buffer (BSA in 0.01 mol L^{-1} PBS) for 1 hour at room temperature to prevent non-specific adsorption, then washed 3 times with PBS. Followed, the microplates were incubated with 100 μL biotinylated aptamer at 37 °C for 30 minutes. Then, 100 μL of the sample solution containing *S. typhimurium* was added into each well, incubating at 37 °C for 30 minutes, followed by washing and air drying. Once again, 100 μL biotinylated aptamer was incubated with each well, for 30 minutes at 37 °C, then washed 3 times with PBS and air dried. Subsequently, 100 μL of avidin-catalase conjugation was added into each treated well, incubating at 37 °C for 30 minutes, followed by washing with PBS 5 times, deionized water once, and air dried. Then, 100 μL of 280 μmol L^{-1} H_2O_2 in MES buffer (1 mmol L^{-1}, pH 6.5) was introduced into each well and incubated at room temperature for 30 minutes. Finally, 100 μL of freshly prepared gold (III) chloride trihydrate (0.2 mmol L^{-1}) in MES buffer was added to each well. After 15 minutes, the absorbance at 550 nm was recorded using a Molecular Devices SpectraMax M5 plate reader. The process of the colorimetric determination method of *S. typhimurium* is schematically illustrated in Fig. 1.

2.4 Determination of *S. typhimurium* in the chicken samples

Raw chicken purchased from local supermarkets was confirmed without *Salmonella spp.* according to ISO 6579:2002. The 18h buffered peptone water (BPW) enrichments of raw chicken was divided into nine sections, and the volume of each was 900 μL. Finally, 100 μL of different concentrations of *S. typhimurium* were added to the 18 h BPW enrichments of raw chicken to prepare the spiked samples. The spiked samples with *S. typhimurium* concentrations between 1.0×10^2 and 1.0×10^4 cfu mL^{-1} were then analyzed by the above colorimetric determination method.

3. Results and discussion

3.1 Optimization of the concentration of BSA

The avidin possesses some hydrophobic patches, which enables it to adsorb on the hydrophobic surfaces such as polystyrene (the basis of the microplates' bottom.[25] In this work, the avidin (1 mg mL^{-1}) was diluted with carbonate buffer (pH 9.6) and the dilution ratio was 1:100. In order to avoid the non-specific binding or adsorption, the microplate was coated with BSA in 0.01 mol mL-1 PBS. In this experiment, the signals from the same blank samples (sterile LB medium) were respectively detected against the different microplates which were blocked by different concentrations (5, 10, 15, 20, 25 mg mL^{-1}) of BSA in PBS. As shown in Fig. 2, the non-specific adsorption had a great influence on the experimental results; the effect of the non-specific binding or adsorption was reduced with increasing BSA concentration from 0 to 20 mg mL^{-1}; the effect of non-specific adsorption on the experiment was minimized when the concentration of BSA was from 20 to 25 mg mL^{-1}. However, for

the concentrations of 20 and 25 mg mL^{-1}, there was a slight difference between the intensity of the signals that correlated with the concentration of BSA. Consequently, 20 mg mL^{-1} was selected as the optimized concentration of BSA.

3.2 Optimization of the concentration of aptamer

The biotinylated aptamer were fixed on the avidin coated on the microplate by the specific binding between biotin and avidin. The amount of the *S. typhimurium* specific aptamer fixed directly influenced the amount of the captured *S. typhimurium* and the intensity of the signals. To obtain the optimized concentration of aptamer, different concentrations ranging from 10^{-4} to 10^{-12} mol L^{-1} were tested by detecting the same sample (10^6 cfu mL^{-1}). And the concentrations of *S. typhimurium* were determinated by the classical counting methods. As shown in Fig. 3, the intensity of the signals decreased dramatically when the concentration of the aptamer increased over the concentration range of 10^{-12} to 10^{-7} molar per liter, and when the concentration of the aptamer increased over the concentration range of 10^{-7} to 10^{-4} molar per liter, the absorbance (A550 nm) decreased moderately. However, for the concentrations of 10^{-5} and 10^{-4} molar per liter, there was only a slight difference between the intensities of the signals. So, the 10^{-5} molar per liter was fixed as the optimized concentration of aptamer.

3.3 Optimization of the dilution of avidin-catalase

In this work, the dilution of avidin-catalase had a great effect on the intensity of the signal and it was an important factor for the experimental results. To obtain the optimal dilution of avidin-catalase, the avidin-catalase was diluted to 1:50, 1:100,

1:150, 1:300, 1:450, and 1:600 with the blocking buffer for the detection of the same sample (the concentration of *S. typhimurium* in the sample was 10^6 cfu mL^{-1}). As shown in Fig. 4, the absorbance at A550 nm was increased significantly as the avidin-catalase dilution ratio increases from 1:150 to 1:600; the signal intensity increased moderately as the avidin-catalase dilution ratio increases from 1:50 to 1:150, but there was a slight difference between the intensities of the signals that correlated with the dilution of avidin-catalase. Thus, 1:150 was determined as the optimized dilution of avidin-catalase in this experiment.

3.4 Analytical performance

Under the optimal conditions, the developed method was conducted against a series of concentrations of *S. typhimurium*. There was a strong linear correlation between the intensity of the signal and the concentration of *S. typhimurium* over the range from 10 to 10^6 cfu mL-1 ($y= -0.0264x+0.3052$, $R^2 = 0.9920$) (Fig. 5). The detection limit of the developed method was calculated based on $3<\sum>/slope$, where $<\sum>$ was the standard deviation of blank samples and slope was obtained from the standard correlation curve between the intensity of the signals and the concentration of *S. typhimurium*. And the statistical analysis revealed that the detection limit of *S. typhimurium* was 10 cfu mL^{-1}. The developed method in this work was further compared with some previous reported methods about the detection of *S. typhimurium* (Table 1). It can be obviously seen that the developed method is more sensitive, with lower detection limit. However, the linear range of the developed method should be improved and it would be taken into account in the future studies.

3.5 Specificity evaluation

To evaluate the specificity of this developed method, control tests were performed for *Vibrio parahemolyticus*, *Staphylococcus aureus*, *Streotococcus*, *Listeria monocytogenes*, *E. coli*, and the blank samples. The analysis of all samples was performed under the same conditions, and the concentrations of all bacteria were 10^6 cfu mL^{-1}. As shown in Fig. 6, the intensity of the signals for *S. typhimurium* were much weaker than the other five bacterial samples and the blank samples. The color of the trial group (*S. typhimurium*) was blue, while the color of the control groups were red. The results suggest that the developed method has good selectivity for *S. typhimurium* detection.

3.6 Analysis of *S. typhimurium* in chicken samples

The effectiveness of the developed method for *S. typhimurium* in raw chicken samples was further studied. The 18 h BPW enrichments of raw chicken samples added with different concentrations of *S. typhimurium* were examined by the developed aptamer-based method and compared with the classic plate counting method. The analytical results (presented in Fig. 7) showed that there was no significant difference between the counting method and the developed method (R^2=0.998, P<0.001), and the linear fit that was obtained was y=0.995x+10107. A recovery between the range of 92 to 107% was observed (Table 2), which would be considered satisfactory. It confirms that the colorimetric detection method based aptamer recognition and gold nanoparticle can be used for the detection of real samples.

4. Conclusions

A sensitive method was established for colorimetric detection of *S. typhimurium* based on aptamer recognition and gold nanoparticle. The developed method was low cost, time-saving, easy to perform, and high specific, compared with the traditional methods, such as culturing, immunological and genetic methods. A linear relationship was present between the absorbance at 550 nm and the concentrations from 10 to 10^6 cfu mL^{-1}, and the detection limit can reach 10 cfu mL^{-1}. The developed method shows high specificity and excellent accuracy, rendering it a promising analytical tool for wide use in the on-site test of *S. typhimurium* in food samples.

Acknowledgements This work was supported by China Agriculture Research System (CARS-42), and National Key Technology Research and Development Program (2014BAD19B01) and Innovation Program of Nanjing Strategic Industry (2014PT030008).

Conflict of Interest Changqing Zhu, Ying Hong, Zhen Xiao, Yang Zhou, Yuan Jiang, Ming Huang, Xinglian Xu, Guanghong Zhou declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Schematic illustration of the colorimetric detection method for *S. typhimurium* based on aptamer recognition and gold-nanoparticle.

Fig. 2 Plot for optimizing the concentration of BSA.

Fig. 3 Plot for optimizing the concentration of aptamer. The concentration of *S. typhimurium* in the sample is 10^6 cfu mL^{-1}.

Fig. 4 Plot used to optimize the dilutions of avidin-catalase. The concentration of *S. typhimurium* in the sample is 10^6 cfu mL^{-1}.

Fig. 5 Standard correlation curve between the intensity of the signals and the concentration of *S. typhimurium*.

Fig. 6 The intensity of the signals measured for (a) *Vibrio parahemolyticus*, (b) *Staphylococcus aureus*, (c) *Streotococcus*, (d) *Listeria monocytogenes*, (e) *E. coli*, (f) the control and (g) *S. typhimurium*. Concentrations of all bacteria were 10^6 cfu mL-1.

Fig. 7 Relationship between the developed method and the plate counting method for the target bacteria measurement in raw chicken samples.

Table 1 Figures of merits of comparable methods for determination of *S. typhimurium*

Table 2 Recovery assays of S. typhimurium in chicken samples

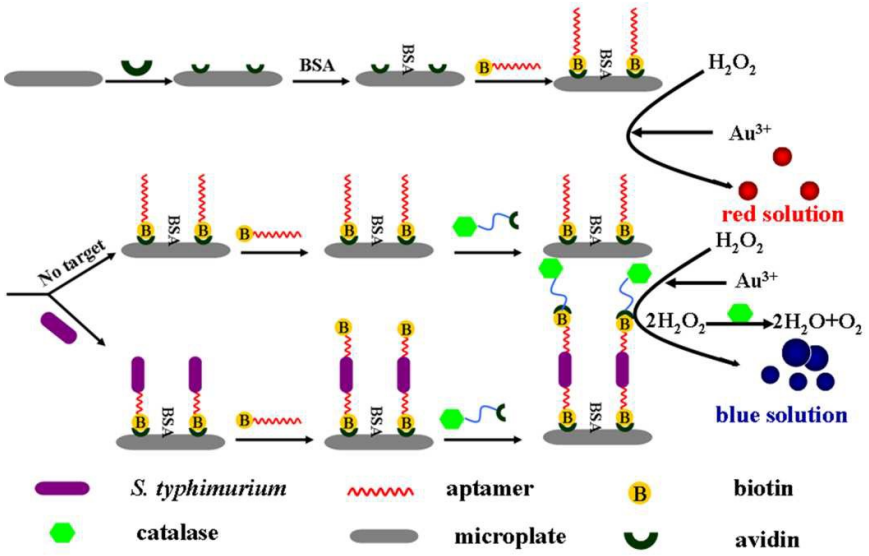


Fig. 1

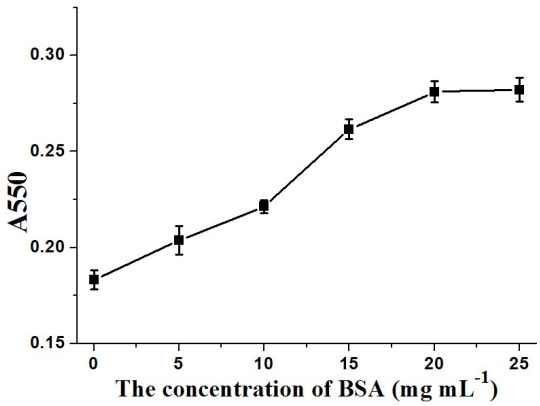


Fig. 2

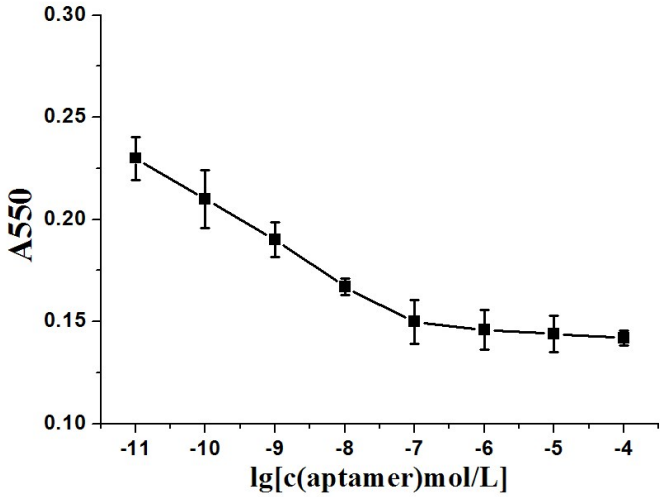


Fig. 3

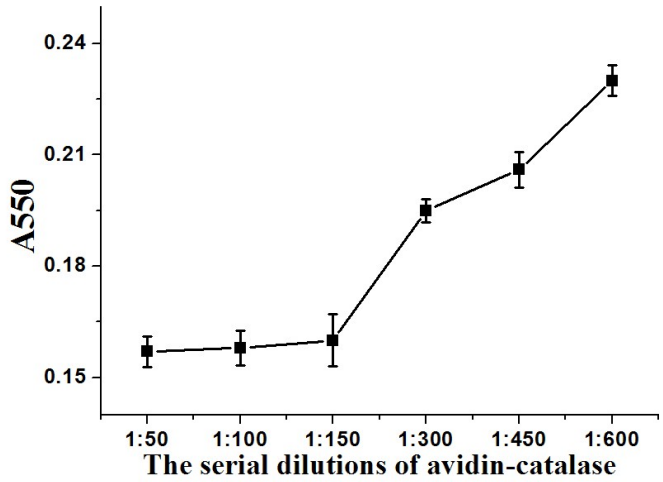


Fig. 4

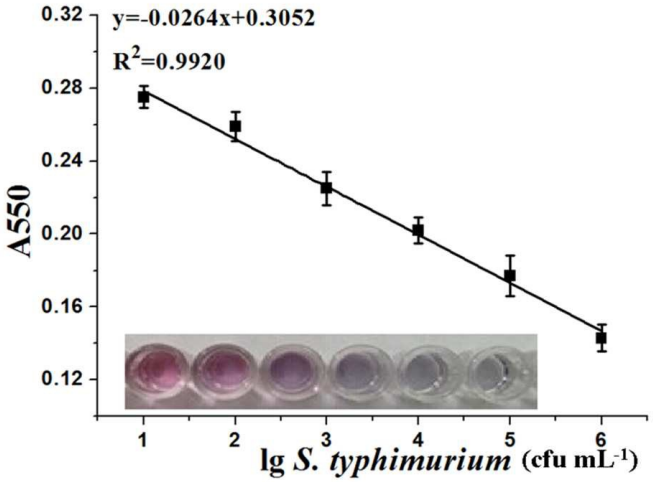


Fig. 5

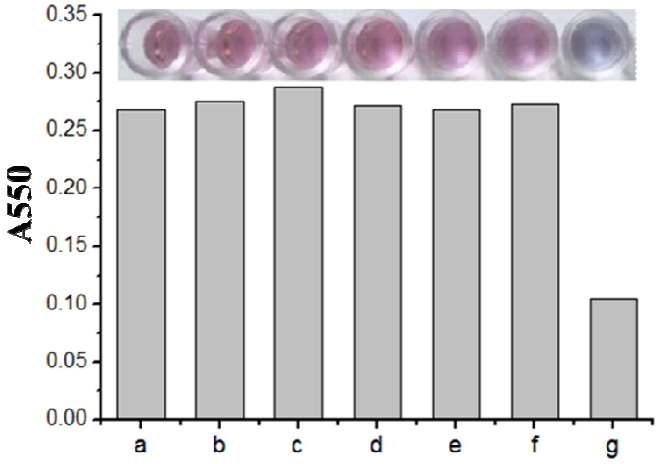


Fig. 6

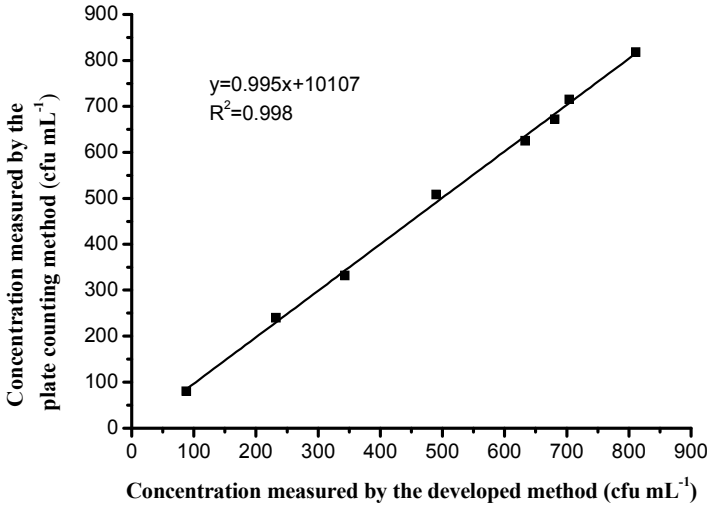


Fig. 7

Table 1

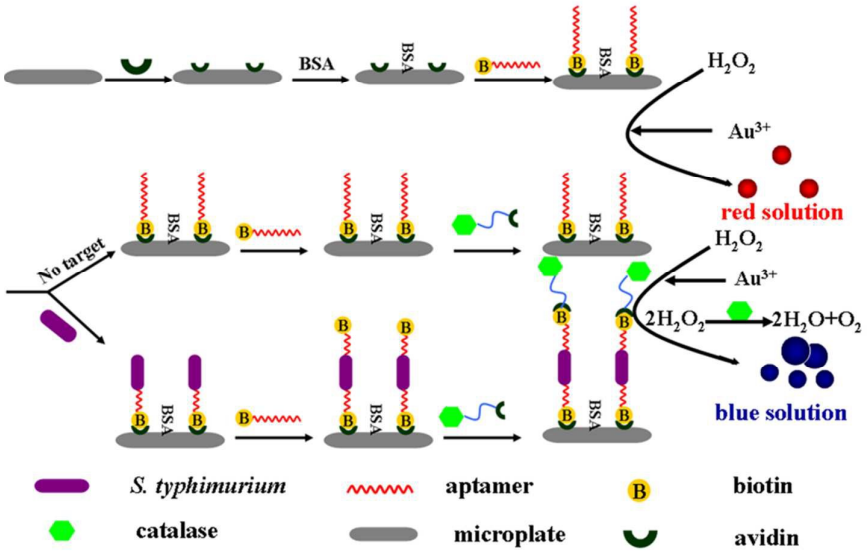
Method used	LOD (cfu mL^{-1})	Application	Linear range (cfu mL^{-1})	Ref.
Fluorescent aptasensor based on a grapheme oxide platform	100	Milk samples	1×10^{3}~1×10^{8}	3
Automated quartz crystal microbalance (QCM) instrument with nanoparticles amplification	10~20	Pre-cooked chicken samples	N. I.	26
A universal fluorescent aptasensor based on AccuBlue dye	25	Shimp and chicken samples	50~10^{6}	27
A label-free electrochemical impedance immunosensor	5×10^{2}	Milk samples	1×10^{3}~1×10^{7}	28

Ultrasensitive chemiluminescent immunoassay with silver enhancement of nanogold labels	5	Poultry, pork and ready to eat food samples	5~1038	29
Visual and colorimetric detection method based on aptamer recognition and gold-nanoparticles	10	Raw chicken samples	1×10^{1}~1×10^{6}	This work

N. I. No information

Table 2

Sample no.	Original value (cfu mL-1)	Added (cfu mL-1)	Found (cfu mL-1)	Recovery (%)
1	0	1.0×10^{2}	$(0.92\pm 0.03)\times 10^{2}$	92
2	0	1.0×10^{3}	$(0.99\pm 0.02)\times 10^{3}$	99
3	0	1.0×10^{4}	$(1.07\pm 0.01)\times 10^{4}$	107



A sensitive colorimetric detection of *S. typhimurium* based on aptamer recognition with detection limit of 10 cfu mL-1.