An Optical and Rapid Sandwich Immunoassay Method for Detection of Salmonella pullorum and Salmonella gallinarum...
An optical and rapid sandwich immunoassay method for detection of Salmonella pullorum and Salmonella gallinarum based on immune blue silica nanoparticles and magnetic nanoparticles

Qian Sun, Guangying Zhao, Wencao Dou*
Food Safety Key Laboratory of Zhejiang Province, College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, 310018, China

A R T I C L E   I N F O
Article history:
Received 22 July 2015
Received in revised form
23 November 2015
Accepted 26 November 2015
Available online 28 November 2015

Keywords:
Optical sandwich immunoassay
Blue silica nanoparticles
Magnetic nanoparticles
Salmonella pullorum and Salmonella
gallinarum

A B S T R A C T
An optical and rapid sandwich immunoassay of Salmonella pullorum and Salmonella gallinarum (S. pullorum and S. gallinarum) was designed using anti-S. pullorum and S. gallinarum antibody-functionalized blue silica nanoparticles (IgG-Blue-SiNPs) and magnetic nanoparticles (IgG-MNPs) as immunosensing probes in this article. The IgG-MNPs were used for enrichment of S. pullorum and S. gallinarum. IgG-Blue-SiNPs were used for signal amplification. The Blue-SiNPs were synthesized by doping C.I. reactive blue 14 into silica nanoparticles using an inverse microemulsion method. The morphology, surface charge and functional groups of Blue-SiNPs were characterized by SEM, Zeta potential and FTIR spectroscopy. S. pullorum and S. gallinarum in sample solution was captured, enriched and separated by IgG-MNPs. Then IgG-Blue-SiNPs were added into the above mixture solution, S. pullorum and S. gallinarum was sandwiched by IgG-MNPs and IgG-Blue-SiNPs, forming a blue plaque. Under optimal conditions, the detection limit for pure S. pullorum and S. gallinarum was from 8.8 × 10^4 CFU/ml. Besides, this qualitative detection method was economic, simple, rapid, specific and good stability. Such a simple optical sandwich immunoassay holds great potential as an on-site tool for clinical diagnosis of bacteria and viruses.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction
Nanoparticle-based sandwich immunoassays, as a promising approach for selective and sensitive analysis, has recently gained increasing attention [1,2]. Various Nanoparticle-based sandwich immunoassays have been developed in different fields including food safety, clinical diagnosis and environmental monitoring [3,4]. Usually, the nanoparticle-based assay consisted of two kinds of nanoparticles: (i) magnetic nanoparticles coated with antibody as capture probe and (ii) multifunctional nanoparticles (including enzyme labeled nanoparticles, quantum dots, gold nanoparticles and TiO_2 nanocrystals) decorated with antibody as recognition probe [5–8].

For the successful development of nanoparticle-based sandwich immunoassays, signal amplification is a key technology, which is very crucial for obtaining low detection limit. Enzyme-labeled nanoparticles are commonly exploited as signal transduction tools in immunoassays [9]. The carried enzyme molecule is entered and participated in the catalytic reaction. High efficiency of enzymes makes them suitable for ultrasensitive bioanalysis. However, short lifetime and the critical operating situation limit enzyme immunoassay applicability [10]. Fluorescent nanomaterials are very sensitive, they require for bulky fluorescence spectrometer, so they are not convenient for real-time on-site application. Gold nanoparticles and TiO_2 nanocrystals can be used for on-site detection with a portable UV–Vis absorption spectrometer. However, color and absorption intensity of gold nanoparticles is substantially affected by testing conditions. TiO_2 nanocrystals are colorless and they can only absorb ultraviolet light, which is difficult to read experiment result with naked eye. As the reported assay is imperfect, we aim to develop another optical immunoassay for pathogenic bacteria detection, which have the potential to fulfill the WHO ASSURED criteria (affordable, sensitive, specific, user friendly, robust and rapid, equipment free, deliverable to those who need them) for detection methodologies [11].

Silica nanoparticles (SiNPs) are widely used in several fields includes disease labeling, drug delivery, and biosensor. But most reported SiNPs are colorless or white, they are not suitable for detection of pathogenic bacteria [12–15]. Organic dye has rich color and good stability, and does not fade in the harsh conditions of light,
heat, acid, alkali and so on. From a chemical point of view, so many kinds of organic dyes and their good chemical reactivity make them very suitable for dyeing silica nanoparticles [16,12].

The aim of this work is to exploit blue silica nanoparticles (Blue-SiNps) and magnetic nanoparticles (MNPs) based optical sandwich immunoassay for the rapid detection of pathogenic bacteria. In this study, Salmonella pullorum and Salmonella gallinarum (S. pullorum and S. gallinarum) was used as a model analyte. S. pullorum and S. gallinarum are common infectious bacteria in poultry breeding [18-19]. Rapid and sensitive detection of S. pullorum and S. gallinarum is of great importance for poultry breeding. We chose organic dye (C.I. Reactive Blue 14) to synthesize the Blue-SiNps using an inverse microemulsion method. C.I. Reactive Blue 14 was modified to the surface of SiNps by silane coupling agent. The antibody-coated magnetic nanoparticles (IgG-MNPs) were used for enrichment and separation of S. pullorum and S. gallinarum. The antibody-coated Blue-SiNps (IgG-Blue-SiNps) were used to detect the S. pullorum and S. gallinarum. The eye-catching color change was used to identify the S. pullorum and S. gallinarum.

2. Experimental

2.1. Materials and Instruments

Triton X-100, cyclohexane, hexanol, and ammonia (25–28 wt%) were obtained from Chengdu Kelong Chemical Reagent Co., Ltd. (Chengdu, China). 3-2-[2-(Aminoethy1amino)ethylamino] propyl-Trimethoxysilane (APTMS), Tetraethyl orthosilicate (TEOS), N-hydroxy-succinimide (NHS), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 2-(N-morpholino)ethanesulfonic acid (MES) were obtained from Aladdin Industrial Inc. (Shanghai, China). Bovine serum albumin (BSA) was obtained from Beijing Dingguo Biotechnology Co. Ltd. (Beijing, China). Magnetic nanoparticles (MNPs) were obtained from Enriching Biotechnology Ltd. (Shanghai, China). C.I. Reactive Blue 14 was supplied by Zhejiang Shunlong Chemical Co., Ltd (Zhejiang, China). Polyclonal antibody against S. pullorum and S. gallinarum (100 μg/mL) was purchased from China Institute of Veterinary Drugs Control (Beijing, China). Other reagents were all of analytical grade and were used as received without further purification. The water used was doubly distilled.

Hitachi SU-70 Scanning electron microscopy (SEM) was purchased from Hitachi Inc. (Japan, Tokyo); Malvern Nano ZS potential laser particle analyzer was provided by Malvern Instruments Co., Ltd. (Worcestershire, UK); Nicolet 380 Fourier transform infrared spectrometer (FTIR) (Thermo, Shanghai, China); 3–18 K high speed refrigerated centrifuge was purchased from Sigma Laborzentrifugen GmbH (Osterode, Germany); All electrochemical experiments were performed at room temperature (25 ± 1 °C).

2.2. Bacterial strains and preparation

A variety of bacteria were employed in this work including S. pullorum and S. gallinarum as the target bacteria, and Enterobacter sakazakii (E. Sakazakii), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Bacillus subtilis (B. Subtilis), Salmonella enteritidis (S. enteritidis) and Salmonella typhimurium (S. typhimurium) as control group. All bacteria were conserved by the laboratory of author. S. pullorum and S. gallinarum was grown at 37 °C in Lysogeny broth (LB) medium with shaking. Cells were harvested in late exponential growth phase by centrifugation at 6000 rpm at 4 °C for 10 min and washed in triplicate using physiological saline aqueous solution and dispersed in 5 mL physiological saline aqueous solution. Concentration of the bacteria was confirmed by the colony counting (CFU/mL). Ten-fold serial dilutions of S. pullorum and S. gallinarum solution were made in physiological saline aqueous solution and 1 mL of dilutions (10⁻², 10⁻⁴, 10⁻⁶) were plated out on agar plates using glass inoculators and a small rotating disk. Plates were incubated at 37 °C overnight before counting of colonies. Counted CFU were marked with a pen on the plate cover to discriminate counted from uncounted colonies. After counting one sector, the count was multiplied with the total number of sectors to estimate whole plate CFU count. The enriched bacterial were inactivated with 0.5% formaldehyde and stored at before 4 °C use.

2.3. Synthesis of Blue-SiNps

Blue-SiNps were synthesized according to an inverse microemulsion method described by a previous paper with little change [20]. The details of the procedure are described in the following: 8 mL cyclohexane, 2 g Triton X-100, 2 mL 1-hexanol, 150 μL C.I. Reactive Blue 14 (100 mg/mL) and 400 μL of water were added into 25 mL Erlenmeyer flask and stirred for 15 min to ensure water completely dispersed into cyclohexane. Afterwards, 100 μL TEOS and 20 μL APTMS were added to this inverse microemulsion system followed by 100 μL ammonia (25–28 wt%) to catalyze the hydrolyzation of TEOS. After stirring 48 h, the microemulsion was broken by adding 10 mL acetone. The blue-SiNps were separated from the supernatant by centrifugation at 8000 rpm for 10 min and washed with ethanol three times and followed by twice wash with ultrapure water.

2.4. Surface functionalization of nanoparticles

2.4.1. Covalent immobilization of the antibody onto Blue-SiNps surface

30 mg Blue-SiNps were suspended in 20 mL ultrapure water. 1.4 mL acetic acid and 200 μL APTMS were added into Blue-SiNps solution for the post coating treatment. The APTMS was allowed to hydrolyze under stirring for 1 h at room temperature. After the hydrolysis reaction of APTMS, amino groups were introduced onto the surface of Blue-SiNps. The amino-modified Blue-SiNps (Blue-SiNps-NH₂) were isolated by centrifuging at 8000 rpm for 5 min and washing three times with DMF.

Carboxyl-modified Blue-SiNps were synthesized by reacting Blue-SiNps-NH₂ with glutaric anhydride. 20 mg Blue-SiNps-NH₂ were dispersed in 5 mL DMF containing 200 mg glutaric anhydride, then the solution was stirred under N₂ gas for 6 h. These carboxyl-modified Blue-SiNps were centrifuged at 8000 rpm for 10 minutes and washed with PBS (10 mM, pH 7.3).

The anti-S. pullorum and S. gallinarum antibody was directly immobilized onto the functionalized Blue-SiNps with well-established EDC/NHS coupling chemistry. The immobilization protocol was described as following: 20 mg Blue-SiNps-COOH were resuspended in 5 mL MES (0.1 M, pH 6.7), the Blue-SiNps-COOH suspension was mixed with 50 μL anti-S. pullorum and S. gallinarum polyclonal antibody solution, followed by the addition of 1 mL 10 mM EDC and 10 mM NHS solution. After 2 h incubation at room temperature, the free S. pullorum and S. gallinarum antibody was removed by centrifugation at 8000 rpm at 4 °C for 5 minutes and washed with PBS. The antibody modified Blue-SiNps were resuspended in 3 mL of PBS containing 1% BSA to block non-specific adsorption sites on the nanoparticles. The prepared resultant antibody-conjugated Blue-SiNps (IgG-Blue-SiNps) were dispersed in 1 mL of PBS and stored at 4 °C for its following use.

2.4.2. Immobilization of antibody onto MNPs surface

The antibody against S. pullorum and S. gallinarum was covalently conjugated to carboxyl-modified MNPs according to the manufacturer’s instructions. 100 μL of magnetic nanoparticles (10 mg/mL) were mixed with 50 μL of antibody against S. pullorum
and *S. gallinarum*. The reaction was allowed to proceed at 4 °C overnight. The mixture was washed for three times with washing buffer in a magnetic field. Unreacted active groups on the MNPs were blocked with 1% BSA. Finally, the antibody modified magnetic nanoparticle (IgG-MNPs) were dispersed in 1 mL of PBS and stored at 4 °C before use.

### 2.5. General Assay Procedure

The detection scheme is given in Fig. 1. For a typical sandwich assay, 20 μL IgG-MNPs (1 mg/mL) were mixed with 1 mL *S. pullorum* and *S. gallinarum* solution in centrifuge tube, after incubation for 30 min at 37 °C with gentle shaking, the IgG-MNPs-*S. pullorum* and *S. gallinarum* complex was separated magnetically and the clear supernatant was discarded. The immune complex was then washed with PBS for three times to remove any unbound species. The IgG-MNPs-*S. pullorum* and *S. gallinarum* complex was then dispersed in 20 μL PBS (pH 7.3) and transferred to a well of 96-well microtiter plate, 20 μL IgG-Blue-SiNps (10 mg/mL) were added to the well and the mixture was subjected to react for 15 min at room temperature, IgG-Blue-SiNps formed a sandwich structure with *S. pullorum* and *S. gallinarum* and IgG-MNPs via immune reaction. The immune sandwich complex was separated magnetically and the supernatant was discarded. The immune complex was then washed with PBS to effectively remove unbound IgG-Blue-SiNps. The final plaque at the bottom of the well was recorded by digital camera.

### 3. Results and discussion

#### 3.1. Characterizations of Blue-SiNps

Inverse microemulsion method was chosen to prepare Blue-SiNps in this study. Inverse microemulsion method is a simple and diverse preparation method for synthesis of silica nanoparticles in laboratory, and it is easy to control the morphology of silica nanoparticles. The physical image of Blue-SiNps showed that they were bright color and good dispersion in aqueous solution (Fig. 2 inset). The size and morphology of Blue-SiNps were characterized by SEM (Fig. 2). The nanoparticles had uniform particle size and all Blue-SiNps showed a spherical shape and smooth surface.

The average diameter of the nanoparticles determined by SEM was approximately 45 ± 5 nm and the size distribution was also quite uniform and the characteristics of colored-SiNps were in accordance with descriptions by Tan et al. [21].

The presence of chemical group on the outermost layer of Blue-SiNps was confirmed by the Zeta potential determination. Zeta potential measurement was carried out using a Zetasizer. For the determination of Zeta potential, pH of sample was adjusted by the addition of 0.01 M HCl or 0.01 M NaOH. All values shown in this work were the average of three measurements. Fig. 3A displays the Zeta potential of Blue-SiNps, Blue-SiNps-NH2 and Blue-SiNps-COOH as a function of pH. The isoelectric point (IEP) of Blue-SiNps was at pH 4.9. When Blue-SiNps were in the environment of a neutral solution, the surface potential of Blue-SiNps was about −9 mV. The particle surface had a negative charge, because of the presence of hydroxyl group; The IEP of Blue-SiNps-NH2 was shifted to pH 9.8. The increase of Zeta potential was attributed to the increasing number of protonated amine group on the surface of Blue-SiNps-NH2. Compared to Blue-SiNps-NH2, the IEP of Blue-SiNps-COOH showed positive charge.

![Image of Blue-SiNps](image-url)

**Fig. 2.** The SEM image of Blue-SiNp, physical image of Blue-SiNp (inset).
was shifted to pH 3.4, which was ascribed to negative charge of the carboxyl group, which made the modification and the bioconjugation of the nanoparticles easier.

Chemical composition on the coating layer of Blue-SiNps was also examined by FTIR (as shown in Fig. 3B). Dried sample was measured using KBr pellet method in the range of 400–4000 cm\(^{-1}\). A strong IR absorption bands at the region 980–1220 cm\(^{-1}\), corresponding to the Si–O–Si of the silica core, was found in both Blue-SiNps and Blue-SiNps-NH\(_2\). A new band at \(\sim 2945\) cm\(^{-1}\) in the Blue-SiNps-NH\(_2\) was assigned to the N–H of the silica surface. Compared to Blue-SiNps, the FTIR spectra of Blue-SiNps-NH\(_2\) had a significantly difference in the region 2900–3450 cm\(^{-1}\). These results were consistent with the results of Zeta potential. APTMS was thus believed successfully introduced onto the surface of the Blue-SiNps. The chemical composition of the Blue-SiNps-COOH was also examined by the FTIR spectrum. The stretching band of C=O (which is the characteristic band of carboxyl group) appears at 1718 cm\(^{-1}\). Both the symmetric and asymmetric bands of COO arise in this spectrum at 1638 cm\(^{-1}\) and 1557 cm\(^{-1}\), respectively. The spectra indicate the presence of C=O (the characteristic band of COOH) which arises from the modification of –COOH by glutaric anhydride.

3.2. Development of sandwich assay method for the identification of \(S.\) pullorum and \(S.\) gallinarum

We used IgG-MNPs and IgG-blue-SiNps to detect \(S.\) pullorum and \(S.\) gallinarum by sandwich assay method. In the sandwich assay method, IgG–MNPs were added to \(S.\) pullorum and \(S.\) gallinarum solution, the \(S.\) pullorum and \(S.\) gallinarum were enriched and isolated in a magnetic field and transferred to well of 96-well microtiter plate. IgG-Blue-SiNps, acted as an indicator of presence of \(S.\) pullorum and \(S.\) gallinarum, were added and reacted for 15 min. When the sample solution contained enough \(S.\) pullorum and \(S.\) gallinarum, IgG-MNPs and IgG-Blue-SiNps were effectively combined on the surface of \(S.\) pullorum and \(S.\) gallinarum, forming blue sandwich complexes through double antibody sandwich binding reaction. The blue plaques were observed at the bottom of the well with the naked eye and Blue-SiNps played as the role of color indicator.

Aimed to prove the superior of Blue-SiNps, IgG-AuNps and IgG-Blue-SiNps were both used to detect \(S.\) pullorum and \(S.\) gallinarum, preparation of IgG-AuNps was shown in Supplementary materials. The procedure of test was the same as mentioned in Section 2.5. \(S.\) pullorum and \(S.\) gallinarum were detected by our sandwich assay method using IgG-AuNps and IgG-Blue-SiNps separately, and PBS was used as negative control. As showed in Fig. 4, when IgG-AuNps was used as signal label the positive and negative results were difficult to distinguish by naked eye. Blue plaque was observed for \(S.\) pullorum and \(S.\) gallinarum strains when IgG-Blue-SiNps were used as signal label. This result showed that Blue-SiNps were more suitable to be used as signal label in optical immunoassay.

We examined the sensitivity of this sandwich assay method and the detection limit for \(S.\) pullorum and \(S.\) gallinarum. The positive and negative results were distinguished by observing at the bottom of the well with the naked eye, blue plaque in the bottom of the well presents positive result, and brown plaque in the bottom of the well presents negative result. The original bacterial solution was diluted to ten concentrations along a gradient from \(10^0\) CFU/mL to \(10^9\) CFU/mL using 0.85% stroke-physiological saline solutions. In the sandwich assay, IgG-MNPs and IgG-Blue-SiNps were sequentially mixed with different concentration \(S.\) pullorum and \(S.\) gallinarum solution. The lowest concentration of \(S.\) pullorum and \(S.\) gallinarum which can produce positive result is defined as the detection limit of this method. Fig. 5A shows that blue plaques were observed when the concentration of \(S.\) pullorum and \(S.\) gallinarum was as low as \(8.8 \times 10^1\) CFU/mL. Brown color of IgG-MNPs was observed in the well contained \(8.8 \times 10^1\) CFU/mL \(S.\) pullorum and \(S.\) gallinarum solution. The detection range of \(S.\) pullorum and \(S.\) gallinarum is \(8.8 \times 10^1\) to \(8.8 \times 10^0\) CFU/mL. The limit of detection of this sandwich assay was much lower than the common immune method such as agglutination test. This is due to the enrichment.
effect of IgG-MNPs, the volume of the sample solution (1 mL) in this sandwich assay is 50 times larger than that in agglutination test method (20 μL). In order to prove this, we made a control experiment, while the other conditions were unchanged, 20 μL S. pullorum and S. gallinarum solutions of different concentration were detected with this sandwich assay. As shown in Fig. 5B, when the concentration of S. pullorum and S. gallinarum was equal or greater than 8.8 × 10^4 CFU/mL, the blue color of Blue-SiNPs started to be observed. In this experiment, the detection limit for S. pullorum and S. gallinarum is 8.8 × 10^4 CFU/mL. By increasing the volume of S. pullorum and S. gallinarum solution, more pullorum and S. gallinarum were enriched by IgG-MNPs, and this made the optical sandwich assay more sensitive. This result proved that the sensitivity of our sandwich immunoassay was significantly increased by increase of sample volume.

3.3. Specificity

To demonstrate the potential of our assay in pathogenic bacteria discrimination, we examined the specificity of this sandwich assay method. The procedure of specificity test was the same as mentioned in Section 2.5. S. pullorum and S. gallinarum, E. Sakazakii, E. coli, S. aureus, B. Subtilis, S. enteritidis and S. typhimurium were all detected by this optical sandwich assay method, the concentrations of pathogenic bacteria were all 10^9 CFU/mL, PBS was used as negative control. As showed in Fig. 6, Blue signal was only observed for S. pullorum and S. gallinarum strains but not for E. Sakazakii, E. coli, S. aureus, B. Subtilis, S. enteritidis, S. typhimurium and PBS, confirming this assay could distinguish S. pullorum and S. gallinarum from other pathogenic bacteria. This result showed that this method had good specificity for S. pullorum and S. gallinarum.

3.4. Stability

We invested the stability of Blue-SiNPs in different pH solution. Fig. 7A shows that negligible changes were observed for the color of Blue-SiNPs in basic conditions, under acidic conditions the color of Blue-SiNPs slightly becomes lighter. In contrast Fig. 7B shows pH dependent variations in the color of gold nanoparticles. The gold nanoparticles (AuNps) are stably dispersed in neutral solution due to the negatively charged citrate groups. The nanoparticles aggregate under acidic or basic conditions due to charge neutralization, resulting in color change of the AuNps under basic conditions and color fade under acidic conditions. This result indicates that Blue-SiNPs are more stable than AuNps in acidic and basic conditions.

The stability of IgG-MNPs and IgG-Blue-SiNPs were studied by the detection of the S. pullorum and S. gallinarum solution.

Fig. 5. The sensitivity results of optical sandwich immunoassay with 1 mL (A) and 20 μL (B) of S. pullorum and S. gallinarum, concentration of S. pullorum and S. gallinarum was changed from 8.8 × 10^4 CFU/mL to 8.8 × 10^5 CFU/mL (from left to right).

Fig. 6. Specificity of the sandwich immunoassay, top from left to right: PBS (0.01 mol/mL), S. pullorum and S. gallinarum (10^9 CFU/mL), E. Sakazakii (10^9 CFU/mL), E. coli (10^9 CFU/mL), S. aureus (10^9 CFU/mL), and B. Subtilis (10^9 CFU/mL); Bottom from left to right: PBS (0.01 mol/mL), S. pullorum and S. gallinarum (10^9 CFU/mL), S. enteritidis (10^9 CFU/mL), S. typhimurium (10^9 CFU/mL).

Fig. 7. The photograph of Blue-SiNPs (A), and AuNps (B), after they were dispersed in different pH solutions.
(8.8 × 10⁸ CFU/mL) with sandwich assay after they were stored at 4 °C for 1, 7, 30, 60 and 90 days. These two kinds of immune nanoparticles were found to be able to retain similar reaction activity after storage at 4 °C for at least 90 days (as shown in Fig. 8).

3.5. Detection of pathogen bacteria in real samples

In this experiment, the commercial milk powder was used to make artificially contaminated samples. Salmonella-free milk powder samples were spiked with S. pullorum and S. gallinarum at the concentration range from 8.8 × 10¹ to 8.8 × 10⁸ CFU/mL. The procedure to detect the milk powder samples by IgG-MNPs and IgG-Blue-SiNps was same as that mentioned in Section 2.5. The results were showed in Fig. 9. This sandwich method was not influenced by complex matrix of real samples, when the concentration of bacteria in milk powder samples was as low as 8.8 × 10² CFU/mL, the blue plaque of IgG-Blue-SiNps in the microtiter plate was observed.

In addition, we also compared the analytical performance of this optical sandwich immunoassay with other S. pullorum and S. gallinarum assay method reported previously including standard culture method, Polymerase Chain Reaction (PCR), electrochemical immunosensor. See from Table 1, this optical immunoassay exhibited rapid detection speed and low detection limit. Significantly, the developed method was capable of continuously carrying out all steps in less than 60 min including incubation, separation, and detection, which was shorter than that of commercial PCR. The comparison suggested superior analytical speed of the present sandwich assay method over some previously reported biosensors. The linear range was an exception, this technology was unable to quantitatively measure the concentration of S. pullorum and S. gallinarum in sample, and this was a defect. Compared with the Gold nanoparticles and enzyme labeled nanoparticles are the most used optical nanoprobes in optical sandwich assay method. Compared with them, Blue-SiNps used in this work are cheaper and more stable than gold nanoparticles and enzyme. For bright color of Blue-SiNps, result of our sandwich assay method can be readout easily by naked eye, which fulfills the equipment free criteria.

4. Conclusions

In summary, we have successfully developed a simple, sensitive, and general optical sandwich immunoassay based on Blue-SiNps, it can be used for rapid detection of S. pullorum and S. gallinarum in PBS and real sample. Different from former nanoparticle-based sandwich immunoassays, the recognition and signal amplification elements of this sandwich immunoassay were IgG-Blue-SiNps, which contained bright blue color and simplified the sandwich assay measurement process. The optical sandwich immunoassay combines the advantage MNPs and Blue-SiNps, target bacteria are efficient captured and separated by MNPs and labeled by Blue-SiNps based great signal amplification. This assay achieved detection of S. pullorum and S. gallinarum with a total assay time less than 60 min (via naked-eye readout). In the process of S. pullorum and S. gallinarum detection, the phenomenon was clear and eye-catching. The detection limit of optical sandwich immunoassay for pure S. pullorum and S. gallinarum was 8.8 × 10¹ CFU/mL. Moreover, this assay was highly specific: it could effectively discriminate S. pullorum and S. gallinarum from other four pathogenic bacteria. This method was not influenced by complex detection matrix in the detection of milk powder sample, the detection limit for S. pullorum and S. gallinarum was 8.8 × 10¹ CFU/mL in milk powder. This economic and rapid optical method can also be extended to other pathogenic microorganisms or even investigated for rapid detection of the virus. However, this optical immune sandwich assay does not allow quantification of the absolute amount of S. pullorum and S. gallinarum present in sample.

### Acknowledgment

This project was supported by the Food Science and Engineering the most important discipline of Zhejiang province (ZYTP20141062). The Talent training provincial superior paper funded project (1110)Y1412001P). Postgraduate Scientific and Technological Innovation Project of Zhejiang Gongshang University (1110XJ1511104) and Plans for college students in Zhejiang Province science and technology innovation activities (acrobatic
tender grass talent programme) project (1110KZN0215112G). Professor Zhongxiu Chen and associate Professor Junli Zhu is acknowledged for their technical help.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2015.11.117.

References


Biographies

Qian Sun received her BE degree in Biological Engineering from Yancheng Teachers University. Now she is a graduate at Zhejiang Gongshang University and will graduate this year.

Guangying Zhao is a Professor and the Deputy Director of School of Food Science and Biotechnology, Zhejiang Gongshang University. Her research interests include the rapid detection of food quality and safety.

Wenxiao Dou was born in Fuxian, Henan Province, China, in 1982. He received his BS degree in Materials Chemistry in 2005 from Jilin University. Then he started his PhD studies in Jilin University under the supervision of Prof. Xingguang Su, obtaining his PhD degree in 2009. He joined School of Food Science and Biotechnology in Zhejiang Gongshang University since July 2009. His current research interests focused on the developments of new bioanalytical methods with an emphasis on optical techniques for food safety, and disease diagnosis.