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Ultra technically-simple and sensitive detection for *Salmonella* Enteritidis by immunochromatographic assay based on gold growth

Tong Bu, Qiong Huang, Lingzhi Yan, Lunjie Huang, Mengyue Zhang, Qingfeng Yang, Baowei Yang, Jianlong Wang*, Daohong Zhang**

College of Food Science and Engineering, Northwest A&F University, Yangling 712100, Shaanxi, China

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ABSTRACT

A signal amplified lateral flow immunoassay (LFIA) based on gold nanoparticles (GNPs) growth and accumulation for rapid detection of *Salmonella* Enteritidis (*S.* Enteritidis) was developed. The signal was amplified on account of the high catalytic activity of GNPs toward the reaction between $HAuCl_4$ and $NH_2OH \cdot HCl$, which could produce new GNPs on the surface of the initial GNPs. The remarkable enhanced signal at the test and control lines can be clearly distinguished by naked eyes even under a lower analyte level. The lowest visible detection limit of the signal enhanced LFIA for *S.* Enteritidis was as low as 10^4 CFU/mL with excellent specificity, achieving a 100-fold improvement in sensitivity compared to the traditional GNP-based LFIA. Finally, for applicability demonstration the method was applied to milk for *S.* Enteritidis analysis. Except for incubation time, the process required only 6 h, which was obvious shorter than that of many reported methods.

1. Introduction

Salmonella infections are serious public health concerns all over the world, and they are contributing to morbidity and mortality in humans and other animal species (Sun, Zhao, & Dou, 2015). Recent survey shows that around 155,000 deaths are caused by *Salmonella* per year (Majowicz, Musto, Scallan, Angulo & Kirk, 2010). *Salmonella* is involved in a variety of foodborne outbreaks associated with animal derived food, dairy products and others (Liu, Yan, Mao, Wang, & Deng, 2016). More than 2500 serovars of *Salmonella enterica* have been recognized, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis are the most prevalent (Humphrey, 2004). *S.* Enteritidis has emerged as one of most common zoonotic agents, leading a major cause of gastroenteritis, vomiting, typhoid fever, watery diarrhea and eventually death worldwide. Infections with *S.* Enteritidis can result in acute systemic disease and a high incidence of mortality in the nursling, elderly, or immunocompromised patient. Because of their fast replication under normal environmental conditions, these organisms pose a serious threat to human health even at a low concentration (Cho & Irudayaraj, 2013). To reduce the risk of *S.* Enteritidis exposure, it is essential to identify the sources of *S.* Enteritidis contamination, which can be properly conducted by using high sensitive, rapid assays.

Up to now, several available methods for *Salmonella* have been reported, including conventional microbiological culture and colony counting (Allen, Edberg, & Reasoner, 2004), enzyme-linked immunosorbent assay (ELISA) (Cho & Irudayaraj, 2013; Uyttendaele, Vanwildemeersch, & Debevere, 2003), polymerase chain reaction (PCR) (Martin, Garriga, & Aymerich, 2012) and electrochemical biosensor (Delibato, Volpe, Romanazzo, De Medici & Toti, 2009). These methods are highly selective and sensitive, accurate and reliable. Yet, these methods are not suitable for real-time screening, since they inherently rely on sophisticated and costly instruments, time consuming sample pre-treatments, and complicated operations. In a sense, it has still remained great technical challenge to achieve easy operation, rapid, portable and low-cost methods for the earlier and sensitive analysis of *S.* Enteritidis, especially for point-of-care (POC) applications.

Recently, the immunochromatographic assay (ICA) has been widely noted due to its outstanding advantages such as fabrication, convenient operation, effective cost, disposable feature, short assay time and facile analytical procedure (Wang, Wang, Zhang, Su, & Luo, 2016), and become a well-established diagnostic tool for

* Corresponding author.
** Corresponding author.
E-mail addresses: wanglong79@nwsuaf.edu.cn (J. Wang), zhangdh@nwsuaf.edu.cn (D. Zhang).

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various POC testing and rapid detection. ICA, usually with colloidal gold served as a label because of unique optical properties, promising chemical stability and easy surface modification, can realize qualitative or semi-quantitative analysis of antigens (Mao, Baloda, Gurung, Lin, & Liu, 2008). Unfortunately, the GNPs-based ICA suffers from a lower detection sensitivity compared to immunoassays based on other probes such as fluorophores, radioactive isotopes, and horseradish peroxidase (Posthuma-Trumpie, Korf, & van Amerongen, 2009), which hinders its further applications.

To meet the demand, there has been a growing interest focusing on improving high sensitive assay capacity of ICA devices by using different labels such as fluorescent microsphere (Xie, Wu, Xiong, Xu & Xiong 2014), gold magnetic bifunctional nanobeads (GMBN) (Xia, Yu, Liu, Xu, & Lai, 2016), quantum dots (Li, Wang, Wang, Tang & Pounds, 2010) and so on. Although the fluorescent and magnetic ICAs offered high sensitivity, the chemical instability and complicated cross-linking with antibodies limit their POC or in-field applications. On the other hand, some novel researches have been developed to improve sensitivity of the GNP-based LFIA. One mode is by using a gold catalyzation method, e.g. silver enlargement technology (Panferov, Safenkova, Varitsev, Drenova & Kornev, 2016) and gold growth method (e.g., $HAuCl_4$ and $NH_2OH{\cdot}HCl$) (Wang, Chen, Sheng, Liu, & Wu, 2015; Wang, Liu, Song, Tang, & Kuang, 2015). Sensitivities in these methods were 10 and 8 times improved than those of the traditional colloidal gold based ICA, regrettably, these approaches needed sophisticated washing and rinsing procedures. Another way is to apply two GNP conjugates (Chen, Yu, Liu, Peng & Liu, 2015) by which the sensitivity was greatly enhanced compared to the traditional GNP-based assay. But this method increased the difficulty of strip preparation due to the introduction of another expensive antibody. Consequently, the composite nanomaterials, which are easy to operate and have no complex synthesis requirement, will be an ideal colored reagent to enhance the ICA sensitivity.

In this study, inspired by the aforementioned signal amplification methods, a gold-coated GNPs based ICA was reported for *S.* Enteritidis detection using $HAuCl_4$ and $NH_2OH{\cdot}HCl$ as the enhanced solution. The high sensitivity was achieved by using colloidal gold to catalyze the reaction between $HAuCl_4$ and $NH_2OH{\cdot}HCl$. The assay was technically-simple and rapid compared to other previous reports (Panferov et al., 2016; Wang & Chen et al., 2015; Wang & Liu et al., 2015) with no repeated washing requirement, higher sensitivity and less background color interference. Under optimal conditions, the system was applied to the *S.* Enteritidis detection in milk samples to evaluate its feasibility and practicability. Results demonstrated that the developed ICA provided a simple, reliable, rapid and sensitive strategy for visual detection of *S.* Enteritidis. We believe that the gold-coated GNPs based method could also blaze a trail in several other fields such as early clinical diagnosis, environmental security monitoring etc.

2. Materials and methods

2.1. Chemicals and equipments

Reagents. A pair of monoclonal antibodies (McAb) (3C5 as the detection antibody, 2B4 as the capture antibody) to target *S.* Enteritidis flagellin were prepared in our laboratory as previously reported (Zhang, Li, Yang, Zhang, & Zhang, 2011), after characterization by a sandwich ELISA, the sensitivity of the paired McAbs for *Salmonella* detection was 10^2 CFU/mL and there were very weak cross-reactions of the paired McAbs with other interfering bacteria of *Salmonella* and non-*Salmonella* strains. Goat anti-mouse IgG was purchased from Luoyang Baiaotong Experimental Materials Center. Goat anti-mouse IgG (H + L)·Horseradish peroxidase (HRP) was obtained from Pioneer Biotechnology. Inc. 3,3′,5,5′-Tetramethylbenzidine (TMB), Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4{\cdot}3H_2O$) were purchased from Sigma-Aldrich. Milk was purchased from a local supermarket in Yangling, China. NC membranes were purchased from Millipore Corp. Glass fibers, sample pads, and absorbent pads were purchased from Shanghai Kingdiag-biotech CO., Ltd. Bovine serum albumin (BSA) and glycerol were purchased from MP Biochina. Ovalbumin (OVA) was purchased from solarbio® Life Sciences. Luria-Bertani (LB) medium and Rappaport-Vassiliadis Mdeium (MM, RV Medium) were purchased from Beijing Land Bridge Technology Co., China. All solvents and other chemicals used in this work were of analytical-reagent grade or better.

Equipments. Guillotine cutter (HGS-201) and Dispensing Platform (HGS510-2D) were purchased from Hangzhou Autokun Technology Co., Ltd. The high speed refrigerated centrifuge (HC-3018R) was obtained from Anhui USTC Zonkia Scientific Instruments Co., Ltd. Freeze dryer (FDS-2.5E) was purchased from SIM International Group Co., Ltd.

Bacterial Strains. S. Enteritidis and non-*S.* Enteritidis strains were kindly provided by Baowei Yang's research group in our college. Among these strains, *Salmonella* bacteria were sampled from Xinjiang, China, they were isolated from food, belonging to wild bacteria, so, there is no standard strain number at present. Specifically, *S.* Enteritidis, *Salmonella* London (*S.* London), *Salmonella* Typhimurium (*S.* Typhimurium), *Salmonella* Paratyphi B (*S.* Paratyphi B) and *Salmonella* Hadar (*S.* Hadar) were sampled from chicken, lamb skewers, chicken gizzards, beef, live chicken, respectively. Other non-*Salmonella* as follows: *Staphylococcus aureus* (*S. aureus*) (ATCC29213), *Escherichia coli* (*E. coli*) (ATCC25922), *Candida albicans* (*C. albicans*) (ATCC10231), *Listeria monocytogenes* (*L. monocytogenes*) (CMCC54004), *Campylobacter coli* (*C. coli*) (ATCC29428) and *Enterobacter sakazakii* (*E. sakazakii*) (ATCC29544). The strains were transferred into LB liquid medium and cultured at 37 °C for 18–24 h before use.

2.2. Preparation of colloidal gold

In this assay, colloidal gold solution was prepared by using trisodium citrate to reduce $HAuCl_4{\cdot}3H_2O$ according to the classical Frens (Zhu, Chen, Lu, & Cheng, 2008). One milliliter of 1% hydrogen tetrachloroaurate trihydrate was added to 96 mL of ultrapure water, stirred, and heated to boiling temperature. At this time the color was yellowish green. Four milliliters of 1% trisodium citrate was added to the mixture immediately and the color changed from yellowish green to colorless. The reaction was maintained about 10 min at boiling temperature until the color changed to stable and transparent red, then stirred for another 15 min. Finally, the solution was cooled at room temperature and stored at 4 °C for further use.

2.3. Synthesis of the gold nanoparticle-antibody (GNP-McAb) conjugate

McAbs against *S.* Enteritidis were produced on the basis of the modified method described elsewhere (Zhang, Li & Yang, 2011). The preparation process and the performance characterization for the paired McAbs were showed in the supplementary materials. Antibody-gold nanoparticle conjugate was prepared according to the method reported previously (Zhang, Li, Zhang, & Zhang, 2011). The pH of the colloidal gold solution was adjusted by addition of 0.01 M K_2CO_3. Under gentle stirring, anti-*S.* Enteritidis McAb was added to colloidal gold solution, agitated for 30 min, then 10% BSA solution was mixed with the colloidal gold solution to achieve a final concentration of 1% BSA to block the residual sites on the