Comparison of 4 label-based immunochromatographic assays for the detection of *Escherichia coli* O157:H7 in milk

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ABSTRACT

Immunochromatographic assays (ICA) are widely used to detect pathogens. In this study, we used traditional gold nanoparticles (GNP), quantum dots (QD), fluorescent nanoparticles (FNP), and europium (Eu) (III) chelate nanoparticles (EuNP) as ICA labels. We first compared the ability of the 4 ICA test strips to quantitatively detect *Escherichia coli* O157:H7 in milk. We then optimized various parameters influencing the ICA. The sensitivity to *E. coli* O157:H7 of the GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA was 2.5×10^4 , 5×10^3 , 1.0×10^3 , and 5.0×10^2 cfu mL⁻¹, respectively. The EuNP-ICA exhibited the highest sensitivity. The amounts of monoclonal antibodies (mAb) per GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA test strip were 0.16, 0.37, 0.04, and 0.10 µg, respectively. The corresponding coefficients of variation were 7.4 to 15.8%, 10.4 to 18.6%, 2.7 to 7.8%, and 6.9 to 10.5%, respectively. The FNP-ICA required the least mAb per test strip and had the best coefficient of variation. The linear ranges of GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA were 1.0×10^4 to 1.0×10^6 , 2.5×10^3 to 1.0×10^6 , 2.5×10^2 to 2.5×10^5 , and 2.5×10^2 to 2.5×10^5 cfu mL⁻¹, respectively. The FNP-ICA and EuNP-ICA had wider linear ranges than GNP-ICA and QD-ICA. Additionally, the FNP-ICA and EuNP-ICA showed better tolerance than GNP-ICA and QD-ICA in the milk samples. The FNP-ICA and EuNP-ICA showed remarkable potential for detection of pathogens in milk. **Key words:** gold nanoparticle, quantum dot, fluorescent nanoparticle, Eu (III) chelate nanoparticle

INTRODUCTION

Pathogens are the major etiological agents of diseases related to dairy product consumption, posing great

threat to public health (Guimarães et al., 2017; Taponen et al., 2017). More than 90% of the diseases related to dairy product consumption are caused by bacteria (Franco et al., 2013). The World Health Organization has emphasized the need to control and prevent the spread of pathogens associated with food consumption (World Health Organization, 2015). Traditional culture and colony-counting methods (Brichta-Harhay et al., 2007) are standard approaches for detecting pathogens, but these methods are inconvenient, because they require considerable time and effort and highly trained personnel. A simple and sensitive pathogen detection method is needed to ensure food safety. The feasibility of methods such as PCR (Bian et al., 2015), surface plasmon resonance (Wang et al., 2013), gene chip (Jiawang et al., 2016), electrochemical strategy (Barreiros dos Santos et al., 2013), and ELISA (Shan et al., 2016) for detecting pathogens has been extensively studied. These methods are more sensitive than traditional detection approaches, but their complex procedures, expensive equipment, and extensive analysis time also restrict their application in the onsite detection of pathogens.

The immunochromatographic assay (ICA) is a rapid, simple, sensitive, specific, user-friendly, affordable detection method (Ju et al., 2016). Gold nanoparticles (GNP) are widely used as labels to detect pathogens, including *Vibrio parahaemolyticus* (Liu et al., 2016), *Escherichia coli* O157:H7 (Zhang et al., 2015), *Salmonella typhi* (Preechakasedkit et al., 2012), and *Listeria monocytogenes* (Ueda et al., 2013). However, traditional GNP-based ICA (GNP-ICA) are limited by their relatively low sensitivity (Liu et al., 2011). Novel ICA based on photoluminescent nanoparticles have been developed to improve sensitivity using quantum dots (QD; Xu et al., 2013; Morales-Narváez et al., 2015), fluorescent nanoparticles (FNP; Zhang et al., 2017), and europium (III) chelate nanoparticles (EuNP; Zhang et al., 2014a) as labels to detect pathogens. The advantages of QD are broad adsorption, size-tunable emission in the visible-near infrared range, high photostability, and strong luminescence (Hildebrandt et al.,

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2017). The advantages of FNP are high fluorescence intensity and photostability (Zhou et al., 2014). The advantages of EuNP are excellent photoluminescence properties, including high emission fluorescence intensity, large Stokes shift, sharp emission profile, and long emission fluorescence lifetime (Lai et al., 2016).

In this study, we applied GNP, QD, FNP, and EuNP as labels in a sandwich ICA system to quantitatively detect pathogens in milk. We selected *E. coli* O157:H7 as the target analyte. To our knowledge, direct comparisons of these 4 ICA labels to detect pathogens in milk have not yet been reported.

MATERIALS AND METHODS

Materials and Instruments

Carboxylic acid-modified CdSSe/ZnS QD (excitation wavelength = 365 nm, emission wavelength = 610 nm) were purchased from Nanjing Technology Co., Ltd. (Hangzhou, China). Fluorescein isothiocyanate (FITC) FNP (excitation wavelength = 470 nm, emission wavelength = 525 nm) were purchased from Merck Co. (Darmstadt, Germany); EuNP (excitation wavelength = 365 nm, emission wavelength = 615 nm) were purchased from Weice Biotech Co. (Nanjing, China). Anti-*E. coli* O157:H7 monoclonal antibody (mAb) and rabbit anti-*E. coli* O157:H7 polyclonal antibody (pAb) were purchased from Meridian Life Science, Inc. (Memphis, TN). Nitrocellulose (NC) membrane, absorbent pad, sample pad, and conjugate pad were purchased from Millipore (Bedford, MA). Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.9%), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC-HCl), *N*-hydroxysulfosuccinimide (sulfo-NHS), and BSA from Sigma-Aldrich Chemical Co. (St. Louis, MO). We obtained glucosamine hydrochloride (glu-NH₂) from Aladdin Chemistry Co. Ltd. (Shanghai, China). Milk was purchased from the Rainbow Supermarket in Nanchang, China. All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

The 4 portable strip readers for the GNP-immuno-chromatographic test strip (ICTS, absorption wavelength = 525 nm), QD-ICTS (excitation wavelength = 365 nm, emission wavelength = 610 nm), FNP-ICTS (excitation wavelength = 470 nm, emission wavelength = 540 nm), and EuNP-ICTS (excitation wavelength = 365 nm, emission wavelength = 610 nm) were all obtained from Fenghang Scientific Instrument Co., Ltd. (Zhejiang, China). We used the delayed time-resolved mode of the EuNP-ICTS portable strip reader with a 200- μs delay time and a 400- μs counting window. The

BioDot XYZ platform, equipped with a motion controller for solution dispensing, was supplied by BioDot (Irvine, CA).

Bacterial Strains

We used *E. coli* O157O157:H7 (ATCC 43888) and 14 non-*E. coli* O157O157:H7 strains: *Listeria welshimeri* (ATCC 35897), *Listeria innocua* (ATCC 33090), *L. monocytogenes* (ATCC 13932), *Micrococcus luteus* (CMCC 28001), *Shigella sonnei* (CMCC 51592), *Shigella flexneri* (CMCC 2457), *Proteus vulgaris* (CMCC 49027), *Enterobacter sakazakii* (CMCC 45407), *Bacillus subtilis* (BD 366), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (CMCC 11997), *Salmonella choleraesuis* (CICC 21493), *S. choleraesuis* (ATCC 10708), and *E. coli* (CMCC 44102). All strains were preserved in our laboratory.

Preparation of GNP

The GNP were prepared according to a previously described citrate reduction method (Li et al., 2013). Gold chloride trihydrate solution [100 mL, 0.01% (wt/vol)] in ultra-pure water was heated to boiling. Then, 1.45 mL of 1% (wt/vol) sodium citrate solution was added under constant stirring. The solution was maintained at a boiling temperature for 5 min and then stored at 4°C for further use.

Preparation of Labeled mAb

To prepare the GNP-mAb, the pH of the colloidal gold solution was adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0 with 0.1 M HCl or 0.2 M K₂CO₃. Then, anti-*E. coli* O157:H7 mAb (1.0, 4.0, 7.0, or 10.0 $\mu\text{g mL}^{-1}$) were added dropwise to 1 mL of the GNP solution to form the GNP-mAb complex. The solution was agitated for 60 min. Then, 100 μL of blocking solution containing 10% (wt/vol) BSA was added to the mixture and agitated for 30 min. Finally, the resulting mixture was centrifuged at $6,948 \times g$ for 30 min at 4°C. The precipitate was dissolved in 0.1 mL of PBS solution containing 5% sucrose, 2% trehalose, 1% polyethylene glycol 20,000, 1% BSA, and 0.25% Tween 20.

To prepare the QD-mAb, 10 μg of QD was added to 200 μL of 0.05 M borate buffer (pH 4.0, 5.0, 6.0, 7.0, or 8.0) containing 1.25 μg of EDC-HCl and 0.31 μg of sulfo-NHS for 30 min to activate the carboxyl group of QD. Then, anti-*E. coli* O157:H7 mAb (23.1, 46.2, 69.3, or 92.4 $\mu\text{g mL}^{-1}$) was added dropwise to the mixture to form the QD-mAb complex. The resulting mixture was agitated for 15 min. Then, 35 μL of blocking solution

containing 1% (wt/vol) glu-NH₂ was added, and the solution was agitated for 45 min. Then, 75 μ L of 10% (wt/vol) glu-NH₂ was added to the solution. Finally, the resulting mixture was centrifuged at $25,038 \times g$ for 10 min at 4°C. The precipitate was dissolved in 200 μ L of 0.01 M PBS at pH 7.4 containing 20% glycerin (vol/vol).

To prepare the FNP-mAb, we used the EDC-HCl-mediated method as previously reported, with some modifications (Liu et al., 2015). The FNP (50 μ g) were added to 1 mL of 0.02 M phosphate buffer (pH 4.0, 5.0, 6.0, 7.0, or 8.0) containing 4.25 μ g of EDC-HCl to activate the carboxyl groups of FNP. Anti-*E. coli* O157:H7 mAb (0.4, 0.7, 1.0, or 3.0 μ g mL⁻¹) was added dropwise to the mixture to form the FNP-mAb complex. The solution was agitated for 2 h. Then, the FNP-mAb solution was blocked with 100 μ L of blocking solution containing 10% (wt/vol) BSA. Finally, the mixture was separated by centrifugation at $10,005 \times g$ for 10 min at 4°C. The precipitate was dissolved in 100 μ L of 0.02 M Na₂HPO₄ containing 1% BSA (wt/vol), 5% sucrose (wt/vol), 3% trehalose (wt/vol), 1% Tween 20 (vol/vol), and 0.1% NaN₃ (wt/vol) at pH 7.4.

To prepare EuNP-mAb, 50 μ g of EuNP were added to 500 μ L of 0.05 M borate buffer (pH 4.0, 5.0, 6.0, 7.0, or 8.0) containing 1.75 μ g of EDC-HCl to activate the carboxyl of EuNP. Anti-*E. coli* O157:H7 mAb (5, 10, 15, or 20 μ g mL⁻¹) was added dropwise to the mixture to form the EuNP-mAb complex. The solution was agitated for 2 h. Then, the EuNP-mAb solution was blocked with 50 μ L of 0.05 M borate buffer containing 10% (wt/vol) BSA and 1% (vol/vol) Tween 20 at pH 7.0. Finally, the mixture was centrifuged at $17,788 \times g$ for 20 min at 4°C. The precipitate was dissolved at pH 7.0 in 100 μ L of 0.05 M borate buffer containing 0.2% (wt/vol) BSA and 0.5% (vol/vol) Tween 20.

Characterization of Labels and Label-mAb Complex

The morphology and size of the 4 labels (GNP, QD, FNP, and EuNP) were analyzed by transmission electron microscope (JEM 2100; Jeol, Tokyo, Japan) and a scanning electron microscope (S-4800; Hitachi, Tokyo, Japan). The UV-visible absorption spectra of GNP were recorded using a UV-visible spectrophotometer (Genesys 10S, Thermo Scientific, Waltham, MA). The fluorescence spectra of QD, FNP, and EuNP were recorded using a fluorescence spectrophotometer (F380; Guangdong Scientific Ltd., Tianjin, China). The dynamic light scattering of the 4 labels and the label-mAb complexes was analyzed using a particle size analyzer (Nano-ZS; Malvern Instruments Ltd., Malvern, UK).

Preparation of ICTS

The ICTS consisted of a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad (Figure 1). The sample pads were pretreated with 20 mM sodium borate buffer [(pH 8.0) containing 1.0% (wt/vol) BSA, 0.05% (wt/vol) NaN₃, and 0.5% Tween 20] and then dried at 60°C for 2 h. The pAb to *E. coli* O157:H7 and goat anti-mouse IgG were sprayed on the nitrocellulose membrane as test and control lines, respectively, and the membranes were dried at 37°C for 16 h. The sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad were assembled, cut into strips 4 mm wide, and packaged in foil bags.

Detection of 4 ICA

The label-mAb complexes were premixed with 100 μ L of sample in ELISA microplate wells for 5 min, and then the mixture was added to the sample pad of the ICTS (Figure 1). After a period of reaction, the absorbance of the test line (**A_T**) was recorded by the GNP-ICTS portable strip reader to quantitatively determine *E. coli* O157:H7, and the fluorescent intensity of the test line (**FI_T**) was recorded by the QD-ICTS, FNP-ICTS, and EuNP-ICTS portable strip readers to quantitatively determine *E. coli* O157:H7. The emission fluorescence signal of the EuNP-ICA was recorded after a 200- μ s delay and a 400- μ s counting window.

Immunological Kinetics Analysis of 4 ICA

The immunological kinetics of GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA were studied for 30, 60, 40, and 30 min, respectively. The signal intensity of the test line was recorded every 30 s. The concentration of *E. coli* O157:H7 was 10⁵ cfu mL⁻¹. The kinetics reaction curve was established by plotting the signal intensity of the test line against the immunoreaction time.

Standard Calibration Curve of 4 ICA

The standard calibration curve was established by plotting the logarithm of A_T or FI_T against the logarithm of the *E. coli* O157:H7 concentration. Different *E. coli* O157:H7 concentrations (0, 1.0 $\times 10^2$, 2.5 $\times 10^2$, 5.0 $\times 10^2$, 1.0 $\times 10^3$, 2.5 $\times 10^3$, 5.0 $\times 10^3$, 1.0 $\times 10^4$, 2.5 $\times 10^4$, 5.0 $\times 10^4$, 1.0 $\times 10^5$, 2.5 $\times 10^5$, 5.0 $\times 10^5$, and 1.0 $\times 10^6$ cfu mL⁻¹) were prepared by diluting 10⁸ cfu mL⁻¹ of *E. coli* O157:H7 with 0.01 M PBS (pH 7.4). Sensitivity was defined as the mean plus 3-fold

standard deviation (SD) of the negative sample (Oh et al., 2009).

Specificity of 4 ICA

The specificity of the 4 ICA was estimated by testing 15 bacterial strains, including the *E. coli* O157:H7 (10^6 cfu mL⁻¹) target strain and 14 non-*E. coli* O157:H7 strains (10^6 cfu mL⁻¹). A negative test was performed with sterile PBS solution. The experiment was repeated in triplicate.

Analysis of Milk Sample

We added 0.1 mL of *E. coli* O157:H7 to 1 mL of milk (1.0×10^4 , 2.5×10^4 , 5.0×10^4 , 1.0×10^5 , and 2.5×10^5 cfu per mL of milk). The spiked milk was then homogenized for 1 min. The accuracy and precision of the 4 ICA were evaluated by analyzing recovery and coefficient of variation (CV). The experiment was repeated in triplicate. Milk samples spiked with *E. coli* O157:H7 at a concentration of 5.0×10^4 , 1.0×10^5 , or 2.5×10^5 cfu mL⁻¹ were quantitatively analyzed using

Figure 1. Schematic illustration of the 4 label-based immunochromatographic assays (ICA) used to quantitatively analyze *Escherichia coli* O157:H7. When the target *E. coli* O157:H7 was included in the sample, the label-monoclonal antibody (mAb) bound with *E. coli* O157:H7 to form the label-mAb-*E. coli* O157:H7 immunocomplex, which then migrated across the nitrocellulose (NC) membrane. The immunocomplex was then captured by polyclonal antibody (pAb) and goat anti-mouse IgG to form signals on both test and control lines. When *E. coli* O157:H7 was excluded from the sample, the label-mAb could not be captured by the pAb to form the sandwich immunocomplex, resulting in an absence of the test line signal. GNP = gold nanoparticle; QD = quantum dot; FNP = fluorescent nanoparticle; EuNP = europium (III) chelate nanoparticle. Color version available online.

the GNP-ICA and QD-ICA. Milk samples spiked with *E. coli* O157:H7 at a concentration of 1.0×10^4 , 2.5×10^4 , or 5.0×10^4 cfu mL⁻¹ were quantitatively analyzed using the FNP-ICA and EuNP-ICA.

Different *E. coli* O157:H7 concentrations (0, 1.0×10^2 , 2.5×10^2 , 5.0×10^2 , 1.0×10^3 , 2.5×10^3 , 5.0×10^3 , 1.0×10^4 , 2.5×10^4 , 5.0×10^4 , 1.0×10^5 , 2.5×10^5 , 5.0×10^5 , and 1.0×10^6 cfu mL⁻¹) were prepared by diluting 10^8 cfu mL⁻¹ of *E. coli* O157:H7 in sterile PBS solution or milk. The spiked samples were then homogenized for 1 min. The standard calibration curves of the 4 ICA of sterile PBS solution and milk samples were established by plotting the logarithm of A_T or FI_T against the logarithm of *E. coli* O157:H7 concentration. The matrix effect of milk on the sensitivity of

the 4 ICA was identified by comparing the differences between the standard calibration curves in sterile PBS solution and milk.

RESULTS AND DISCUSSION

Characterization of Free Labels and Label-mAb Complexes

We characterized the morphology and size of the 4 labels (GNP, QD, FNP, and EuNP) using a transmission electron microscope (Figures 2A to D) and a scanning electron microscope (Figures 2E to H). The transmission and scanning electron micrographs showed that GNP, QD, FNP, and EuNP were relatively

Figure 2. Characterization of the 4 labels. Transmission electron micrographs of (A) gold nanoparticle (GNP); (B) quantum dot (QD); (C) fluorescent nanoparticle (FNP); and (D) europium (III) chelate nanoparticle (EuNP). Scanning electron micrographs of (E) GNP; (F) QD; (G) FNP; and (H) EuNP. The UV-visible absorption spectrum of (I) GNP and the fluorescence spectra of (J) QD; (K) FNP; and (L) EuNP.

uniform in size and had average diameters of 21, 11, 182, and 155 nm, respectively. Figures 2C and D (inset) illustrated that numerous fluorescein isothiocyanate molecules and Eu (III) chelates were tightly embedded in the polystyrene matrices of FNP and EuNP. Figure 2I showed that the UV-visible absorption spectrum of GNP exhibited a maximum surface plasmon resonance absorption at 521 nm at concentrations of 0.5 mg mL⁻¹. Fluorescence spectra showed that the maximum excitation wavelength (λ_{ex}) and maximum emission wavelength (λ_{em}) of QD, FNP, and EuNP (Figure 2 J to L) were 397.2 and 617.6, 468.2 and 527.4, and 373.6 and 616.2 nm, respectively, at concentration of 0.5 mg mL⁻¹. The maximum emission fluorescence

intensities were 3,655, 2,115, and 4,397 for QD, FNP, and EuNP, respectively. The EuNP had the highest fluorescence intensity. This characteristic contributed remarkably to improvements in the sensitivity of the ICA. Furthermore, the Stokes shifts of QD, FNP, and EuNP were 220.4, 59.2, and 242.6, respectively, and the corresponding emission profiles with the full width at half-maximum were 37.8, 63.9, and 10 nm. The EuNP showed the largest Stokes shift and sharpest emission profile, facilitating discrimination against light scattering background autofluorescence associated with QD and FNP (Zhang et al., 2014b). Finally, EuNP had the longest emission fluorescence lifetime (1 ms) compared with QD (12 to 28 ns; Labeau et al., 2003) and FNP

Figure 3. Hydrodynamic diameters of (A) gold nanoparticle (GNP) and GNP-monoclonal antibody (mAb) (GNP-mAb); (B) quantum dot (QD) and QD-mAb; (C) fluorescent nanoparticle (FNP) and FNP-mAb; and (D) europium (III) chelate nanoparticle (EuNP) and EuNP-mAb.

(<10 ns; Santra et al., 2006). This characteristic could also eliminate background autofluorescence, resulting in higher sensitivity (Yang et al., 2016).

We formed the GNP-mAb by electrostatic adsorption between GNP and mAb, but synthesized the QD-mAb, FNP-mAb, and EuNP-mAb by coupling the carboxyl group of the labels with the amino group of the mAb using the EDC·HCl-mediated method. We compared the dynamic light scattering results of the 4 labels and label-mAb complexes to confirm that the mAb were coupled on the surface of the labels. Figure 3 shows that the hydrodynamic diameters of GNP, QD, FNP, and EuNP were 23 ± 0.1 , 55 ± 1.1 , 206 ± 1.3 , and 178 ± 4.2 nm, respectively. The hydrodynamic diameters of GNP-mAb, QD-mAb, FNP-mAb, and EuNP-mAb were 76 ± 1.8 , 133 ± 1.3 , 227 ± 3.1 , and 190 ± 1.9 nm, respectively. These results indicated that the mAb were successfully coupled on the surface of the 4 labels. Additionally, we studied the polydispersity index (PDI) to analyze the size distribution of the 4 labels and label-mAb complexes (Figure 4). The PDI of GNP, QD, FNP, and EuNP were 0.24 ± 0.01 , 0.35 ± 0.04 , 0.06 ± 0.01 , and 0.04 ± 0.01 nm, respectively. The PDI of GNP-mAb, QD-mAb, FNP-mAb, and EuNP-mAb increased to 0.26 ± 0.01 , 0.36 ± 0.01 , 0.11 ± 0.03 , and 0.10 ± 0.03 nm, respectively. A lower PDI indicated better size distribution and resulted in better sensitivity to the ICA (Cui et al., 2015). The EuNP and EuNP-mAb showed the lowest PDI, which created better conditions for the application of the label in the ICA.

Optimization of ICA

We labeled the 4 ICA under different pH values (4.0, 5.0, 6.0, 7.0, and 8.0) and analyzed them by studying the signal intensities of their test lines. We prepared sterile PBS solution without *E. coli* O157:H7 (negative sample) and sterile PBS solution spiked with 10^5 cfu mL⁻¹ of *E. coli* O157:H7 (positive sample). For GNP-ICA, when the label pH was adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0, the A_T of the positive sample were 544 ± 10 , 984 ± 91 , $1,224 \pm 217$, 317 ± 22 , and 218 ± 48 , respectively (Supplemental Figure S1A; <https://doi.org/10.3168/jds.2017-12554>). We determined that the optimal pH for GNP-ICA was 6.0 because of the strong A_T of the positive sample and the low A_T of the negative sample. As labeling pH increased, the $-\text{COOH}$ groups on the surfaces of the carboxylic acid-modified photoluminescent label-based ICA (QD-ICA, FNP-ICA, and EuNP-ICA) gradually transformed into $-\text{COO}^-$. The optimal labeling pH values for QD-ICA, FNP-ICA, and EuNP-ICA were pH 6.0, 5.0, and 6.0, respectively, because of the strong FI_T of the positive sample and the

low FI_T of the negative sample (Supplemental Figure S1B–D).

We also investigated the effect of the amount of mAb on the signal intensity of the proposed assay. We analyzed the 4 ICA labeled with different amounts of mAb by studying the signal intensities of their test lines. We prepared sterile PBS solution without *E. coli* O157:H7 (negative sample) and sterile PBS solution spiked with 10^6 cfu mL⁻¹ of *E. coli* O157:H7 (positive sample). The optimal labeling amounts of mAb for GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA were 4.0, 46.2, 1.0, and $10.0 \mu\text{g mL}^{-1}$, respectively, because of the strong A_T or FI_T of the positive sample and the weak A_T or FI_T of the negative sample (Supplemental Figure S2; <https://doi.org/10.3168/jds.2017-12554>).

We investigated the immunological kinetics of the 4 ICA by recording A_T or FI_T every 30 s. Supplemental Figure S3 (<https://doi.org/10.3168/jds.2017-12554>) illustrates that A_T of GNP-ICA remained stable after 20 min, and the FI_T of QD-ICA, FNP-ICA, and EuNP-ICA remained stable after 50, 30, and 25 min, respectively.

Analytical Performance of ICA

We constructed the calibration curve of the 4 label-based ICA under optimal experimental conditions by plotting the logarithm of A_T or FI_T against the logarithm of *E. coli* O157:H7 concentration. We then fitted the data using linear equations. The regression equations were $\log(Y) = 0.7197 \log(X) - 0.9694$, $\log(Y)$

Figure 4. Polydispersity index (PDI) of the 4 labels before and after coupling with monoclonal antibodies (mAb). EuNP = europium (III) chelate nanoparticle; FNP = fluorescent nanoparticle; GNP = gold nanoparticle; QD = quantum dot. Vertical bars indicate the SD ($n = 3$).

Table 1. Comparison of the immunochromatographic assays (ICA)¹

ICA	Sensitivity (cfu·mL ⁻¹)	Linear range (cfu·mL ⁻¹)	Amount of mAb (μg) ²
GNP-ICA	2.5×10^4	1.0×10^4 – 1.0×10^6	0.16
QD-ICA	5.0×10^3	2.5×10^3 – 1.0×10^6	0.37
FNP-ICA	1.0×10^3	2.5×10^2 – 2.5×10^5	0.04
EuNP-ICA	5.0×10^2	2.5×10^2 – 2.5×10^5	0.10

¹GNP = gold nanoparticle; QD = quantum dot; FNP = fluorescent nanoparticle; EuNP = europium (III) chelate nanoparticle.

²Per test strip.

$= 0.6549 \log (X) - 0.0017$, $\log (Y) = 0.8154 \log (X) - 0.6419$, and $\log (Y) = 0.8127 \log (X) + 0.2821$, with the reliable correlation coefficients R^2 of 0.9653, 0.9743, 0.9897, and 0.9897 for GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA, respectively (Figure 5).

We also compared the sensitivity and linear ranges of the 4 ICA (Table 1). The calculated sensitivities were 2.5×10^4 , 5.0×10^3 , 1.0×10^3 , and 5.0×10^2 cfu mL⁻¹ for the GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA, respectively. These results indicated that the EuNP-ICA possessed the highest sensitivity compared with the GNP-ICA, QD-ICA, and FNP-ICA. This was due to the unique photoluminescent properties of lanthanide chelates, including high emission fluorescence

Figure 5. Calibration curves to quantitatively analyze *Escherichia coli* O157:H7. (A) Gold nanoparticle-based immunochromatographic assay (GNP-ICA); (B) quantum dot-based ICA (QD-ICA); (C) fluorescent nanoparticle-based ICA (FNP-ICA); and (D) europium (III) chelate nanoparticle-based ICA (EuNP-ICA). Sensitivity was defined as the mean plus 3-fold SD of the negative sample. Vertical bars indicate SD ($n = 3$).

intensity, large Stokes shift, sharp emission profile, and long emission fluorescence lifetime (1 ms). Additionally, the delayed time-resolved mode of the EuNP-ICTS portable strip reader eliminated the background autofluorescence noise and achieved a high signal-to-noise

ratio. Moreover, the low PDI of the EuNP increased sensitivity. We also studied the linear ranges of the 4 ICA and found that the ranges of the FNP-ICA and the EuNP-ICA (2.5×10^2 to 2.5×10^5 cfu mL⁻¹) were broader than those of the GNP-ICA (1.0×10^4 to 1.0

Figure 6. Specificity of the proposed 4 immunochromatographic assays (ICA) over other non-*Escherichia coli* O157:H7. EuNP-ICA = europium (III) chelate nanoparticle-based ICA; FNP-ICA = fluorescent nanoparticle-based ICA; GNP-ICA = gold nanoparticle-based ICA; QD-ICA = quantum dot-based ICA. Vertical bars indicate SD (n = 3).

$\times 10^6$ cfu mL⁻¹) and the QD-ICA (2.5×10^3 to 1.0×10^6 cfu mL⁻¹).

We calculated the amount of mAb per test strip to compare the amount of mAb of the 4 ICA (Table 1). The amounts of mAb per GNP-ICA, QD-ICA, FNP-ICA, and EuNP-ICA test strip were 0.16, 0.37, 0.04, and 0.10 μ g, respectively. The FNP-ICA required the least amount of mAb, presumably because the amount of mAb was inversely proportional to the surface area of the label. The FNP had the largest size, resulting in the largest surface area and least amount of mAb per test strip.

Specificity of ICA

We tested 15 bacterial strains to estimate the specificity of the 4 proposed ICA. The strains included *E. coli* O157:H7 (10^6 cfu mL⁻¹) and 14 non-*E. coli* O157:H7 strains (10^6 cfu mL⁻¹). We also performed a negative test using sterile PBS solution (Figure 6). The A_T or FI_T of ICA with the *E. coli* O157:H7 sample exhibited a strong signal, and the A_T or FI_T of the ICA with non-*E. coli* O157:H7 sample exhibited a weak signal. These results clearly demonstrated the high specificity of the 4 ICA to detect *E. coli* O157:H7.

Accuracy and Precision of ICA

We performed recovery experiments to evaluate the accuracy and precision of the 4 ICA using milk samples spiked with *E. coli* O157:H7. Table 2 shows that the recoveries of the 4 ICA ranged from 83.3 to 125.3%, with CV in the range of 2.7 to 18.6%. The proposed 4 ICA satisfied the requirements for the quantitative detection of *E. coli* O157:H7 in milk. The CV for the FNP-ICA was 2.7 to 7.8%, better than those of for the

GNP-ICA (7.4 to 15.8%), QD-ICA (10.4 to 18.6%), and EuNP-ICA (6.9 to 10.5%).

Tolerance of ICA in Milk Sample

We investigated the influence of the milk sample on the sensitivity of the 4 ICA (Figure 7). The respective sensitivities of the 4 ICA in the spiked PBS and milk were as follows: GNP-ICA, 2.5×10^4 and 1.0×10^5 cfu mL⁻¹; QD-ICA, 5.0×10^3 and 2.5×10^4 cfu mL⁻¹; FNP-ICA, 1.0×10^3 and 1.0×10^3 cfu mL⁻¹; and EuNP-ICA, 5.0×10^2 and 5.0×10^2 cfu mL⁻¹. The FNP-ICA and EuNP-ICA showed better tolerance to the milk matrix than the GNP-ICA and QD-ICA. The improved tolerance was attributed to the good mechanical and chemical stability of the FNP and EuNP (Ma et al., 2009; Saraf et al., 2015; Wang et al., 2016). The chemical and colloidal instability of QD in biological environments adversely affected the sensitivity of QD-ICA (Aldana et al., 2001). The FNP-ICA and EuNP-ICA may have satisfactory tolerance to other matrices.

CONCLUSIONS

We developed 4 nanoparticle-based ICA for the quantitative and sensitive detection of *E. coli* O157:H7. All 4 ICA showed practical quantitative detection under optimal conditions. The EuNP-ICA showed the highest sensitivity, and the FNP-ICA required the least amount of mAb and had the best CV. Additionally, the FNP-ICA and EuNP-ICA had wider linear ranges and showed better tolerance than GNP-ICA and QD-ICA in milk samples. The FNP-ICA and EuNP-ICA have potential practical applications for the detection of pathogens in milk.

Table 2. Recoveries of *Escherichia coli* O157:H7 detected by the immunochromatographic assays (ICA)¹

ICA	Spiked concentration ² (cfu·mL ⁻¹)	Detected (cfu·mL ⁻¹)	Recovery ³ (%)	CV (%)
GNP-ICA	5.0×10^4	44,450	88.9	15.8
	1.0×10^5	97,147	97.1	7.6
	2.5×10^5	313,365	125.3	7.4
QD-ICA	5.0×10^4	58,865	117.7	12.6
	1.0×10^5	114,057	114.1	18.6
	2.5×10^5	234,737	93.9	10.4
FNP-ICA	1.0×10^4	10,038	100.4	4.0
	2.5×10^4	29,859	119.4	2.7
	5.0×10^4	58,295	116.6	7.8
EuNP-ICA	1.0×10^4	8,334	83.3	6.9
	2.5×10^4	24,560	98.2	8.3
	5.0×10^4	54,353	108.7	10.5

¹GNP = gold nanoparticle; QD = quantum dot; FNP = fluorescent nanoparticle; EuNP = europium (III) chelate nanoparticle.

²Mean value of 3 replicates at each spiked concentration.

³Recovery = (detected concentration/spiked concentration) $\times$ 100.

Figure 7. Influence of the milk matrix on the sensitivity of (A) gold nanoparticle-based immunochromatographic assay (GNP-ICA); (B) quantum dot-based ICA (QD-ICA); (C) fluorescent nanoparticle-based ICA (FNP-ICA); and (D) europium (III) chelate nanoparticle-based CA (EuNP-ICA). Vertical bars indicate SD ($n = 3$).

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