Fluorescence immunoassay through histone-ds-poly(AT)-templated copper nanoparticles as signal transducers for the sensitive detection of *Salmonella choleraesuis* in milk

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ABSTRACT

The rapid and sensitive detection of foodborne pathogens is one of the most important issues in food safety control. In this work, we developed a novel fluorescence immunoassay method for the sensitive detection of *Salmonella choleraesuis*. The method uses the fluorescent signals of histone-ds-poly(AT)-templated copper nanoparticles (His-pAT CuNP) as signal transducers and glucose oxidase as an alternative for horseradish peroxidase for the generation of hydrogen peroxide (H₂O₂) through the catalysis of glucose. The H₂O₂ is then further converted into hydroxyl radical (·OH) by Fenton reagents. Owing to the ultrahigh sensitivity of His-pAT CuNP synthesis toward ·OH, the proposed fluorescence immunoassay method exhibited excellent sensitivity for *S. choleraesuis*, with a limit of detection of 8.04×10^1 cfu/mL, which is 3 orders of magnitude lower than that of the tetramethylbenzidine-based traditional immunoassay. The reliability of the proposed method was evaluated by using spiked milk samples with *S. choleraesuis* concentration ranging from 8.8×10^1 to 8.8×10^4 cfu/mL. The average recoveries for the intra- and inter-assay ranged from 73.52 to 96.59% and from 66.99 to 98.24% with a coefficient of variation from 6.85 to 31.26% and 5.46 to 17.99%, respectively. These results indicated that the proposed fluorescence immunoassay possesses a great potential for ultra-sensitive detection of foodborne pathogens in food safety control.

Key words: *Salmonella choleraesuis*, fluorescence immunoassay, DNA-templated copper nanoparticle, milk

INTRODUCTION

Foodborne pathogens pose important public health concern worldwide because they can contaminate foods through any food chains from farm to fork (Shan et al., 2015; Shang et al., 2018). The Centers for Disease Control and Prevention estimated that 1 in 6 Americans (or 48 million people) each year becomes adversely affected by foodborne diseases (CDC, 2018). The World Health Organization estimated that approximately a million people suffer from foodborne disease each year in the United Kingdom (WHO, 2011). The traditional culture-based approach used for foodborne pathogen detection usually takes up 5 to 6 d to obtain determined results, failing to meet the requirement of rapid screening detection (Abdulai et al., 2014, 2015). Various simple, specific, and rapid methods, such as real-time multiplex PCR (Hiitiö et al., 2018), immunochromatographic strips (Luo et al., 2018), and ELISA have been developed (Chen et al., 2016). Among them, ELISA is one of the most popular methods for high-throughput screening detection of foodborne pathogen-polluted samples. However, the sensitivity of traditional ELISA for foodborne pathogen detection employing horseradish peroxidase (HRP) to generate colored molecules as signal outputs usually ranges from 10^4 to 10^6 cfu/mL (Chen et al., 2015). Thus, a highly sensitive ELISA method for detecting low level of foodborne pathogens is still required.

In recent years, methods for synthesizing DNA-templated fluorescent metal nanoparticles, such as gold and silver nanoclusters and copper nanoparticles (CuNP; Petty et al., 2004; Chakraborty et al., 2015; Mao et al., 2015; Lian et al., 2017), have been developed. These novel DNA-templated fluorescent nanoclusters or nanoparticles show great potential as signal reporters because of their excellent biocompatibility and superior fluorescence properties (Liu, 2014). Among them,

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DNA-templated CuNP have distinct advantages, such as instantaneous reaction, low toxicity and price, and large Stokes shifting (>200 nm; Qing et al., 2013). Long ss-DNA templates can be easily cleaved into mono-oligonucleotides or short oligonucleotides by the $\cdot\text{OH}$, thereby reducing fluorescence signal because the synthesis of DNA-templated CuNP is damaged (Tang et al., 2006). Thus, DNA-templated CuNP are ideal signal transducers for fabricating $\cdot\text{OH}$ -dependent biosensors. To our best knowledge, fluorescence immunoassay by using $\cdot\text{OH}$ to regulate the synthesis of DNA-templated CuNP as a signal transducer for the ultrasensitive detection of foodborne pathogens has not been reported.

In the present study, the histone-ds-poly(AT) templated (**His-pAT**) CuNP were ingeniously integrated into a sandwich immunoassay platform as fluorescent signal reporter. *Salmonella choleraesuis* was selected as the model target because it is an important non-typhoidal *Salmonella* bacterium and narrowly infects humans, causing diseases in all ages. Glucose oxidase (**Gox**) was used as the substitute of HRP for hydrogen peroxide (H_2O_2) generation through glucose oxidation, and H_2O_2 was then converted into $\cdot\text{OH}$ by Fenton reagent. Owing to the high sensitivity of His-pAT CuNP growth toward $\cdot\text{OH}$, the limit of detection (**LOD**) of the proposed method reached 8.04×10^1 cfu/mL, which is approximately 1,000-fold lower than that of tetramethylbenzidine (**TMB**)-based conventional immunoassay (8.7×10^4 cfu/mL). The detection performances of the developed method were evaluated on the basis of specificity, precision, and practicability by using other common foodborne pathogens and *S. choleraesuis*-spiked milk samples. The results demonstrated that the proposed fluorescence immunoassay showed great potential for sensitive detection of *S. choleraesuis* in real milk samples.

MATERIALS AND METHODS

Materials and Apparatuses

Magnetic beads (180 nm) were purchased from Allrunnano Technology Co. Ltd. (Shanghai, China). *N*-Hydroxysuccinimide sodium salt activated long-chain biotin (**bio**) and streptavidin (**SA**) were purchased from Hualan Technology Co. Ltd. (Shanghai, China). Copper sulfate pentahydrate, iron dichloride tetrahydrate, ascorbic acid, histone (from calf thymus, type III-S), 3-(*N*-morpholino)-propanesulfonic acid (**MOPS**), TMB, BSA, HRP, Gox, and glucose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The designed ss-poly(AT)-1 (5'-AATAATAATCTATA-ATAATT-3') and ss-poly(AT)-2 (5'-AATTATTATA-

GATTATTATT-3') were commercially synthesized by Sangon Biological Engineering Technology and Service Co. Ltd. (Shanghai, China). The electron microscopy supplies were purchased from Beijing Zhongjinkeyi Technology Co. Ltd. (Beijing, China). A pair of monoclonal antibodies against *S. choleraesuis*, labeled as 11D8-D4 and 5F11-B11, were used as the detection antibodies (**dAb**) and the capture antibodies (**cAb**), respectively. Pasteurized milk was purchased from the local supermarket. The other reagents used were of analytical grade and purchased from Sinopharm Chemical Corp. (Shanghai, China). Chemicals and materials were employed without further purification. Ultrapure water was obtained from a Milli-QA apparatus (Molsheim, France). The 96-well plates (high binding and white or black) were obtained from Corning Inc. (Corning, NY). The H_2O_2 kit was purchased from Beijing ComWin Biotech Co. Ltd. (Beijing, China). Fluorescence signals were measured on Varioskan LUX (Thermo Fisher, Vantaa, Finland). Transmission electron microscopy analyses were performed on a JEM-2100 electron microscope operating at 100 kV (Tokyo, Japan).

Bacteria and Culture Conditions

Salmonella choleraesuis (ATCC 10708), *S. enteritidis* (ATCC 13076), and other common pathogenic bacterial strains, including *Escherichia coli* O157:H7 (ATCC 43888), *Listeria monocytogenes* (ATCC 13932), *Listeria welshimeri* (ATCC 35897), *Enterobacter sakazakii* (CMCC 45401), *Vibrio parahaemolyticus* (PVPA 0146), *Proteus vulgaris* (CMCC 49027), and *Bacillus subtilis* (collection), were grown in Luria-Bertani medium overnight at 37°C. The harvested bacterial solutions were continuously diluted with sterile PBS to different concentrations for the following experiments. The cell number of bacteria was determined by counting the colonies grown on the plates.

Synthesis of His-pAT CuNP

The CuNP were synthesized according to our previously described method (Xiong et al., 2018). First, the ss-poly(AT)-1 and ss-poly(AT)-2 were annealed at 90°C in MOPS buffer (10 mM MOPS and 150 mM NaCl at pH 7.6) for 10 min, then quickly cooled down to room temperature (25°C). Second, both 0.63 μM of ss-poly(AT)-1 and ss-poly(AT)-2 were mixed for the assembly of ds-poly(AT). After the solution was mixed for 1 min, 10 mg/mL of histone was added into solution for another 1 min for the reaction to form histone-ds-poly(AT) complex, which is a template for CuNP synthesis. Finally, 5 mM ascorbic acid and 2.5 mM Cu^{2+}

were sequentially added. In a typical run, the added volume of ss-poly(AT)-1, ss-poly(AT)-2, ascorbic acid, and Cu^{2+} were 20 μL individually, and the histone was 40 μL .

His-pAT CuNP-Based Fluorescence Immunoassay for *S. choleraesuis* Detection

The 96-well microplates were coated with 100 μL of cAb (2.5 $\mu\text{g}/\text{mL}$) in bicarbonate buffer (0.01 M at pH 8.6) at 4°C overnight and then blocked with 1 mg/mL of BSA solution at 37°C for 1 h. After washing 3 times with PBS with Tween 20 (PBST), 100 μL of the desired final concentrations of *S. choleraesuis* was added and incubated at 37°C for 1 h. After washing 3 times with PBST, 100 μL of biotinylated dAb (dAb@bio, 2.5 $\mu\text{g}/\text{mL}$) was added into the plates for 1 h at 37°C. After washing the plates 3 times with PBST, 100 μL of SA (1.25 $\mu\text{g}/\text{mL}$) was added. Following the 30-min reaction at 37°C and after washing the plates 3 times with PBST, 100 μL of biotinylated Gox (Gox@bio; 10 $\mu\text{g}/\text{mL}$) was added, and the plates were incubated for 45 min at 37°C. After washing the plates 4 times with PBST and twice with ultrapure water, 140 μL of the solution containing 0.5 M of glucose, 0.63 μM of ss-poly(AT)-1, and 0.5 mM of Fe^{2+} were added to each well. After reacting at 37°C for 60 min of the above solution, 20 μL of the ss-poly(AT)-2 (0.63 μM) was added to each well for 1 min hybridization, and 40 μL of histone (10 mg/mL) was added for another 1 min reaction. Finally, 20 μL of ascorbic acid (5 mM) and 20 μL of Cu^{2+} (2.5 mM) were sequentially added. The fluorescence intensity (FI) from the formed CuNP was determined (excitation and emission at 340 and 600 nm, respectively) by using a microplate reader within 10 min. A TMB-based conventional immunoassay was developed for comparison with detailed procedure provided in the Supplemental Information (<https://doi.org/10.3168/jds.2019-16472>).

Detection of *S. choleraesuis* in Spiked Milk Samples

Pasteurized milk was spiked with *S. choleraesuis* at different concentrations and detected through the proposed method. *Salmonella choleraesuis* was isolated, and its concentration was determined according to our previously developed 2-step large-volume immunomagnetic separation method (Luo et al., 2017). In brief, 10 mL of milk was added into 90 mL of 0.01 M sterilized PBS to form a 10% homogenate. The *S. choleraesuis* solutions were spiked to the above homogenates with *S. choleraesuis* to final concentrations ranging from 8.8×10^1 to 8.8×10^4 cfu/mL. Approximately 3 μg of dAb@bio was added into *S. choleraesuis*-spiked homogenate

milk. After the homogenized milk was incubated at 37°C for 1 h with gentle shaking, 700 μg of SA-modified magnetic beads was added, and the mixture solution was incubated for another 1 h at 37°C with gentle shaking. Finally, the target of *S. choleraesuis* was separated from the mixture with an external magnetic field. The separated *S. choleraesuis* was dissociated from the magnetic beads by heating at 90°C for 15 min and re-suspended in 1 mL of PBS. The supernatant containing *S. choleraesuis* was analyzed by the proposed method.

RESULTS AND DISCUSSION

Principle of Fluorescence Immunoassay for *S. choleraesuis* Detection

The detailed principle of the proposed fluorescence immunoassay is depicted in Figure 1. The anti-*S. choleraesuis* monoclonal antibody and biotinylated anti-*S. choleraesuis* monoclonal antibody were employed as the cAb and dAb, respectively. The Gox@bio was used as an alternative for the HRP enzyme. The SA-bio system was used as a bridge to connect dAb@bio and Gox@bio. When *S. choleraesuis* was present in the sample solution, Gox was immobilized on the plate wells by the SA-bio system. Glucose was oxidized by Gox for H_2O_2 production, and H_2O_2 was converted into $\cdot\text{OH}$ with Fenton reagent (Fe^{2+}). Then, ss-poly(AT)-1 was cleaved into mono-oligonucleotides or short oligonucleotides by $\cdot\text{OH}$, resulting in a weak FI on the plate wells because of a failed ds-poly(AT) template for CuNP synthesis. Decrease in H_2O_2 generation increased His-pAT CuNP synthesis and high FI. The concentration of *S. choleraesuis* can be quantitatively determined by recording the fluorescence retention of His-pAT CuNP because *S. choleraesuis* concentration is inversely proportional to the amount of His-pAT CuNP.

His-pAT CuNP-Based Fluorescent Signal Transducer

The His-pAT CuNP were synthesized by using a ds-poly(AT) DNA as template, ascorbic acid was used for reducing Cu^{2+} to CuNP, and histone was used for enhancing the FI and stability of the pAT CuNP. The optimum concentrations of ds-poly(AT), ascorbic acid, Cu^{2+} , and histone for His-pAT-CuNP synthesis were based on our previous report (Xiong et al., 2018). Figure 2A shows that the as-prepared His-pAT CuNP showed a bright red fluorescence under UV lamp excitation at 365 nm. In the fluorescence spectra of the His-pAT CuNP, the maximum excitation and emission wavelengths were 340 and 600 nm, respectively (Figure 2B). The transmission electron microscopy images indi-

cated that most of CuNP are approximately spherical with good mono-dispersity (Figure 2C).

The sensitivity of His-pAT CuNP growth to $\cdot\text{OH}$ was evaluated by incubating ss-poly(AT)-1 with different concentrations of H_2O_2 (0–50 μM) in the presence of 0.5 mM Fe^{2+} . Fluorescence retention of resultant His-pAT CuNP was calculated with the formula $F/F_0 \times 100\%$, where F and F_0 represent the fluorescence intensities of His-pAT CuNP solution with or without the desired H_2O_2 concentration, respectively. Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-16472>) indicates that the fluorescence retention of the obtained His-pAT CuNP decreased sharply as the H_2O_2 concentration increased from 0 to 12.5 μM and then plateaued when the H_2O_2 concentration further increased to 50 μM . The inset in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-16472>) shows that the change in the fluorescence retention of formed His-pAT CuNP displayed a favorable linearity against the logarithm of H_2O_2 concentration ranging from 0.39 to 12.5 μM . The LOD of the formed His-pAT CuNP to the H_2O_2 was 0.15 μM , demonstrating the growth of His-pAT CuNP was extremely sensitive to the $\cdot\text{OH}$. We then further

investigated the feasibility of using Gox to regulate His-pAT CuNP synthesis by mixing His-pAT CuNP growing system with different Gox concentrations under 0.5 M of glucose. Figure 2D shows that the fluorescence retention of formed His-pAT CuNP decreased when Gox concentration increased from 0 to 44.64 fM and then reached a plateau with the further increase in Gox. The fitted linear equation can be described as $y = -19.43\ln(x) + 61.27$ ($R^2 = 0.9803$). The LOD of Gox to the fluorescence retention of His-pAT CuNP was calculated to be 0.23 fM. The LOD value was defined as the lowest Gox concentration that generated evident fluorescence retention (90%) and was approximately 30-fold lower than that of HRP to TMB (Supplemental Figure S2; <https://doi.org/10.3168/jds.2019-16472>). The above results indicated that an ultralow Gox concentration could produce sufficient amounts of H_2O_2 and lead to a remarkable fluorescence signal variation by disrupting the His-pAT CuNP synthesis. These results suggested that the Gox regulating the synthesis of His-pAT CuNP as a signal transducer can improve the sensitivity of sandwich immunoassay for *S. choleraesuis* detection.

Figure 1. The principle of the proposed fluorescence immunoassay. Gox@bio = biotinylated glucose oxidase; dAb@bio = biotinylated detection antibodies; cAb = capture antibodies; *S. choleraesuis* = *Salmonella choleraesuis*; CuNP = copper nanoparticles; SA = streptavidin; ASA = ascorbic acid; hv = irradiation with light; λ_{em} = wavelength emission.

Development of Fluorescence Immunoassay Based on His-pAT CuNP as Signal Transducers

To obtain the best performance of sandwich fluorescence immunoassay, we optimized various parameters that can affect detection sensitivity for *S. choleraesuis*, including cAb, dAb@bio, Gox@bio, and SA concentrations. The detailed preparation of dAb@bio and Gox@bio is shown in the Supplemental Information (<https://doi.org/10.3168/jds.2019-16472>).

The synthesized dAb@bio and Gox@bio were characterized by adding different concentrations of dAb@bio and Gox@bio (0–4 $\mu\text{g/mL}$) into the SA coated plate wells. Supplemental Figures S3A and 3B (<https://doi.org/10.3168/jds.2019-16472>) show that the optical density values increased with increased concentrations of dAb@bio and Gox@bio, indicating that biotin was successfully coupled on the dAb and Gox molecules, respectively. The concen-

Figure 2. Characterization of histone-ds-poly(AT)-templated copper nanoparticles (His-pAT CuNP). (A) Photographs of His-pAT CuNP solution under UV light (365 nm, left: without Cu^{2+} , right: with Cu^{2+}). (B) Excitation and emission spectra of His-pAT CuNP. (C) Left: transmission electron microscopy (TEM) images of His-pAT CuNP; right: high-resolution TEM images of His-pAT CuNP. (D) Sensitivity of glucose oxidase (Gox) toward the His-pAT CuNP synthesis. The Gox was incubated with 0.5 M of glucose, 0.5 mM of Fe^{2+} , and 0.63 μM of ss-poly(AT)-1 for 1 h at 37°C before the typical synthesis run of His-pAT CuNP. F_0 and F represented the fluorescence intensities (FI) in the absence and presence of the desired concentration of Gox, respectively. The limit of detection (LOD) of Gox toward the His-pAT CuNP synthesis was defined as the lowest concentration of Gox that FI retained 90% of F_0 . The error bars represent the SD of 3 measurements. a.u. = arbitrary units.

trations of cAb and dAb@bio were optimized by the “chessboard titration” method with different cAb concentrations under a series concentration of dAb@bio on the plate well. The fluorescence retention was used to confirm the optimum parameters. The *S. choleraesuis* concentration for the evaluation of fluorescence retention was set as 8.8×10^7 cfu/mL. Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-16472>) shows that the fluorescence retention gradually decreased

Figure 3. Optimization of the experimental conditions for fluorescence immunoassay. (A) Optimization of biotinylated glucose oxidase (Gox@bio) concentration. The streptavidin (SA) concentration was set as 0.5 μg/mL. (B) Optimization of SA concentration. F_0 and F represented the fluorescence intensities (FI) in the absence and presence of 8.8×10^7 cfu/mL of *Salmonella choleraesuis*, respectively. The error bars represent the SD of 3 measurements.

Figure 4. (A) Quantitative assay of *Salmonella choleraesuis* by using the developed fluorescence immunoassay in PBS solution with *S. choleraesuis* concentration ranging from 8.8×10^0 to 8.8×10^8 cfu/mL. F_0 and F represented the fluorescence intensities (FI) in the absence and presence of the desired *S. choleraesuis* concentration, respectively. LOD = limit of detection. (B) Specificity of the developed fluorescence immunoassay. The concentrations of *S. choleraesuis* was at 8.8×10^6 cfu/mL, whereas other pathogenic bacteria strains were 1×10^8 cfu/mL. Control is the sterile PBS solution. The error bars represent the SD of 3 measurements.

with increased cAb and dAb@bio concentration from 0.31 to 2.5 µg/mL and 0.31 to 2.5 µg/mL, respectively. Thus, 2.5 µg/mL of cAb and dAb@bio was regarded as the optimal concentration. The SA-bio system was used to bridge dAb@bio with Gox@bio. The concentration of Gox@bio and SA were also optimized by determining fluorescence retention for the detection of *S. choleraesuis* at 8.8×10^7 cfu/mL. Figure 3A shows that fluorescence retention decreased from 44.73 to 28.87% when Gox@bio increased from 0.04 to 5 µg/mL and then plateaued when Gox@bio concentrations further increased. Therefore, 5 µg/mL of Gox@bio was used for subsequent experiments. Figure 3B shows that the fluorescence retention decreased from 82 to 18% with the SA increasing from 0.16 to 1.25 µg/mL and then reached a plateau by further increasing SA concentrations. Thus, 1.25 µg/mL of SA was considered the optimal amount for producing the best detection signal.

Under optimal conditions, the sensitivity of the proposed fluorescence immunoassay was evaluated by detecting different concentrations of *S. choleraesuis* (from 8.8×10^0 to 8.8×10^8 cfu/mL). The calibration curve was constructed by plotting the fluorescence retention with $F/F_0 \times 100\%$ values against the logarithmic concentration of *S. choleraesuis*. As presented in Figure 4A, the regression equation of the proposed method for quantitative *S. choleraesuis* detection can be described as $y = -2.836\ln(x) + 88.379$ ($R^2 = 0.9935$), with a good linear detection ranging from 8.8×10^1 to 8.8×10^6 . In the equation, y is fluorescence retention, and x is *S. choleraesuis* concentration. The error bars were based on triplicate measurements. The LOD was calculated as 8.04×10^1 cfu/mL in accordance with the mean of the determined fluorescence retention of blank samples plus a 3-fold standard deviation. This value is 1,000 times lower than that of the conventional TMB-based ELISA (8.7×10^4 cfu/mL, Supplemental Figure S4; <https://doi.org/10.3168/jds.2019-16472>). Compared with other recently developed methods for *Salmonella* detection,

the proposed method is significantly better and slightly lower than that of quantum dot-based fluorescence and electrochemical response-based immunoassays, respectively (Table 1).

Specificity was determined by evaluating the cross-reaction of the proposed fluorescence immunoassay with *S. choleraesuis*, *S. enteritidis*, and other common pathogenic bacterial strains, including *E. coli* O157:H7, *L. monocytogenes*, *L. welshimeri*, *E. sakazakii*, *V. parahaemolyticus*, *P. vulgaris*, and *B. subtilis*. Sterile PBS was used as the negative control. Compared with the negative control, a lower fluorescence retention was observed when the *S. choleraesuis* concentration was 8.8×10^6 cfu/mL, whereas no significant differences of fluorescence retention were observed when other non-target bacteria were detected at concentrations of 1×10^8 cfu/mL (Figure 4B). These findings demonstrate the high selectivity of the proposed fluorescence immunoassay for *S. choleraesuis*.

The accuracy and precision of our proposed method were evaluated by analyzing 4 different concentrations (8.8×10^1 , 8.8×10^2 , 8.8×10^3 , and 8.8×10^4 cfu/mL) of *S. choleraesuis*-spiked pasteurized whole milk samples. The effects of sample matrix were removed through the 2-step large-volume immunomagnetic separation method developed by our group for the isolation and enrichment of *S. choleraesuis* from milk samples before fluorescence analysis. Table 2 shows that average recoveries for intra- and inter-assay ranged from 73.52 to 96.59% and 66.99 to 98.24% with a coefficient of variation ranging from 6.85 to 31.26% and 5.46 to 17.99%, respectively. These results signified that the proposed fluorescence immunoassay method can be used for detection of *S. choleraesuis* in real milk samples.

CONCLUSIONS

We demonstrated that His-pAT CuNP can be used as signal outputs of immunoassay for the sensitive de-

Table 1. Comparison of the sensitivity of different methods for *Salmonella* detection

Method	Signal output	Linear range (cfu/mL)	LOD ¹ (cfu/mL)	Reference
Fluorescence immunoassay	Quantum dot-based fluorescence response	2.50×10^3 to 1.95×10^8	500	Kuang et al., 2013
Aptamer-based assay	Electrochemical response	2.4×10^0 to 2.4×10^3	3	Ma et al., 2014
Microfluidic immunoassay	Quantum dot-based fluorescence response	0 to 10^6	1,000	Kim et al., 2015
Microfluidic immunoassay	Electrochemical response	10^1 to 10^7	1.56	Singh et al., 2018
Aptamer-based assay	Electrochemical response	6.5×10^2 to 6.5×10^8	1	Ranjbar et al., 2018
Phage-based assay	Capacitive response	2×10^2 to 1×10^7	200	Niyomdech et al., 2018
Fluorescence immunoassay	Copper nanoparticle-based fluorescence response	8.8×10^1 to 8.8×10^6	80.4	This work

¹LOD = limit of detection.

Table 2. Recovery and precision of the proposed fluorescence immunoassay

<i>Salmonella choleraesuis</i> added (cfu/mL)	<i>S. choleraesuis</i> detected (cfu/mL)	Recovery (%)	RSD ¹ (%)
Proposed fluorescence immunoassay, intra-day assay (n = 3)			
8.8 × 10 ¹	6.47 × 10 ¹	73.52	20.56
8.8 × 10 ²	7.82 × 10 ²	88.86	31.26
8.8 × 10 ³	7.09 × 10 ³	80.57	6.85
8.8 × 10 ⁴	8.50 × 10 ⁴	96.59	9.06
Proposed fluorescence immunoassay, inter-day assay (n = 3)			
8.8 × 10 ¹	5.90 × 10 ¹	66.99	9.75
8.8 × 10 ²	7.11 × 10 ²	80.80	9.99
8.8 × 10 ³	8.64 × 10 ³	98.24	17.99
8.8 × 10 ⁴	8.06 × 10 ⁴	91.59	5.46

¹RSD = relative standard deviation.

tection of foodborne pathogens. Given the ultra-high sensitivity of His-pAT CuNP synthesis toward ·OH, the proposed fluorescence immunoassay exhibited outstanding sensitivity for the detection of *S. choleraesuis*, having an LOD of 8.04 × 10¹ cfu/mL. This result is 3 orders of magnitude lower than that obtained by TMB-based traditional immunoassay. The proposed immunoassay showed excellent specificity, good precision, and suitable practicability, and His-pAT CuNP exhibited good stable fluorescence signal in the whole process of immunoassay. In brief, our developed fluorescence immunoassay provided a new potential for developing rapid and highly sensitive detection in food safety control, environment monitoring, and clinical diagnosis.

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