# Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium using quantum dots as fluorescence labels

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In this study, we explored the use of semiconductor quantum dots (QDs) as fluorescence labels in immunoassays for simultaneous detection of two species of foodborne pathogenic bacteria, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. QDs with different sizes can be excited with a single wavelength of light, resulting in different emission peaks that can be measured simultaneously. Highly fluorescent semiconductor quantum dots with different emission wavelengths (525 nm and 705 nm) were conjugated to anti-*E. coli* O157 and anti-*Salmonella* antibodies, respectively. Target bacteria were separated from samples by using specific antibody coated magnetic beads. The bead–cell complexes reacted with QD–antibody conjugates to form bead–cell–QD complexes. Fluorescent microscopic images of QD labeled *E. coli* and *Salmonella* cells demonstrated that QD–antibody conjugates could evenly and completely attach to the surface of bacterial cells, indicating that the conjugated QD molecules still retain their effective fluorescence, while the conjugated antibody molecules remain active and are able to recognize their specific target bacteria in a complex mixture. The intensities of fluorescence emission peaks at 525 nm and 705 nm of the final complexes were measured for quantitative detection of *E. coli* O157:H7 and *S.* Typhimurium simultaneously. The fluorescence intensity (*FI*) as a function of cell number (*N*) was found for *Salmonella* and *E. coli*, respectively. The regression models can be expressed as: $FI = 60.6\log N - 250.9$ with $R^2 = 0.97$ for *S.* Typhimurium, and $FI = 77.8\log N - 245.2$ with $R^2 = 0.91$ for *E. coli* O157:H7 in the range of cell numbers from $10^4$ to $10^7$ cfu ml$^{-1}$. The detection limit of this method was $10^4$ cfu ml$^{-1}$. The detection could be completed within 2 hours. The principle of this method could be extended to detect multiple species of bacteria (3–4 species) simultaneously, depending on the availability of each type of QD–antibody conjugates with a unique emission peak and the antibody coated magnetic beads specific to each species of bacteria.

## Introduction

Foodborne disease has been a serious threat to public health for decades and remains a major public health challenge. Foodborne pathogenic bacteria are the major causes of foodborne diseases, accounting for 91% of the total outbreaks of foodborne illness in the USA.[1] *Escherichia coli* O157:H7 and *Salmonella* Typhimurium are two of the most common foodborne pathogenic bacteria.[2] The development of rapid, sensitive, and specific detection methods is extremely important to implementing an effective response to foodborne illness, ensuring food safety and security. In recent years, immunoassays and molecular methods based on DNA probes or polymerase chain reaction (PCR) have been extensively studied to rapidly detect and identify foodborne bacterial pathogens. However, most of these methods are designed for detection of a single species of pathogens in one assay. The challenge therefore exists to develop rapid, sensitive and specific methods which are capable of simultaneously detecting multiple pathogens of interest. Simultaneous detection reduces the number of reactions to be performed in order to detect the possible presence of individual pathogens, thus saving considerable time and cost. Researchers have addressed this issue by developing molecular methods, including multiplex PCR,[3–7] DNA microarray techniques,[8,9] and array based immunosensors,[10,11] to detect 2–3 foodborne pathogens in one assay. Multiplex PCR allows the simultaneous amplification of more than one target sequence in a single reaction, which makes it possible to detect multiple pathogens by amplifying target sequences from multiple target pathogens. Array based immunosensors usually contain an array of antibodies with different specificity for recognizing different analytes. In a sandwich immunoassay form, after the binding of the target analyte, fluorescent tracer antibodies are added in a subsequent step for fluorescent detection of the binding events. However, there are significant limitations of conventional fluorescent dyes for their use in multi-color immunoassays: 1) typical fluorescence dyes used in immunoassays are susceptible to photo bleaching; 2) their narrow excitation spectra make simultaneous excitation difficult in most cases, and 3) their broad emission spectrum introduces spectral cross talk between different detection channels, making

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quantitative measurement of the relative amount of different probes difficult.

Ideal fluorescent probes for multi-color detection should emit at spectrally resolvable wavelength and have a narrow, symmetric emission spectrum, and the whole group of probes should be excitable at a single wavelength. Fluorescent semiconductor quantum dots (QDs) have recently emerged as a novel and promising class of fluorescent labels due to their advantages over conventional fluorophores, including resistance to photodegradation, improved brightness, and size-dependent, narrow-emission spectra.[10] More importantly, QDs can be excited efficiently at any wavelength shorter than the emission peak yet will emit with the same characteristic narrow, symmetric spectrum regardless of the excitation wavelength. Therefore, many sizes of QDs may be excited with a single wavelength of light, which makes it possible to detect many emission peaks simultaneously. Because of this unique property, the use of QDs as fluorescent labels has enabled multicolor imaging in demanding biological environments. Several groups have reported the applications of QDs in cellular and tissue imaging analysis for biological detection.[12–15] For example, Bruchez *et al.*[15] demonstrated the use of QDs as fluorescent labels in a dual-emission, single-excitation labeling experiment on mouse fibroblasts. Their results indicated that QDs are complementary and in some cases may be superior to existing fluorophores. The use of QDs has also been extended to multi-color detection based on immunoassay formats for toxin analysis[16] and microorganism detection.[17] For example, QDs were used as fluorescent labels for dual color imaging of two species of microorganisms, *Cryptosporidium parvum* and *Giardia lamblia*.[17] Our recent research also demonstrated the feasibility of quantitative detection of *E. coli* O157:H7 using QDs 605 as fluorescent labels.[18]

In this study, we investigated the use of QDs as fluorescence labels in immunoassays coupled with magnetic separation for quantitative detection of two major species of foodborne pathogenic bacteria, *E. coli* O157:H7 and *S.* Typhimurium in one assay simultaneously. Highly fluorescent semiconductor quantum dots with different emission wavelengths (525 nm and 705 nm) were conjugated to anti-*E. coli* O157 and anti-*Salmonella* antibodies, respectively. Target bacteria were separated from samples by using specific antibody coated magnetic beads. The bead–cell complexes reacted with QD–antibody conjugates to form bead–cell–QD complexes. The intensities of the emission peaks at 525 nm and 705 nm of the final complexes were measured for determination of *E. coli* O157:H7 and *S.* Typhimurium.

## Experimental

### Chemicals and biochemicals

Phosphate-buffered saline (PBS, 0.01 M of the phosphate, pH 7.4) was purchased from Sigma-Aldrich (St Louis, MO). Biotinylated rabbit anti-*Salmonella* antibody (4–5 mg $ml^{-1}$) and anti-*E. coli* O157 antibody (4–5 mg $ml^{-1}$) were obtained from Biodesign International (Saco, ME). According to the product manual from the company, the biotinylated anti-*E. coli* antibody is highly specific for *E. coli* O157:H7. Cross-reactivity to other *E. coli* strains has been minimized through extensive adsorption using non-O157:H7 serotypes of *E. coli*. The biotinylated anti-*Salmonella* antibody may show some cross-reactivity to related *Enterobacteriaceae*. Dilutions of anti-*Salmonella* antibody and anti-*E. coli* antibody were prepared with PBS, pH 7.4 for further use. Magnetic beads (MB) coated with rabbit anti-*Salmonella* antibody and MB coated with anti-*E. coli* antibody were from Dynal, Inc. (Lake Success, NY). Qdot® 525 streptavidin conjugate (2 μM) and Qdot® 705 streptavidin conjugate (2 μM) were purchased from Quantum Dot Corporation (Hayward, CA). Dilutions of QDs were made with the buffer supplied with the QDs.

### Bacterial cultures and surface plating methods

Stock cultures of *Salmonella* Typhimurium (ATCC 14028) and *Escherichia coli* O157:H7 (ATCC 43888) were obtained from American Type Culture Collection (Rockville, MD). Cultures were grown 18–20 h at 37 °C in brain heart infusion (BHI) broth (Remel, Lenexa, KS). Serial 10-fold dilutions were made in physiological saline solution. The cell numbers of *S.* Typhimurium and *E. coli* O157:H7 were determined by surface plating 0.1 ml proper dilutions onto XLT4 agar (Remel) and MacConkey sorbitol agar (Remel) agar, respectively. After incubation at 37 °C for 24 h, colonies on the plates were counted to determine the number of viable cells in the cultures in terms of colony forming units per millilitre (cfu $ml^{-1}$). Bacterial cell numbers in the cultures were determined by colony forming units per millilitre (cfu $ml^{-1}$) obtained from the surface plating. For safety considerations, the cultures were placed in a boiling water bath for 20 min to kill bacterial cells for further use.

Bacterial samples containing single species of bacteria were prepared by diluting 0.1 ml of the culture with 0.9 ml PBS (pH 7.4) to desired concentrations. Samples containing the mixture of bacterial cells were prepared by mixing 0.1 ml of each of the same serial dilutions of the two cultures and 0.8 ml of PBS. According to the plating counts, the ratio of the final concentrations of *E. coli* O157:H7 and *S.* Typhimurium in the mixture was $\sim 1 : 10$.

### QD–antibody conjugation

QD–antibody conjugates were developed using the coupling strategy between streptavidin and biotin. The coupling reaction is shown in Fig. 1. In detail, 10 μl of biotinylated anti-*Salmonella* antibody (1 : 10 dilution) and 10 μl of biotinylated anti-*E. coli* antibody (1 : 10 dilution) were added to 100 μl of 0.2 μM of QDs 705 and 100 μl of 0.2 μM QDs 525, respectively. After 30 min incubation at room temperature, QD705–anti-*Salmonella* antibody conjugates and QD525–anti-*E. coli* antibody conjugates were formed and ready for further use. All QD–antibody conjugates were made up fresh everyday for tests.

### Immunoassay procedure

The entire immunoassay procedure for detection of the two species of bacteria is outlined in Fig. 2. First, *S.* Typhimurium cells and *E. coli* O157:H7 cells were captured by magnetic

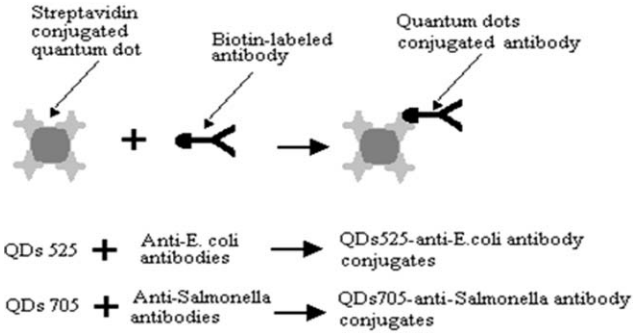


**Fig. 1** Schematic diagram of the coupling strategy to form QD–antibody conjugates. Streptavidin coated QDs 525 and streptavidin coated QDs 705 were conjugated with biotinylated anti-*E. coli* antibody and biotinylated anti-*Salmonella* antibody, respectively.

beads coated with antibodies that are selective for each species of the bacterial cells and separated from the sample matrix. In this step, 20 μl of each type of immunomagnetic beads were added to 1 ml of the sample containing the two species of bacterial cells in a micro-centrifuge tube. The mixture was shaken on a RKVSD 10101 mixer (ART, Inc., Laurel, MD) at a speed of 10 rpm for 40 min at room temperature. The bead–cell complexes were then separated from the solution by putting the tube on a magnetic separator for 2 min. After removing the liquid, the bead–cell complexes were washed with 0.5 ml PBS (pH 7.4). Secondary, QD–antibody conjugates were added to the above bead–cell complexes to form bead–cell–QD "sandwich" conjugates. To perform this step, 50 μl of each type of QD–antibody conjugates were added to the bead–cell complexes. After 30 min reaction, the "sandwich" conjugates were separated from the solution using the magnetic separator again. The "sandwich" conjugates were washed with 0.5 ml PBS (pH 7.4) twice, and resuspended with 150 μl PBS, pH 7.4. The fluorescence intensity produced by these QDs attached to bacterial cells was measured.

### Fluorescence measurement

The fluorescence measurement was performed using a laptop-controlled portable system which included a USB2000 miniature fiber-optic spectrometer, a LS-450 LED light source module, a R400-7 UV-vis optical probe (Ocean Optics, Inc., Dunedin, FL), and a probe/cuvette holder housed in a dark box. The spectrometer contained a low-cost 2048-element linear CCD-array detector in a working range from 360 to 900 nm. The LED module contained a 395 nm wavelength VIS LED in place of the 470 nm bulb in the LS-450. The optical probe was composed of a tight bundle of seven optical fibers (400 μm in diameter for each fiber) in a stainless steel ferrule where six illumination fibers surround one reading fiber.

### Microscopy

Transmission electron microscopy (TEM) was used to observe QDs before and after antibody conjugation. TEM tests were performed on a JEOL 100CX transmission electron microscope (JEOL, Inc., Peabody, MA) with an acceleration voltage of 100 kV. Five microlitres of the original QDs 705 solution and 5 μl of QD705 anti-*Salmonella* antibody conjugates were spread on the carbon grid and dried in air overnight. The prepared samples were tested using TEM.

Fluorescence microscopy was used to capture images of bacterial cells with QDs labels. Five microlitres of *S.* Typhimurium culture ($10^9$ cfu $ml^{-1}$) and *E. coli* O157:H7 culture ($10^8$ cfu $ml^{-1}$) were spotted on each glass slide and were heat dried to fix cells on the glass slides. The slides were washed with water and dried with air. They were then incubated with 10 μl of their QD–antibody conjugates for 30 min at room temperature. After washing with water, the

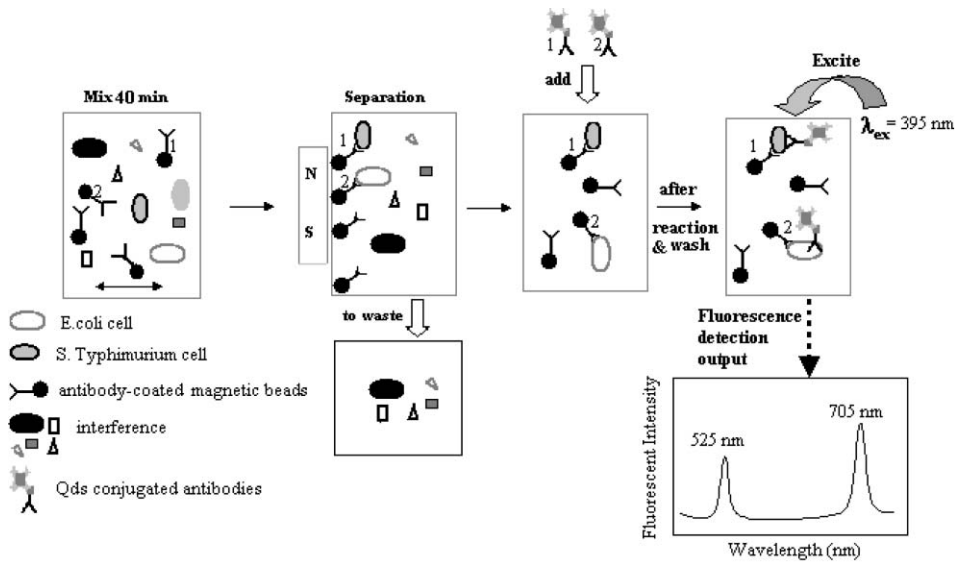


**Fig. 2** The entire immunoassay procedure for detection of the two species of bacteria. *S.* Typhimurium cells and *E. coli* O157:H7 cells in the mixture were captured by magnetic beads coated with antibodies that are selective for each species of the bacterial cells and separated from the sample matrix. QD–antibody conjugates were used as fluorescence labels through the immuno-recognition between antibodies and target bacterial cells.

 

slide was observed under a Zeiss Axioplan II imaging microscope (Carl Zeiss, Jena, Germany) equipped with an ORCA-ER digital camera (Compix, Inc., Cranberry Township, PA).

## Results and discussion

### QD–antibody conjugation

QD–antibody conjugates were developed using the coupling strategy between streptavidin and biotin using streptavidin coated QDs and biotin labeled antibodies (Fig. 1). In practice, the biotinylated antibodies have multiple biotins. Fig. 1 showing 1 biotin per antibody is for simplicity. It is worth emphasising here that the selection of an appropriate ratio between of the amounts of streptavidin coated QDs and biotinylated antibody is critical to get detectable signals in a simultaneous detection system. Based on the user manual of the Qdot streptavidin conjugates, the loading of streptavidin is typically 15–25 streptavidins per Qdot conjugate. The probes should, however, be used as if there were one streptavidin per quantum dot. In our experiments, we considered and controlled the antibody : QD ratio to achieve a proper ratio. Two experiments were performed to select an appropriate ratio of streptavidin coated QDs and biotin labeled antibodies. A two-step procedure (stepwise addition of biotin labeled antibodies and streptavidin coated QDs to the bacteria sample) was performed to determine the minimum required amount of biotinylated anti-*E. coli* antibody. A one-step procedure (one time addition of antibody–QD conjugates to the bacteria sample) was performed to determine an appropriate ratio between biotin labeled antibodies and streptavidin coated QDs for preparing the antibody–QD conjugates to get a detectable signal in a single species detection system.

Table 1 shows the results for detection of $6.0 \times 10^6$ cfu ml$^{-1}$ *E. coli* O157:H7 using two-step addition of biotinylated anti-*E. coli* antibody and streptavidin coated QDs 525 and one-step addition of QD525–anti-*E. coli* antibody conjugates. In the two-step addition tests, the concentration of QDs, 10 nM, was selected based on our previous study.[18] The results indicated that the use of 1/1000 and 1/10 000 dilution of biotinylated anti-*E. coli* antibody was not able to produce detectable signal. The minimum required amount of biotinylated anti-*E. coli* antibody to get a detectable signal was 200 μl of 1/100 dilution.

From the results of the one-step addition of QD–antibody conjugates, it was observed that QD–antibody conjugates prepared by simply mixing of the same amount of the two compositions used in the two-step test (20 μl 1/10 dilution of antibody + 200 μl 10 nM QDs) did not produce a detectable signal. QD–antibody conjugates prepared by increasing the concentration of antibody (20 μl of 4–5 mg ml$^{-1}$ antibody + 200 μl of 10 nM QDs) also could not produce a detectable signal. The possible reason of the failure to produce detectable signal is due to the improper ratio between the biotinylated anti-*E. coli* antibody and streptavidin coated QDs 525. In 200 μl of the conjugates solution, taking 150 000 Dalton as the molecular weight of the antibody, the molar concentration of the 1/100 dilution of antibody was approximately 0.3 μM. Compared with the concentration of QDs, 10 nM, the concentrations of antibody were far more than those of QDs. The improper ratio of the two compositions led to a large number of excess antibody molecules being present in the resulting conjugates solution. As a result, in the conjugates solution, only a small number of QD–antibody conjugates were present in a large amount of free antibody solution. When this solution reacts to bacterial cells, the competitive binding of free antibody molecules to antigens on bacterial surfaces led to insufficient binding of QD–antibody conjugates to cell surfaces, thus no detectable fluorescence signal could be observed. For the next two combinations, 20 μl of 1/100 dilution of antibody + 200 μl of 10 nM QDs and 20 μl of 1/1000 dilution of antibody + 200 μl of 10 nM QDs, even though the concentrations of the two components were close, the concentrations of antibodies were too low to produce effective binding to the antigens on bacterial cell surfaces. Considering that one streptavidin molecule has four binding sites for biotin, the concentration of the biotinylated antibody could be a little higher than that of the streptavidin coated QDs. As shown in Table 1, to prepare effective QD–antibody conjugates, the minimum level of the concentration of the biotinylated antibody was 0.3 μM. And this level of biotinylated antibody requires approximately 0.2 μM of streptavidin conjugated QDs 525 to form QD–antibody conjugates, to get a detectable signal. The appropriate ratio between the concentrations of streptavidin conjugated QDs and biotinylated antibody is very important not only to produce a detectable signal, but also to avoid cross-reaction between an excess of antibodies and undesirable QDs in the later steps.

### Characteristics of QD–antibody conjugates

Fig. 3 shows transmission electron microscopic photographs of (A) the original streptavidin coated QDs 705 and (B) after their

**Table 1** Results of the two-step procedure and one-step procedure for determination of the required minimal concentration of biotinylated antibody and the ratio between biotinylated antibody and streptavidin coated QDs to generate detectable fluorescence signal for detection of *E. coli* O157:H7 and *S.* Typhimurium

| Two-step procedure | | | One-step procedure | | |
|---|---|---|---|---|---|
| Biotinylated antibody (4–5 mg ml$^{-1}$) | Streptavidin coated QDs | Signal | Biotinylated antibody (4–5 mg ml$^{-1}$) | Streptavidin coated QDs | Signal |
| **200 μl 1/100 ×** | **200 μl 10 nM** | **Yes** | 20 μl original | 200 μl 10 nM | No |
| 200 μl 1/1000 × | 200 μl 10 nM | No | **20 μl 1/10 ×** | **200 μl 10 nM** | **No** |
| 200 μl 1/10 000 × | 200 μl 10 nM | No | 20 μl 1/100 × | 200 μl 10 nM | No |
| — | — | — | 20 μl 1/1000 × | 200 μl 10 nM | No |
| — | — | — | ***20 μl 1/10 ×*** | ***200 μl 0.2 μM*** | ***Yes*** |

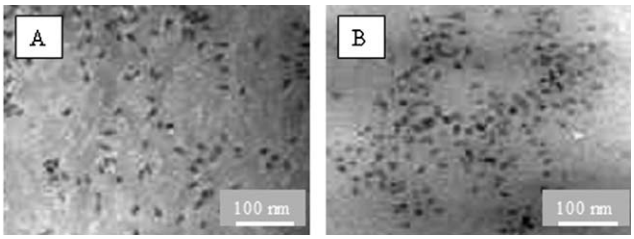


**Fig. 3** Transmission electron microscopic pictures of (A) the original streptavidin coated QDs 705 and (B) after their conjugation to biotinylated anti-*Salmonella* antibodies.

conjugation to biotinylated anti-*Salmonella* antibodies. The core of streptavidin coated QD 705 used in this experiment is made of CdTe. The core is coated with an additional semiconductor shell (ZnS) to improve the optical properties. This core-shell is further coated with a polymer shell which allows the QDs to be coated with streptavidin while retaining their optical properties. The streptavidin coated QDs 705 are rod-shaped, they are about 20 nm long and 10 nm in diameter. The TEM photograph (Fig. 3B) indicated that the conjugation step did not result in aggregation and the QD–antibody conjugates were primarily single particles. This property allowed these QD–antibody conjugates to keep their efficient reactivity in solution.

Fig. 4 shows the excitation spectrum and the fluorescent spectra of mixtures of the original streptavidin coated QDs 705 and QDs 525 and after their conjugation with biotinylated antibodies. QDs can be excited efficiently at any wavelength shorter than the emission peak and emit with a characteristic narrow, symmetric spectrum peak. As shown in Fig. 4, both QDs 525 and QDs 705 could be excited by a single excitation light source at 395 nm and emitted efficiently at their characteristic emission peaks at 525 nm and 705 nm, respectively. There was no cross-talk between the two emission peaks of the selected types of QDs, which provided sufficient spectral resolution for quantitative detection of the fluorescence intensity of each emission peak. Generally, when using conventional organic dyes as fluorescent labels, the ability to simultaneously measure different fluorescences has two requirements: multiple non-colinear excitation beams and a spectral resolution that minimizes emission overlap.[19] Their narrow excitation spectra and broad emission spectra make simultaneous measurements very difficult. QDs allow the use of a single excitation wavelength, which greatly simplified the optical system used to make these measurements and makes simultaneous detection easier. Besides, QDs have improved brightness compared with typical fluorescence dyes, which makes it easier for quantitative measurement of fluorescence intensity. For example, zinc sulfide-capped cadmium selenide quantum dots are 20 times as bright, 100 times as stable against photobleaching, and one-third as wide in spectral linewidth compared with the organic dye rhodamine.[20] Further, unlike typical fluorescence dyes that always have their optimal excitation wavelength close to the emission peak, the excitation wavelength of QDs is much shorter than the emission wavelength, which results in a large Stokes shift and greatly increases the possibility of multiplexed detection of more QD bioconjugates. In addition, the combination of different materials used for nanocrystals and different sizes of the nanocrystals provides a wide spectral range of 400 nm to 2 μm in the peak emission,[13] which greatly increases the capacity of one assay in detecting multiple foodborne pathogens.

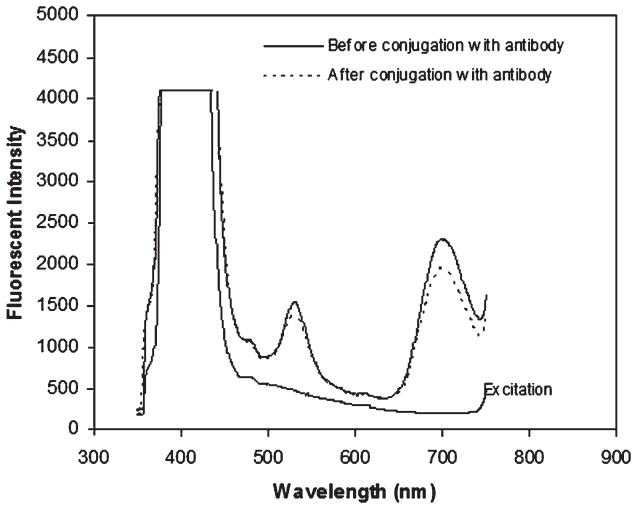


**Fig. 4** The excitation spectrum and the fluorescence spectra of the mixtures of original streptavidin coated QDs 705 and streptavidin coated QDs 525 and after their conjugation to biotinylated antibodies. Integration time: 512 ms.

It can also be seen that the fluorescence spectrum obtained from the mixture of QD705–anti-*Salmonella* antibody conjugates and QD525–anti-*E. coli* antibody conjugates is similar to that obtained from the mixture of QDs 705 and QDs 525 in terms of emission wavelength and peak characteristics (peak shape and spectral width). The slight difference in the fluorescence intensity before and after antibody conjugation was due to the different concentration caused by dilution and mixing. The comparison of the two spectra indicated that the optical properties of QDs remain unchanged after their conjugation to antibody molecules.

### Fluorescence spectra of single species of bacteria and mixed bacteria

Fig. 5 represents fluorescence spectra obtained from the samples containing no bacteria (background), (A) *Salmonella* Typhimurium only, (B) *E. coli* O157:H7 only, and (C) the mixture of the two species. The background fluorescence spectrum was obtained from the sample that contained 20 μl anti-*E. coli* antibody coated magnetic beads, 20 μl anti-*Salmonella* antibody coated magnetic beads, and PBS. As shown in Fig. 5, no evident fluorescence peaks at 525 nm and at 705 nm were observed in the background spectrum. This result indicated that there was no evident non-specific binding between magnetic beads and QD–antibody conjugates without the presence of bacterial cells.

Fig. 5A shows the spectrum produced by the sample containing $2.9 \times 10^6$ cfu $ml^{-1}$ of *S.* Typhimurium. It shows a well-shaped fluorescence peak at 705 nm with a net peak intensity of 70 counts (255 − 185 counts). Apparently, the

 

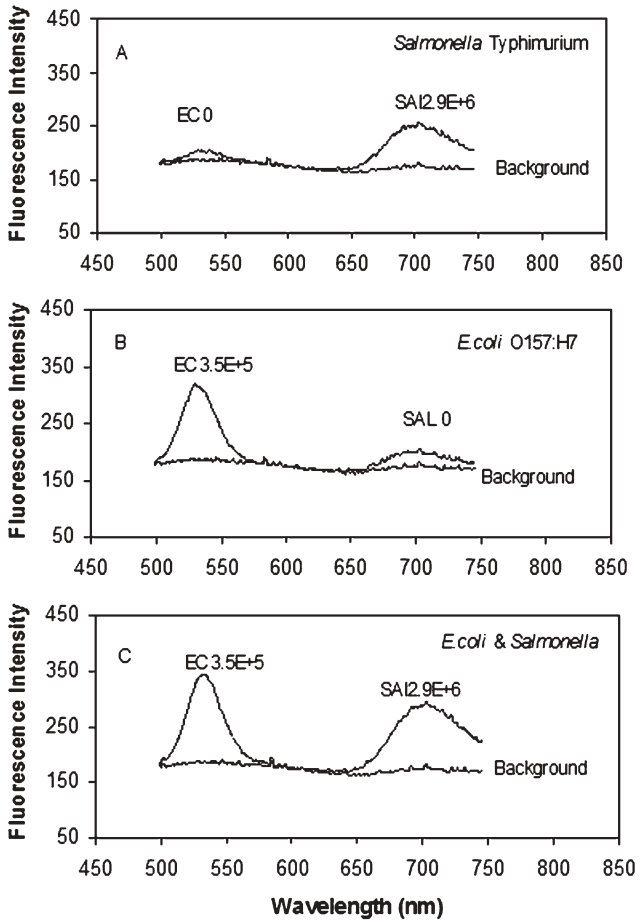


**Fig. 5** Representative fluorescence spectra obtained from the samples containing no bacteria (background), (A) *Salmonella* Typhimurium only, (B) *E. coli* O157:H7 only, and (C) the mixture of the two species. Cell numbers of *E. coli* O157:H7 and *Salmonella* Typhimurium were $3.5 \times 10^5$ cfu ml$^{-1}$ and $2.9 \times 10^6$ cfu ml$^{-1}$, respectively, in the single bacteria sample and the mixture sample. Integration time: 512 ms.

fluorescence peak at 705 nm was produced by QDs 705 that were attached to *Salmonella* cells through the reaction between QD705–anti-*Salmonella* antibody conjugates and *Salmonella* cells. At the same time, a small peak at 525 nm was observed on this fluorescence spectrum. This small fluorescence peak was due to the non-specific binding between the *Salmonella* cells and the QD525–anti-*E. coli* antibody conjugates. The net intensity of this peak was approximately 17 counts (202 − 185 counts), which was much lower than that of the peak at 705 nm. Similar results were observed for the sample containing $3.5 \times 10^5$ cfu ml$^{-1}$ *E. coli* O157:H7 as shown in Fig. 5B. The fluorescence spectrum shows a strong peak at 525 nm and a small peak at 705 nm. The peak at 525 nm was produced by QDs 525 which were introduced to *E. coli* cells through the specific reaction between the *E. coli* cells and QD525–anti-*E. coli* antibody conjugates, while the peak at 705 nm was produced by QDs 705 which was bound to *E. coli* cells due to the non-specific binding between *E. coli* cells and QD705–anti-*Salmonella* antibody conjugates.

In Fig. 5C, the fluorescence spectrum was obtained from the sample that contained both *E. coli* O157:H7 and *S.* Typhimurium. As seen on the spectrum, two strong peaks at 525 nm and 705 nm represent the fluorescence signals produced by *E. coli* O157:H7 cells and *S.* Typhimurium cells, respectively. This result indicated that each type of QD–antibody conjugates could bind to their own target bacteria in the mixture of the two species and produced fluorescence signals.

Comparison of Fig. 5C with Fig. 5A and B shows that the intensity of the fluorescence peak at 525 nm of the mixture sample (Fig. 5C, 339 counts) is higher than that of the peak at 525 nm of the single species sample (Fig. 5B, 317 counts). Similarly, the emission peak at 705 nm in Fig. 5C (294 counts) is higher than the peak at 705 nm in Fig. 5A (255 counts). This comparison indicated that the sample of mixed bacteria produced higher fluorescence peaks than those samples with single species of bacteria even when the concentrations of the target bacteria are the same. The most likely reason for the elevated emission peaks at 525 nm and 705 nm for the mixed sample are cross reactions between QD525–anti-*E. coli* antibody and *Salmonella* cells and between QD705–anti-*Salmonella* antibody and *E. coli* cells. This is experimentally consistent with the small peaks seen in the single bacteria samples (Fig. 5A and B) that were due to nonspecific binding. Cross reactions might also occur between bacterial cells and antibodies on the magnetic beads. Nevertheless, these non-specific bindings are limitations common to immunoassays that use antibodies to provide selectivity. In spite of these low level nonspecific bindings, these results indicated this method provided a way to get fluorescence signals of the two species of target bacteria in one assay.

Fig. 6 displays the fluorescent image of (A) QDs 525-labeled *E. coli* O157:H7 cells and (B) QDs 705-labeled *S.* Typhimurium cells. Clearly, these images showed that QD–antibody conjugates could evenly and completely attach to the surface of bacterial cells, indicating that the conjugated QDs molecules still retain their effective fluorescence, while the conjugated antibody molecules remain active and are able to recognize their specific target bacteria. This result also suggested that QDs could be a new class of fluorescent labels for imaging and detecting bacterial cells.

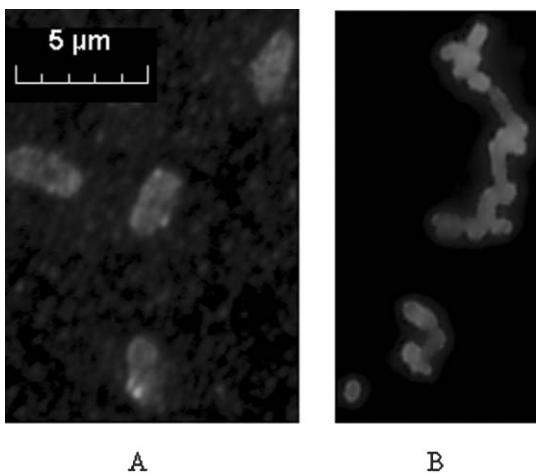


**Fig. 6** Fluorescent images of (A) QDs 525-labeled *E. coli* O157:H7 cells and (B) QDs 705-labeled *Salmonella* Typhimurium cells.

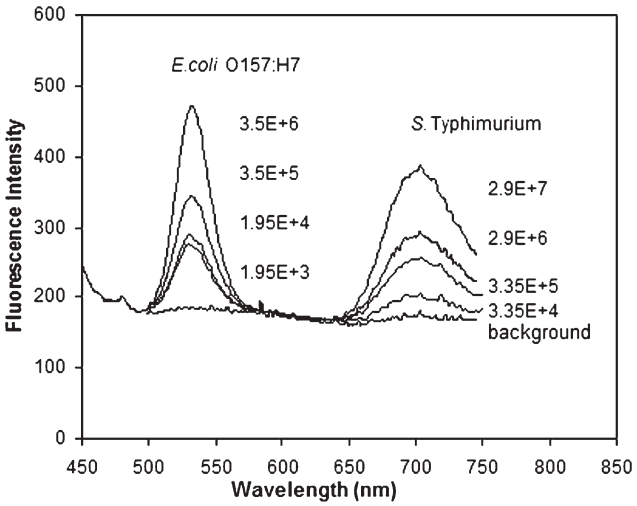


**Fig. 7** A group of fluorescence spectra obtained from the samples containing different bacterial cell numbers of *E. coli* O157:H7 and *S.* Typhimurium. Cell number of *S.* Typhimurium: $3.35 \times 10^4$ to $2.9 \times 10^7$ cfu ml$^{-1}$; cell number of *E. coli* O157:H7: $1.95 \times 10^3$ cfu ml$^{-1}$ to $3.5 \times 10^6$ cfu ml$^{-1}$. Integration time: 512 ms.

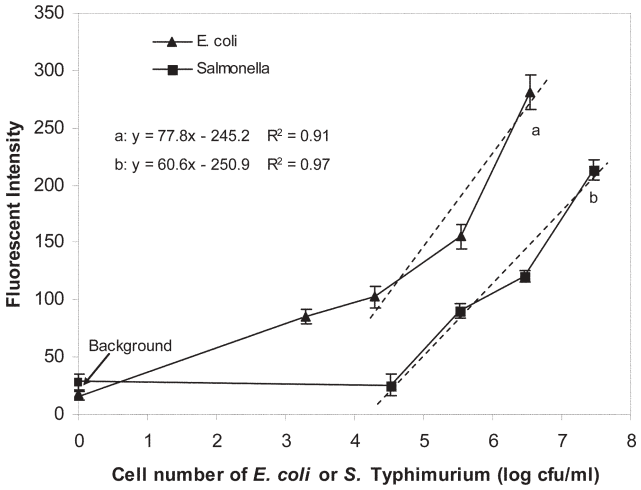


**Fig. 8** The intensities of fluorescence peaks at 525 nm and 705 nm as functions of the concentrations of *E. coli* O157:H7 and *Salmonella* Typhimurium in the mixture of the two species of bacteria. Integration time: 512 ms. Error bars = ±SD (n = 3–4).

### Simultaneous detection of *E. coli* O157:H7 and *Salmonella* Typhimurium

Fig. 7 shows a representative group of fluorescence spectra obtained from the samples containing the mixture of different bacterial cell numbers of *E. coli* O157:H7 and *S.* Typhimurium. It can be seen that the intensity of the fluorescence peak at 705 nm increased with the increasing cell number of *S.* Typhimurium in the range of $3.35 \times 10^4$ to $2.9 \times 10^7$ cfu ml$^{-1}$ in the mixture of the two bacteria samples, demonstrating that the more *Salmonella* cells in the sample, the more QDs they could bind, and thus the stronger fluorescence they could produce. It is also shown that the sample containing the mixture of $3.35 \times 10^4$ cfu ml$^{-1}$ *Salmonella* and $1.95 \times 10^3$ cfu ml$^{-1}$ *E. coli* has an emission peak at 705 nm with a net peak intensity of 23 counts. However, this emission peak is indistinguishable from the non-specific peak (~20 counts) produced by $3.5 \times 10^5$ cfu ml$^{-1}$ of *E. coli* at 705 nm (as shown in Fig. 5B).

Similarly, as shown in Fig. 7, when *E. coli* cell number increased from $1.95 \times 10^3$ cfu ml$^{-1}$ to $3.5 \times 10^6$ cfu ml$^{-1}$, the intensity of the fluorescence peak at 525 nm increased from 271 counts to 462 counts, implying that the higher the concentration of *E. coli* cells in the mixture, the stronger fluorescence signal they could generate. The sample containing $1.95 \times 10^3$ cfu ml$^{-1}$ of *E. coli* O157:H7 in the mixture presents a well-shaped peak at 525 nm with the peak intensity of 85 counts, while the background spectrum did not show a shaped peak at 525 nm. The signal produced by $1.95 \times 10^3$ cfu ml$^{-1}$ of *E. coli* O157:H7 in the mixture is also much higher than that produced by the non-specific peak of $2.9 \times 10^6$ cfu ml$^{-1}$ *S.* Typhimurium at 525 nm (17 counts, as seen in Fig. 5A). This result indicated that $1.95 \times 10^3$ cfu ml$^{-1}$ of *E. coli* O157:H7 could result in a detectable signal in the presence of 1000 times higher concentration of *S.* Typhimurium in a mixture of the two bacteria samples.

The fluorescence intensity (*FI*) at 525 nm and 705 nm as functions of cell numbers (*N*) of *E. coli* and *Salmonella* in the mixed bacterial sample are presented in Fig. 8. The regression models can be expressed as: $FI = 60.6\log N - 250.9$ with $R^2 = 0.97$ for *S.* Typhimurium in the cell number ranging from $10^4$ to $10^7$ cfu ml$^{-1}$, and $FI = 77.8\log N - 245.2$ with $R^2 = 0.91$ for *E. coli* O157:H7 in the range of cell numbers from $10^4$ to $10^7$ cfu ml$^{-1}$. These results indicated that it is possible to determine bacterial cell numbers of *E. coli* O157:H7 and *S.* Typhimurium simultaneously based on the measurement of the intensities of the fluorescence peaks at 525 nm and 705 nm, respectively. The non-specific signals are 17 ± 4.58 at 525 nm and 28 ± 7.5 at 705 nm. The detection limits of this method for *E. coli* O157:H7 and *S.* Typhimurium were *ca.* $10^4$ cfu ml$^{-1}$ by setting the signal level higher than the nonspecific signal. The detection could be completed within 2 hours.

With regard to the detection limit of individual bacteria in the mixture, the detection limits of this method for both bacteria are comparable with several methods for the detection of single species of bacteria based on immuno-reaction strategies, such as immunomagnetic separation-flow injection analysis-mediated amperometric detection which has detection limits of ~$10^5$ cfu ml$^{-1}$ for *E. coli*,[21] immunosensors for *E. coli* O157:H7 with detection limits of $10^6$–$10^7$ cfu ml$^{-1}$,[22,23] and several immunosensors for *Salmonella* with detection limits of $10^5$–$10^6$ cfu ml$^{-1}$.[24–26] Taitt *et al.* achieved a detection limit of ~$8 \times 10^3$ cfu ml$^{-1}$ for *Salmonella* using an array based biosensor with a 1 hour assay.[11] Moreover, the great advantage of this method is that it allows simultaneous detection of the two main foodborne pathogenic bacteria in one sample, which would effectively save time and cost for sample preparations and tests for individual species of bacteria. A number of PCR-based methods have been used for simultaneous detection of pathogenic bacteria.[3,5–7] For example, Bhagwat[5] reported a real-time PCR method that could simultaneously detect *E. coli* O157:H7 and *S.* Typhimurium to the predicted level of 1 to 10 cells ml$^{-1}$ and

 

*Listeria monocytogenes* at 1000 cells $ml^{-1}$. But these methods are intrinsically much slower because of multiple reaction and purification steps and a long pre-enrichment step (16–48 h) to achieve low detection limits. In addition to PCR-based methods, a method based on optical measurement during the growth of bacteria in media was reported for simultaneous detection of *listeriae* and *salmonellae* in foods.[27] However, it required a much longer detection time. The detection of 10–50 salmonellae and 10–50 listeriae in 25 g of the tested foods required a total of 24 h. Positive samples required confirmation of the pathogen by PCR-based assay (6 h), taking a total of $\leqslant$30 h for detection and confirmation.

## Conclusions

Our study demonstrated a simple and rapid method for simultaneous detection of *E. coli* O157:H7 and *S.* Typhimurium using immuno-magnetic beads for separation and QDs as fluorescence labels. The use of QDs overcomes the limitations of typical fluorescence dyes used in immunoassays and enables the detection of multiple emission peaks simultaneously when excited with a single wavelength of light. Conjugation of QDs (different colors) with antibodies specific to individual species of bacteria provides selective labels with different colors for individual species of bacteria, which was successfully used for simultaneous detection of *E. coli* O157:H7 and *S.* Typhimurium in this study. The detection limits for both bacteria in the mixture are comparable to several methods for single species bacteria detection. The detection time is much shorter than PCR-based methods for simultaneous detection of pathogens. The principle of this method can be extended to detect multiple species of bacteria simultaneously, depending on the availability of QD–antibody conjugates that have emission peaks separated from one another and the effective antibody coated magnetic beads to capture each species of target bacteria. Currently, by using commercially available QDs with emission in the range of 525 nm to 705 nm, it is expected that 3–4 species could be detected simultaneously with well separated emission spectra by using this method.

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