SCIENCE CHINA Materials

ARTICLES

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Published online 18 August 2016 | doi: 10.1007/s40843-016-5077-0
Sci China Mater 2016, 59(8): 665–674

Gold nanoparticle-based strip sensor for multiple detection of twelve *Salmonella* strains with a genus-specific lipopolysaccharide antibody

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ABSTRACT In this study, an innovative competitive immunochromatographic strip sensor was developed for rapid detection of *Salmonella* based on a genus-specific anti-lipopolysaccharide (LPS) monoclonal antibody (mAb) and the heterogeneous coating antigen of a LPS-bovine serum albumin conjugate. Gold nanoparticles labeled anti-LPS mAb specifically reacted with the conserved outer core of the *Salmonella* LPS in the sample and the color formed on the T line was negatively correlated with the number of *Salmonella* cells. The sensitivity of Ra mutant LPS (without O-specific chains but has the conserved outer core) was 25 ng mL^{-1}, which explained the detection of *Salmonella* at the genus level. Based on the gray values on the test line, the limit of detection of *Salmonella* was 10^3 colony-forming unit (CFU) for all twelve typical strains of *Salmonella*. The analysis of common Gram-negative and Gram-positive bacteria demonstrated that the strip assay was specific to *Salmonella*. A milk sample test showed that *Salmonella* at a low level (1–5 CFU mL^{-1}) was detected without complex biochemical confirmation steps, sophisticated instruments and professional training.

Keywords: *Salmonella*, lipopolysaccharide, monoclonal antibody, immunochromatographic strip, Au nanoparticles

INTRODUCTION

Food poisoning and foodborne diseases, which tend to occur in a wide area and lead to gastrointestinal disease, are a major concern worldwide [1,2]. Among the foodborne pathogens related to poisoning, *Salmonella* is reported to be the leading cause of poisoning in many countries including China [3,4]. *Salmonella* is a genus of Gram-negative bacteria that has more than 2000 pathogenic serotypes including the well-known serotypes *S. typhimurium* and *S. enteritidis* [5,6]. The main antigens of *Salmonella* are lipopolysaccharide (O antigen), flagellin (H antigen), and capsular polysaccharide (Vi antigen). The difference in O antigen and H antigen between strains is the basis for *Salmonella* serotyping [7]. The standard detection method for *Salmonella* is culture-based and requires multiple steps of enrichment and biochemical confirmation. The results are usually obtained after 4–7 d. Therefore, rapid detection methods are highly desirable to overcome the disadvantages of time-consuming and labor-intensive traditional methods.

Currently, polymerase chain reaction (PCR)-based methods, immunoassays, isothermal amplification methods, and aptamer-based methods have been developed for the rapid detection of *Salmonella*. PCR-based methods including real-time PCR, which are robust but rely on sophisticated instruments and professional training, are more suitable as confirmation methods in the laboratory than as pre-screening tools for large samples [8,9]. Compared with PCR, nucleic acid hybridization including loop-mediated isothermal amplification is portable, rapid and easy to operate [10]. However, the dependence on a relatively high temperature (63°C) and DNA extraction step still limit the practical application of these methods [11,12]. Due to the specificity of the selected aptamer, aptamer-based sensors are mainly used for rapid detection of common serotypes such as *S. typhimurium* and *S. enteritidis*, rather than detection of the genus [5,13,14]. Immunoassays based on the specific antigen and antibody reaction have been widely used in *Salmonella* detection either with the classic enzyme-linked immunosorbent assay (ELISA) [15–18] or novel nanomaterials-based biosensors

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[19–22]. However, due to the drawbacks of multiple steps and high-cost of these methods there is a huge demand for a low cost, portable, rapid and stable method.

The lateral flow-based immunochromatographic strip sensor, which takes advantage of the stable immunoreaction of the monoclonal antibody (mAb) and the rapid chromatographic procedure, is a very powerful point of care test for pathogens, and environmental pollutants in food samples [23–26]. Some immunochromatographic strip assays based on serotype specific or serogroup specific mAbs have been used to detect common *Salmonella* strains including *S. typhimurium* [6,27,28], *S. enteritidis* [29], *S. choleraesuis* [30] and *S. typhi* [31]. Bautista *et al*. [32] reported the detection of 19 out of 22 tested *Salmonella* strains using a commercial immunochromatographic strip. The detection of *Salmonella* at the genus level with the traditional sandwich-based strip assay is mainly limited by the preparation of a genus-specific antibody and the effective pair that works on the strip test.

Therefore, we developed a novel competitive immunochromatographic strip sensor based on a genus-specific anti-lipopolysaccharide (LPS) mAb and the heterogeneous coating antigen of the LPS-bovine serum albumin (BSA) conjugate, to overcome the difficulties associated with rapid detection of *Salmonella* at the genus level. The mAb against the conserved outer core of the *Salmonella* LPS was prepared with the LPS-BSA conjugate as the immunogen for BALB/c mice. Furthermore, our novel strategy eliminated the need for paired mAbs based on the competition of *Salmonella* and the coating antigen with the gold nanoparticle (Au NP) labelled anti-LPS mAb. This strip sensor was evaluated using twelve typical *Salmonella* strains with different O antigens and eight common strains of Gram-negative and Gram-positive bacteria. In addition, milk samples contaminated with a low level of four common *Salmonella* serotypes were analyzed after 12 h enrichment.

EXPERIMENTS

Salmonella strains and growth conditions

The strains of *Salmonella* spp. used in this study are listed in Table S1. Non-*Salmonella* strains included *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 19111), *Escherichia coli* O157:H7 (*E. coli* O157:H7, CICC 21530), *E. coli* O6 (ATCC 25922), *Cronobacter sakazakii* (ATCC 29544), *Vibrio parahemolyticus* (CMCC 20017), *Campylobacter jejuni* (ATCC 33291) and *Campylobacter coli* (ATCC 43478). *Campylobacter jejuni* and *Campylobacter coli* were cultured in Brain-Heart Infusion broth (Oxoid, Basingstoke, UK) at 37°C for 2–3 d in a micro-aerobic environment (4% O_2, 10% CO_2 and 86% N_2) in a three gas incubator (Binder CB210, Tuttlinger, Germany). *Vibrio parahemolyticus* was cultured in tryptone soya broth (Oxoid, Milan, Italy) with 1% NaCl at 37°C. The other bacteria were cultured overnight at 37°C in Brain-Heart Infusion broth.

Production of genus-specific LPS monoclonal antibody

S. typhimurium LPS (Sigma, St Louis, MO, USA) was conjugated to BSA using the active ester method [33] to prepare the immunogen. Briefly, 1 mL of LPS (5 mg mL^{-1}, water) was first added with borate saline buffer (0.2 mol L^{-1}, 50 μL) to maintain the pH at 5 and was activated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (1 mg, *N*,*N*-dimethylformamide) and *N*-hydroxysuccinimide (1 mg, water) at room temperature for 2 h. Then, 100 μL of BSA (50 mg mL^{-1}, water) was added and the pH of the solution was adjusted to 9.0 with bicarbonate buffer (0.05 mol L^{-1}, 50 μL). After overnight reaction, the solution was dialyzed.

BALB/c mice aged 6–8 weeks were immunized with the immunogen to produce the anti-LPS antibody. Spleen cells from the mice were then fused with Sp2/0 myeloma cells. After selection against Ra LPS which lost the O-specific chain but reserved the outer core structure from mutant *S. typhimurium* (Sigma, St Louis, MO, USA) and confirmation using *Salmonella* strains with different O antigens, cell lines with homogenous cross-reactivity within the *Salmonella* genus were obtained. The produced mAbs were purified using the caprylic acid-ammonium sulfate precipitation method. The half maximal inhibitory concentration (IC50) of the mAbs were characterized by indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) [34].

Au NPs labeled monoclonal antibody and coating antigens

Au NPs (15 nm) were synthesized by the classic citrate sodium reduction method [35]. The Au NPs and LPS mAb conjugates were prepared as follows: 1 mL of Au NPs was added to 7 μL of 0.1 mol L^{-1} Na_2CO_3 and the pH was adjusted to 7.5. Then, 10 μL of the anti-LPS mAb (1 mg mL^{-1}) was added to the Au NPs solution and reacted for 2 h at room temperature with gentle mixing. BSA (50 μL of a 100 mg mL^{-1} solution) was then added to the solution and reacted for another 2 h in room temperature to block the non-binding sites. Finally, the Au NPs-modified LPS mAb was centrifuged twice (6000×g, 20 min) and preserved in 10 mmol L^{-1} PBS containing 0.02% NaN_3 at 4°C until use.

The coating antigens of LPS and BSA conjugate were synthesized by the active ester method and the periodate oxidization method [36]. Briefly, 1 mL of LPS (5 mg mL^{-1}, water) was oxidized with 75 μL sodium periodate (10 mg mL^{-1}, water) at room temperature for 30 min with magnetic stirring. Then, 100 μL ethanediol (0.16 mol L^{-1}, water) was added and reacted for another 30 min to neutralize the excess sodium periodate. BSA (100 μL of 50 mg mL^{-1}, water) was then added and the pH of the solution was adjusted to 9.0 with bicarbonate buffer (0.05 mol L^{-1}, 50 μL). After 24 h reaction at room temperature, the solution was dialyzed with 10 mmol L^{-1} PBS at 4°C.

Establishment of the competitive immunochromatographic strip sensor

The prepared coating antigen (1 mg mL^{-1}) and goat anti-mouse IgG antibody (0.5 mg mL^{-1}) were sprayed onto the test line (T line) and the control line (C line) on the nitrocellulose (NC) membrane using a BioJet Quanti3000 dispenser. After air-drying at 37°C for 2 h, the NC membrane was cut into strips (4 mm wide) with a CM4000 guillotine cutting module. The fabricated strips were stored with a desiccant at room temperature until use.

The analysis of *Salmonella* was as follows: a pure cultured *Salmonella* strain was boiled for 15 min. The sample (100 μL) was added to the solution and mixed with 7 μL of the Au NP-mAb probe and 43 μL of suspension buffer (10 mmol L^{-1} PBS, 2% BSA, 0.1% Tween, 0.2% sucrose). The antigen-antibody reaction was allowed to continue for 5 min at 37°C. The strip was then loaded and the results were judged with the naked eye after 10 min. Weakend red color on the T line compared with that of the negative control indicated that the sample was positive for *Salmonella*. The same red color on the T line compared with that of the negative control indicated the sample was negative for *Salmonella*. Red band on the C line should present for both positive and negative sample. The visual limit of detection (vLOD) is defined as the lowest concentration of *Salmonella* that produces the color on the test line significantly weaker than that of the negative control [23,37]. The optical density of the test line was recorded with a strip reader.

Coating antigens, anti-LPS mAbs, and sample dilution buffer were successively optimized to improve the sensitivity of the competitive immunochromatographic strip.

Sensitivity and specificity of the immunochromatographic strip sensor

The sensitivity of the competitive strip was tested with Ra LPS and the twelve *Salmonella* strains which had different serogroups of O antigen. Ra LPS from *Salmonella* was serially diluted to 100, 50, 25, 10, 5 ng mL^{-1} and 0 with PBS (10 mmol L^{-1}, pH 7.2) before analysis. The twelve pure cultured *Salmonella* strains were boiled and serially diluted to 10^7, 10^6, 10^5, 10^4 CFU mL^{-1}, and 0 with PBS for determination. Specificity of the strip was evaluated using Gram-positive bacteria including *Staphylococcus aureus* and *Listeria monocytogenes*, and Gram-negative bacteria including *E. coli* O157:H7, *E. coli* O6, *Cronobacter sakazakii*, *Vibrio parahemolyticus*, *Campylobacter jejuni*, *Campylobacter coli* and *S. enteritidis*. The pure cultured strains as described above were boiled and diluted to 2×10^8 CFU mL^{-1} for the test.

Milk sample detection using the immunochromatographic strip sensor

A pure milk sample was purchased from a local market and confirmed to be free of *Salmonella* spp. by the culture-based method [38]. To simulate a real sample contaminated with a low level of *Salmonella* spp., the milk was individually spiked with fresh cultured *S. paratyphi A*, *S. typhimurium*, *S. enteritidis*, and *S. arizona* at 1–5 CFU mL^{-1} by serial dilution. The spike level and the original concentration of the culture (10^9 to 5×10^9 CFU mL^{-1}) were further confirmed by the plate counting method. Then, 25 mL of the spiked samples were added to 225 mL buffered peptone water (Oxoid, Basingstoke, UK) and cultured at 37°C for 12 h. Samples of the culture were boiled for 15 min before analysis with the immunochromatographic strip.

RESULTS AND DISCUSSION

Principle of the competitive immunochromatographic strip sensor

The principle of the immunochromatographic strip for detection of *Salmonella* spp. is shown in Fig. 1. The T line and C line were coated with *Salmonella* LPS-BSA conjugate and Goat anti-mouse IgG antibody, respectively. When no *Salmonella* was present in the sample, the Au NPs labeled anti-LPS mAbs reacted with the LPS-BSA conjugate on the T line and the remainder was captured by the anti-mouse IgG antibodies on the C line. Red bands due to the Au NPs were observed both on the T line and the C line. When *Salmonella* was present in the sample, the Au NPs labeled anti-LPS mAbs first reacted with the LPS on the surface of *Salmonella* cells and then reacted with the T line and the C line. The color of the T line decreased at this time. The color intensity on the T line was negatively correlated with the amount of *Salmonella* in the sample.

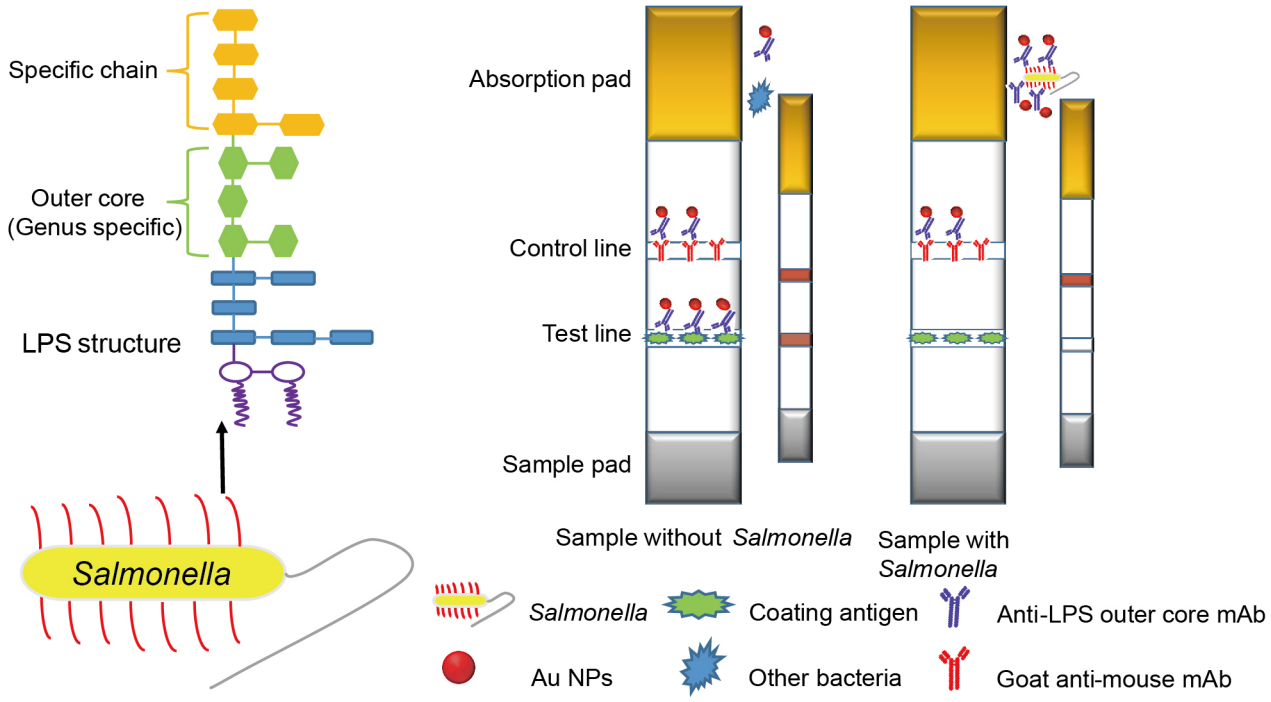

Figure 1 Scheme of the immunochromatographic strip sensor for *Salmonella* spp. Weakened red color on the T line compared with that of the negative control and a red band on the C line indicated that the sample was positive for *Salmonella*.

Compared to the commonly used sandwich-based strategy [39,40], this LPS mAb and coating antigen-based paper sensor for competitive detection of *Salmonella* is innovative and greatly simplifies the development of the strip assay. This is because sandwich-based strategy usually needs two mAbs which must be both paired in ELISA and still work in lateral flow assay. In our previous work, we developed a sandwich ELISA based on a genus specific LPS mAb and the sensitivity was determined to be 10^6 CFU mL^{-1} [36]. This indicated that sandwich based strip assay may not be suitable for this mAb because the detection limit of immunochromatographic strip is usually higher than ELISA. Furthermore, Compared with the insufficient cross-reactivity among *Salmonella* spp. in previous studies of the sandwich-based immuno-strip assay [28,31], using of the *Salmonella*-specific anti-LPS mAb enabled the rapid detection of *Salmonella* at the genus level with broad cross-reactivity and high specificity.

Optimization of the competitive immunochromatographic strip sensor

The transmission electron microscopy (TEM) image (Fig. S1) shows that the synthesized Au NPs have uniform diameter (15±2.82 nm) and good dispersity. The ultraviolet-visible (UV-vis) spectra (Fig. S2) indicates the maximal absorbance of the Au NP red-shift from 520 to 526 nm after the reaction with the mAb 5H12, which reflects the increased diameter caused by successful conjugation.

The coating antigen, anti-LPS mAb and sample dilution buffer significantly affected the performance of the strip and thus were optimized. First, the two prepared coating antigens of the LPS-BSA conjugates (Fig. S3) were evaluated using the anti-LPS mAbs. Fig. S4 shows that the color intensity formed on the T line with the homologous coating antigen synthesized by the active ester method is too strong and the color of the C line is very light. In contrast, the colors of the T line and C line with the heterogeneous conjugate synthesized by the periodate oxidization method are comparable. This is because the antibody affinity against the homologous coating is usually higher than the heterogeneous coating [41]. We chose the heterogeneous coating antigen by the periodate oxidization method for further study as moderate affinity was suitable for the competition between the sample and the coating antigen.

The anti-LPS mAbs were then tested with the competitive immunochromatographic strip with Ra LPS as standard. Fig. S5 shows that these anti-LPS mAbs reacted with the Ra LPS which retained the conserved outer core of *Salmonella* and clearly inhibited the color on the T line. Among these mAbs, 5H12 was selected due to complete inhibition of the color on the T line with 100 ng mL^{-1} Ra LPS and relatively higher color intensity on the T line for the negative control.

In addition, 10 mmol L^{-1} PBS containing 3.3 mol L^{-1} ethylenediaminetetracetic acid (EDTA) and 0.1% Triton 100 was used as a sample dilution buffer to break the steric hindrance caused by the buried outer core structure in the inner side of LPS on the cell surface of *Salmonella* [42]. Fig. S6 indicates that the sample dilution buffer completely inhibited 10^5 CFU *S. kentucky* and no color was observed on the T line. In contrast, the color was still visible on the T line with PBS as the sample dilution buffer. This was because EDTA chelated the bivalent cations that can stabilize the micelle-like structure of LPS on the cell surface and Triton 100 is a mild surfactant that can improve the membrane permeability of the cell [43].

Sensitivity and cross-reactivity of the immunochromatographic strip sensor

Fig. 2a shows that the vLOD of Ra LPS using the immunochromatographic strip was 25 ng mL^{-1} and the IC50 of the 5H12 against Ra LPS in the IC-ELISA was 6.5 ng mL^{-1} (Fig. 2b). Ra LPS from *S. typhimurium* loses the repetitive O specific chain which has high diversity between different serogroups of *Salmonella* and retains the conserved outer core structure in *Salmonella* [18]. The sensitive detection of Ra LPS clearly indicated the mechanism involved in the detection of *Salmonella*.

To validate the sensitivity and cross-reactivity in the genus *Salmonella*, twelve strains belonging to serogroups O: 2, O: 4, O: 7, O: 8, O: 9 O: 3, 10 and IIIa were analyzed using the competitive immunochromatographic strip. Fig. 3a shows that all the tested strains including *S. paratyphi A, S. agona, S. paratyphi B, S. typhimurium, S. thompson, S. blockley, S. kentucky, S. enteritidis, S. typhi, S. dublin, S. anatum,* and *S. arizona* inhibited the color on the T line with an increasing number of *Salmonella* cells. The vLOD of these *Salmonella* strains was 10^5 CFU, except for *S. arizona* with a vLOD of 10^6 CFU. With the strip reader, area of the gray values on the T lines were recorded and plotted. Fig. 3b indicate that the gray values decreased at 10^3 CFU for all the tested strains (5%–30%) except *S. thompson* (10^4 CFU). The significant improvement of the sensitivity is due to the competitive scheme and the decrease of the gray value is more sensitive than the visual change of the color. These tested strains represent the typical serotypes of *Salmonella* (serogroup A, B, C, D and E) that lead to the majority of *Salmonella*-related foodborne diseases [44]. *S. arizona* is characterized by a special biochemical reaction (fermentation of lactose to produce acids and CO_2) and unique outer core structure of the O antigen (IIIa) in the genus of *Salmonella* [45]. Unlike the *N*-acetyl-D-glucosamine on the outer core of the LPS in other *Salmonella* strains, the D-glucosamine in *S. arizona* was not *N*-acetylated. The detection of *S. arizona*, although at a relatively higher vLOD, demonstrated the homogenous cross-reactivity in the *Salmonella* genus.

In the previous works, the majority of the reported ELISA and immunochromatographic strips were specific to only

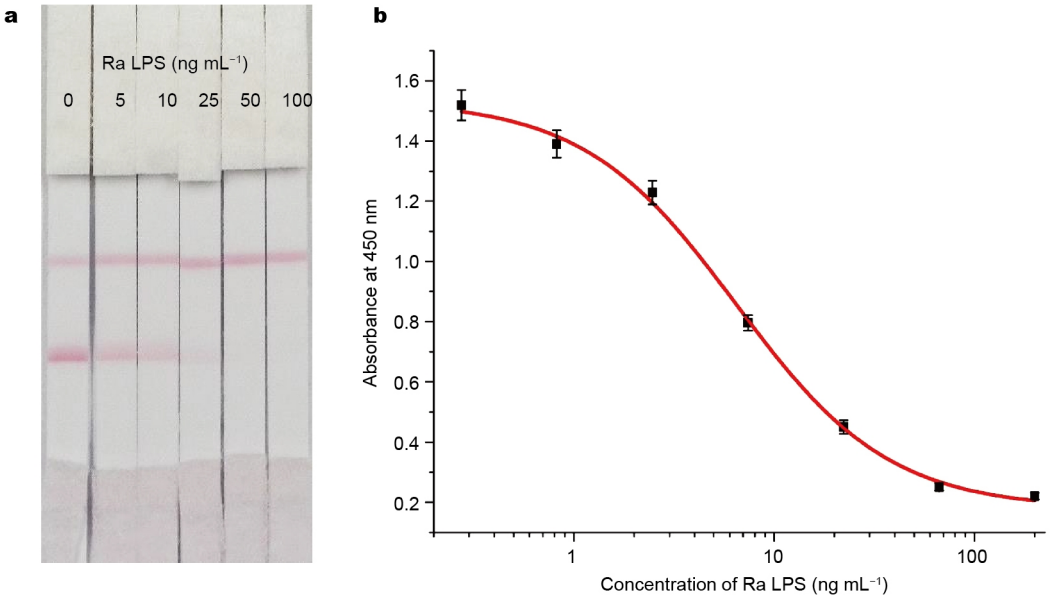

Figure 2 Detection of Ra LPS with the mAb 5H12 based competitive Immunochromatographic strip sensor (a). Standard curve of the competitive ELISA with the mAb 5H12 (b).

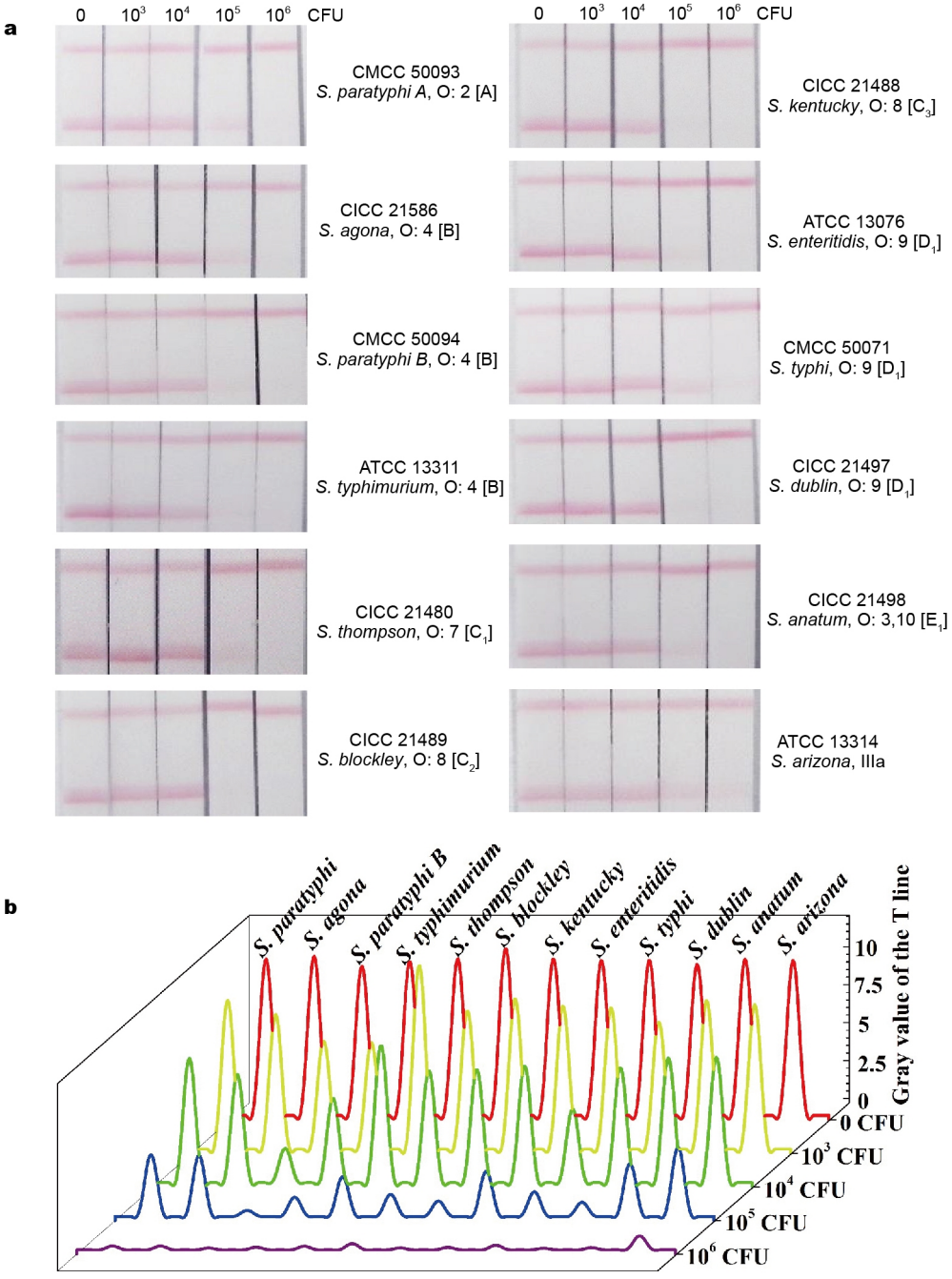

Figure 3 (a) Images of the Immunochromatographic strip sensor for detection of twelve typical strains of *Salmonella*; (b) gray values of the T lines for detection of the twelve typical strains.

one or two serotypes of *Salmonella*, with sensitivities ranged from 10^3 to 10^5 CFU mL^{-1} [15,31,46]. Although the tests were very sensitive, the applications for detection of the genus of *Salmonella* were limited. The genus specific sandwich ELISA methods of *Salmonella*, reported by Wu *et al.* [36] and Choi *et al.* [42] respectively, all have a sensitivity of 10^6 CFU mL^{-1}. The lateral flow assay of *Salmonella* developed by Bautista *et al.* [32] has a sensitivity ranged from 10^4 to 10^5 CFU mL^{-1}, but failed to detect the *S. worthington, S. choleraesuis var. kunzendorf*, and *S. johannesburg*. Therefore, compared with the previous works, our paper sensor is both sensitive and accurate for the detection of *Salmonella*. The future of our work will focus on further improving the sensitivity of the *Salmonella*

biosensor based on lanthanide (Ln^{3+})-doped nanoparticles [47], ZnO nanomaterials [48], and surface-enhanced Raman scattering active nanomaterials [49].

Specificity and long-term stability of the immunochromatographic strip sensor

Six common Gram-negative bacteria that also have the LPS structure and two important Gram-positive pathogens were tested to evaluate the specificity of the strip assay. As shown in Fig. 4, the Gram-negative *E. coli* O157:H7, *E. coli* O6, *Cronobacter sakazakii*, *Vibrio parahemolyticus*, *Campylobacter jejuni*, *Campylobacter coli* and Gram-positive *Staphylococcus aureus* and *Listeria monocytogenes* did not inhibit the color on the T line, even at a very high concentration (2×10^8 CFU mL^{-1}). However, *S. enteritidis* completely inhibited the color on the T line. These data indicate that the developed strip assay showed excellent specificity within *Salmonella* and did not react with other common bacteria including non-pathogenic *E. coli*. Muldoon *et al.* [50] reported an O antigen mAb-based immunochromatographic strip and a bacteriophage-based method to eliminate the cross-reaction between *Citrobacter* spp. and *E. coli*. In our study no cross-reaction with these bacteria was observed, which may be attributed to the mAb that specifically recognized the conserved outer core of *Salmonella*.

In addition, the long-term stability and repeatability of the strip sensor were evaluated. The prepared strips were stored at room temperature with desiccant and the Au NPs labeled LPS mAb was stored in 0.02 mol L^{-1} PBS containing 0.02% NaN_3 at 4°C. Based on our results, the sensitivity and specificity did not changed during six months preservation. This was because the mAb on the NC membrane, which was air-dried and kept in dry environment at 25°C, was stable and free of the bacteria. On the other hand, adsorbed BSA and mAb on the Au NPs prevented the Au NPs from aggregation and the NaN_3 inhibited the growth of bacteria.

Milk sample analysis with the immunochromatographic strip sensor

To apply the novel strip assay to the detection of *Salmonella* in a real sample, pure milk was spiked with *S. paratyphi A*, *S. typhimurium*, *S. enteritidis*, and *S. arizona* at a low level (1–5 CFU mL^{-1}) and analyzed using the strip after enrichment. Fig. 5 shows that *S. paratyphi A*, *S. typhimurium*, and *S. enteritidis* completely inhibited the color on the T line and *S. arizona* clearly inhibited the color on the T line. Therefore, the four typical strains of *Salmonella* at a low level in the pure milk sample were detected by visual judgement after 12 h enrichment. Analysis of these four *Salmonella* strains with different O antigens validated the effectiveness of the developed competitive immunochromatographic strip.

Table S2 shows that the results of the analysis are consistent with those of the classic culture-based method. However, the developed strip assay greatly decreased the detec-

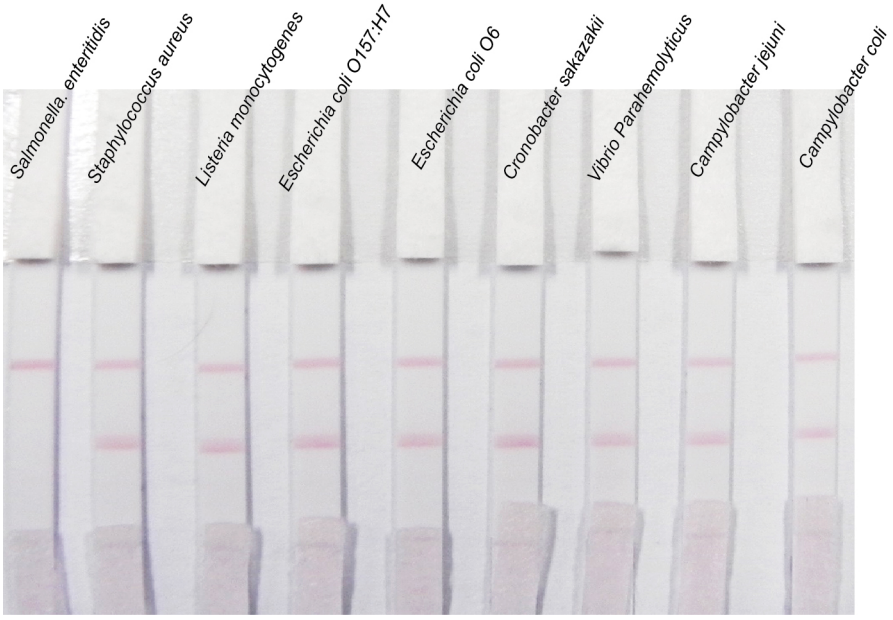

Figure 4 Specificity of the immunochromatographic strip sensor with the Gram-negative and Gram-positive bacteria.

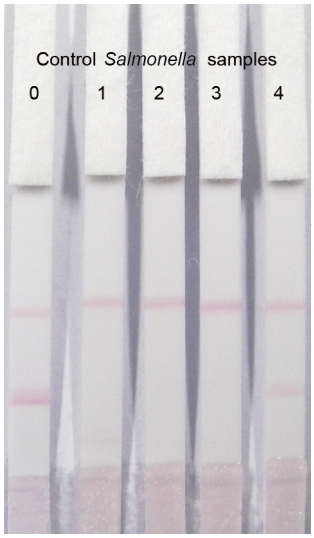

Figure 5 Analysis of *Salmonella* at low level in pure milk sample after 12 h enrichment: 0, Negative control; 1, *S. paratyphiA*; 2, *S. typhimurium*; 3, *S. enteritidis*; 4, *S. arizona*.

tion time of *Salmonella* spp. including *S. arizona*, without the need for complex biochemical confirmation steps, sophisticated instruments, and professional training [9].

CONCLUSION

In summary, we report a novel competitive immunochromatographic strip sensor based on a genus-specific anti-LPS mAb for the rapid detection of twelve typical strains of *Salmonella*. Murine mAbs against the conserved outer core structure of *Salmonella* LPS were produced with LPS-BSA conjugates as immunogens. Based on the heterogeneous conjugate as the coating antigen for the T line, and the Au NPs labeled outer core specific mAb as the detection probe, the innovative competitive immunochromatographic strip was established. Our results showed that 25 ng mL^{-1} of the Ra LPS from *Salmonella* inhibited color on the T line and the sensitivity based on gray values was at 10^3 CFU for all the tested *Salmonella* strains except *S. thompson* (10^4 CFU). The broad cross-reaction in the *Salmonella* genus and excellent specificity with the other tested Gram-negative and Gram-positive bacteria indicated that this novel strip assay was accurate for the detection of *Salmonella* at the genus level. In addition, real sample analysis demonstrated that a milk sample contaminated with *Salmonella* at a low level (1–5 CFU mL^{-1}) was detected after 12 h enrichment. The analysis of *Salmonella* using this strip assay not only saved time, but also simplified the detection which did not require complex biochemical confirmation steps, sophisticated instruments and professional training. Therefore, the novel competitive strip sensor developed in this study is a promising portable and rapid platform for the detection of *Salmonella* spp. in food and clinical samples.

Received 6 June 2016; accepted 12 July 2016; published online 18 August 2016

1 Singh J, Sharma S, Nara S. Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water. Food Chem, 2015, 170: 470–483
2 Zhu D, Yan Y, Lei P, *et al.* A novel electrochemical sensing strategy for rapid and ultrasensitive detection of *Salmonella* by rolling circle amplification and DNA-Au NPs probe. Anal Chim Acta, 2014, 846: 44–50
3 Zaki MH, Man SM, Vogel P, *et al.* *Salmonella* exploits NLRP12-dependent innate immune signaling to suppress host defenses during infection. Proc Natl Acad Sci USA, 2014, 111: 385–390
4 Yang X, Huang J, Wu Q, *et al.* Prevalence, antimicrobial resistance and genetic diversity of *Salmonella* isolated from retail ready-to-eat foods in China. Food Control, 2016, 60: 50–56
5 Fang Z, Wu W, Lu X, *et al.* Lateral flow biosensor for DNA extraction-free detection of *Salmonella* based on aptamer mediated strand displacement amplification. Biosens Bioelectron, 2014, 56: 192–197
6 Park S, Kim YT, Kim YK. Optical enzyme-linked immunosorbent assay on a strip for detection of *Salmonella typhimurium*. Biochip J, 2010, 4: 110–116
7 Yoshida C, Lingohr EJ, Trognitz F, *et al.* Multi-laboratory evaluation of the rapid genoserotyping array (SGSA) for the identification of *Salmonella* serovars. Diagn Microbiol Infect Dis, 2014, 80: 185–190
8 Hein I, Flekna G, Krassnig M, *et al.* Real-time PCR for the detection of *Salmonella* spp. in food: an alternative approach to a conventional PCR system suggested by the FOOD-PCR project. J Microbiol Method, 2006, 66: 538–547
9 Park SH, Ricke SC. Development of multiplex PCR assay for simultaneous detection of *Salmonella* genus, *Salmonella* subspecies I, *Salm. Enteritidis*, *Salm. Heidelberg* and *Salm . Typhimurium*. J Appl Microbiol, 2015, 118: 152–160
10 Shao Y, Zhu S, Jin C, *et al.* Development of multiplex loop-mediated isothermal amplification-RFLP (mLAMP-RFLP) to detect *Salmonella* spp. and *Shigella* spp. in milk. Int J Food Microbiol, 2011, 148: 75–79
11 Liu CC, Yeung CY, Chen PH, *et al.* *Salmonella* detection using 16S ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. Food Chem, 2013, 141: 2526–2532
12 Zhuang L, Gong J, Li Q, *et al.* Detection of *Salmonella* spp. by a loop-mediated isothermal amplification (LAMP) method targeting *bcfD* gene. Lett Appl Microbiol, 2014, 59: 658–664
13 Jyoti A, Vajpayee P, Singh G, *et al.* Identification of environmental reservoirs of nontyphoidal Salmonellosis: aptamer-assisted bioconcentration and subsequent detection of *Salmonella typhimurium* by quantitative polymerase chain reaction. Environ Sci Tech, 2011, 45: 8996–9002
14 Ma X, Jiang Y, Jia F, *et al.* An aptamer-based electrochemical biosensor for the detection of *Salmonella*. J Microbiol Method, 2014, 98: 94–98
15 Jain S, Chattopadhyay S, Jackeray R, *et al.* Highly sensitive detection of *Salmonella* typhi using surface aminated polycarbonate membrane enhanced-ELISA. Biosens Bioelectron, 2012, 31: 37–43
16 Kong D, Liu L, Xing C, *et al.* Sensitive and highly specific detection

of *Cronobacter sakazakii* based on monoclonal sandwich ELISA. Food Agricultural Immunol, 2015, 26: 566–576
17 Wang W, Feng M, Kong D, *et al.* Development of an immunochromatographic strip for the rapid detection of *Pseudomonas syringae pv. maculicola* in broccoli and radish seeds. Food Agric Immunol, 2015, 26: 738–745
18 Wang W, Liu L, Song S, *et al.* A highly sensitive ELISA and immunochromatographic strip for the detection of *Salmonella typhimurium* in milk samples. Sensors, 2015, 15: 5281–5292
19 Chen Y, Xie M. A colorimetric and ultrasensitive immunosensor for one-step pathogen detection via the combination of nanoparticle-triggered signal amplification and magnetic separation. RSC Adv, 2015, 5: 100633–100637
20 Dai F, Zhang M, Hu B, *et al.* Immunomagnetic nanoparticles based on a hydrophilic polymer coating for sensitive detection of *Salmonella* in raw milk by polymerase chain reaction. RSC Adv, 2015, 5: 3574–3580
21 Fei J, Dou W, Zhao G. A sandwich electrochemical immunoassay for *Salmonella pullorum* and *Salmonella gallinarum* based on a AuNPs/SiO_2/Fe_3O_4 adsorbing antibody and 4 channel screen printed carbon electrode electrodeposited gold nanoparticles. RSC Adv, 2015, 5: 74548–74556
22 Pandey SK, Rishi P, Suri CR, *et al.* Anodic stripping voltammetry of anti-Vi antibody functionalized CdTe quantum dots for the specific monitoring of *Salmonella enterica* serovar typhi. RSC Adv, 2015, 5: 88234–88240
23 Kong D, Liu L, Song S, *et al.* A gold nanoparticle-based semi-quantitative and quantitative ultrasensitive paper sensor for the detection of twenty mycotoxins. Nanoscale, 2016, 8: 5245–5253
24 Wang L, Chen W, Ma W, *et al.* Fluorescent strip sensor for rapid determination of toxins. Chem Commun, 2011, 47: 1574–1576
25 Wang W, Liu L, Xu L, *et al.* Gold-nanoparticle-based multiplexed immunochromatographic strip for simultaneous detection of staphylococcal enterotoxin A, B, C, D, and E. Part Part Syst Charact, 2016, 33: 388–395
26 Xing C, Liu L, Song S, *et al.* Ultrasensitive immunochromatographic assay for the simultaneous detection of five chemicals in drinking water. Biosens Bioelectron, 2015, 66: 445–453
27 Leem H, Shukla S, Song X, *et al.* An efficient liposome-based immunochromatographic strip assay for the sensitive detection of *Salmonella typhimurium* in pure culture. J Food Saf, 2014, 34: 239–248
28 Shukla S, Leem H, Kim M. Development of a liposome-based immunochromatographic strip assay for the detection of *Salmonella*. Anal Bioanal Chem, 2011, 401: 2581–2590
29 Moongkarndi P, Rodpai E, Kanarat S. Evaluation of an immunochromatographic assay for rapid detection of *Salmonella enterica* serovars *typhimurium* and *Enteritidis*. J Vet Diagn Invest, 2011, 23: 797–801
30 Xia S, Yu Z, Liu D, *et al.* Developing a novel immunochromatographic test strip with gold magnetic bifunctional nanobeads (GMBN) for efficient detection of *Salmonella choleraesuis* in milk. Food Control, 2016, 59: 507–512
31 Preechakasedkit P, Pinwattana K, Dungchai W, *et al.* Development of a one-step immunochromatographic strip test using gold nanoparticles for the rapid detection of *Salmonella typhi* in human serum. Biosens Bioelectron, 2012, 31: 562–566
32 Bautista DA, Elankumaran S, Arking JA, *et al.* Evaluation of an immunochromatography strip assay for the detection of *Salmonella* sp. from poultry. J Vet Diagn Invest, 2002, 14: 427–430
33 Yin Y, Liu L, Song S, *et al.* Development of a highly sensitive icELISA to detect semicarbazide based on a monoclonal antibody. Food Agric Immunol, 2015, 26: 356–365
34 Guan D, Guo L, Liu L, *et al.* Development of an ELISA for nitrazepam based on a monoclonal antibody. Food Agric Immunol, 2015, 26: 611–621
35 Frens G. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nat Phys Sci, 1973, 241: 20–22
36 Wu X, Wang W, Liu L, *et al.* Monoclonal antibody-based cross-reactive sandwich ELISA for the detection of *Salmonella* spp. in milk samples. Anal Methods, 2015, 7: 9047–9053
37 Li X, Li P, Zhang Q, *et al.* Multi-component immunochromatographic assay for simultaneous detection of aflatoxin B1, ochratoxin A and zearalenone in agro-food. Biosens Bioelectron, 2013, 49: 426–432
38 Shukla S, Leem H, Lee JS, *et al.* Immunochromatographic strip assay for the rapid and sensitive detection of *S. typhimurium* in artificially contaminated tomato samples. Can J Microbiol, 2014, 60: 399–406
39 Cho IH, Irudayaraj J. Lateral-flow enzyme immunoconcentration for rapid detection of Listeria monocytogenes. Anal Bioanal Chem, 2013, 405: 3313–3319
40 Zhao Y, Wang H, Zhang P, *et al.* Rapid multiplex detection of 10 foodborne pathogens with an up-converting phosphor technology-based 10-channel lateral flow assay. Sci Rep, 2016, 6: 21342
41 Ranganathan A, Paradise GA, Hansen CA, *et al.* Indirect homologous competitive enzyme-linked immunosorbent assay for the detection of a class of glycosylated dihydrochalcones. J Agric Food Chem, 2013, 61: 6964–6970
42 Choi D, Tsang RSW, Ng MH. Sandwich capture ELISA by a murine monoclonal antibody against a genus-specific LPS epitope for the detection of different common serotypes of *Salmonella*. J Appl Bacteriology, 1992, 72: 134–138
43 Petsch D, Anspach FB. Endotoxin removal from protein solutions. J Biotech, 2000, 76: 97–119
44 Sasaki Y, Tsujiyama Y, Asai T, *et al. Salmonella* prevalence in commercial raw shell eggs in Japan: a survey. Epidemiol Infect, 2011, 139: 1060–1064
45 Heinrichs DE, Yethon JA, Whitfield C. Molecular basis for structural diversity in the core regions of the lipopolysaccharides of *Escherichia coli*and *Salmonella enterica*. Mol Microbiol, 1998, 30: 221–232
46 Wu W, Li J, Pan D, *et al.* Gold nanoparticle-based enzyme-linked antibody-aptamer sandwich assay for detection of *S. typhimurium*. ACS Appl Mater Interf, 2014, 6: 16974–16981
47 Huang P, Tu D, Zheng W, *et al.* Inorganic lanthanide nanoprobes for background-free luminescent bioassays. Sci China Mater, 2015, 58: 156–177
48 Zhang Y, Kang Z, Yan X, *et al.* ZnO nanostructures in enzyme biosensors. Sci China Mater, 2015, 58: 60–76
49 Duan J, Zhan J. Recent developments on nanomaterials-based optical sensors for Hg^{2+} detection. Sci China Mater, 2015, 58: 223–240
50 Muldoon MT, Teaney G, Li J, Onisk DV, Stave JW. Bacteriophage-based enrichment coupled to immunochromatographic strip-based detection for the determination of *Salmonella* in meat and poultry. J Food Prot, 2007, 70: 2235–2242

Acknowledgments This work was supported by the National Natural Science Foundation of China (21471068), the National Key Technologies R&D Program from Ministry of Science and Technology of China (2012BAK08B01), Special Fund for Argo-scientific Research in the Public Interest (201513006), the Natural Science Foundation of Jiangsu Province (BK201501, BK20140003, BE2013613, BE2013611 and CSE11N1310), and the Graduate Innovation Project in Jiangsu Province of China

(KYLX15_1137).

Author contributions Kuang H and Xu C conceived and designed the experiments. Wang W, Liu L, and Song S performed the experiments. Xu L, Kuang H and Zhu J analyzed the data. Wang W wrote the paper. Kuang H, Xu L, and Xu C revised and approved the final version of the paper. All authors reviewed the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Supplementary information is available in the online version of this article.

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基于菌属特异性抗体的金标试纸传感器同时检测12种沙门氏菌

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摘要 沙门氏菌属是全球关注的重要食源性致病菌之一. 本文以沙门氏菌属特异性脂多糖抗体与胶体金纳米粒子进行偶联, 并进行固相化, 装配了适合于快速、可视化分析的金标试纸传感器. 裸眼观察下, 金标试纸条可以识别浓度低至 25 ng mL^{-1}的沙门氏菌属脂多糖. 结合灰度扫描分析, 金标试纸对受试的12种沙门氏菌的敏感性可达到10^3 菌落形成单位(CFU). 对牛奶样品进行12 h的富集孵育, 该金标试纸条对沙门氏菌的检测灵敏度可达 1个CFU. 金标试纸传感器无需复杂的前处理过程和专业设备, 操作简单, 有效地提高了致病菌的检测效率.