Detection of *Staphylococcus Aureus* using quantum dots as fluorescence labels

Hu Yaohua[1], Wang Chengcheng[1], Bai Bing[1], Li Mintong[1*], Ronghui Wang[2], Yanbin Li[2]

(1. *College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, Shaanxi* 712100, *China;* 2. *Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR* 72701, *USA*)

Abstract: *Staphylococcus aureus* (*S. aureus*) has been identified as one of the major foodborne pathogenic bacteria. The development of rapid detection methods for *S. aureus* is needed for assuring food safety. In this study, quantum dots were used as fluorescent labels in an immunoassay for quantitative detection of *S. aureus*. Firstly, biotin-labeled anti-*S. aureus* antibody was conjugated with streptavidin-coated magnetic nanobeads (180 nm diameter) and used to separate *S. aureus* cells. Then streptavidin coated quantum dots (QDs) were conjugated with biotin-labeled anti-*S. aureus* antibody and used as the fluorescence labels to mix with the separated *S. aureus*. Finally the fluorescence intensity of the bead-cell-QD complexes was measured at a wavelength of 620 nm. A linear relationship between *S. aureus* cell number (X) and fluorescence intensity (Y) was found for cell numbers ranging from 10^3 to 10^6 CFU (Colony Forming Unit)/mL, and the detection limit was 10^3 CFU/mL. The regression model can be expressed as $Y = 7.68X + 35.06$ with $R^2 = 0.94$. The detection of *S. aureus* in food sample was explored initially. The fluorescence intensity of food sample was close to the background, so it was not satisfied. Further study will focus on the application of the method for detection of *S. aureus* in food sample.

Keywords: *staphylococcus aureus*, fluorescence measurement, biological detection, quantum dots, microbial monitoring, food safety

DOI: 10.3965/j.ijabe.20140701.009

Citation: Hu Y H, Wang C C, Bai B, Li M T, Wang R H, Li Y B. Detection of *Staphylococcus Aureus* using quantum dots as fluorescence labels. Int J Agric & Biol Eng, 2014; 7(1): 77－83.

1 Introduction

Foodborne disease caused by microorganisms has been the main matter of food safety. In recent years, a series of serious food safety problems have been occurred due to foodborne pathogenic bacteria. In the USA, there are more than 200 known diseases transmitted through food, which caused approximately 8 000 deaths annually[1]. *Staphylococcus aureus* (*S. aureus*) can produce enterotoxin. Once food is contaminated by *S. aureus*, the bacteria may breed rapidly and produce enterotoxin, consequently cause food poisoning[2]. According to Centers of Disease Control (CDC), the food poisoning caused by *S. aureus* ranked only the second to *Escherichia coli*. It is necessary to develop rapid and effective methods for the detection of foodborne pathogenic bacteria. Immunoassay is one of the rapid methods that have been widely used for detecting bacteria in food, environmental and medicinal samples. Most immunological methods are based on optical detection of

Received date: 2013-06-23 **Accepted date:** 2014-01-02

Biographies: Hu Yaohua, PhD, Associate Professor, Research interests: Nondestructive detection, biosensor and grain storage; Email: huyaohua@nwsuaf.edu.cn. **Wang Chengcheng,** Master, Research interests: Biosensor of microbial detection; Email: cc1213@163.com. **Bai Bing**, Master, Research interests: Biosensor of microbial detection; Email: 335341058@qq.com. **Wang Ronghui,** PhD, Professor, Research interests: Biosensor of food safety inspection; Email: rwang@uark.edu. **Li Yanbin,** PhD, Professor, Research interests: Biosensor of food safety inspection; Email: yanbinli@uark.edu.

***Corresponding author: Li Mintong,** PhD, Associate Professor, Research interests: Testing & signal processing and biosensor. College of Mechanical and Electronic Engineering, Northwest A&F University, No. 22, Xinong road, Yangling District, Shaanxi 712100, China. Tel: +86-29-87902391; Email: lmtyd@nwsuaf.edu.cn.

enzyme-linked absorbent[3], or luminescent products[4], or fluorescent label molecules[5,6]. Traditional synthesized fluorescent dyes used in immunoassays have some limitations. They cannot fluoresce for a long time for their susceptibility to photobleaching, and their broad excitation and emission spectra often makes the signals from different fluorophores overlap[7].

With the development of biological material and nanotechnology, it is possible to exploit magnetic nanobeads (MNBs) and semiconductor nanocrystal quantum dots (QDs) to improve the efficiency of immunoassays. Immunomagnetic nanobeads (IMBs) are homogeneous and superparamagnetic microballoon. By coating the magnetic beads with specific antibodies, the IMBs can capture and separate the target bacteria[8,9]. The IMBs could diffuse adequately in the liquid so as to enlarge their superficial area, so they could be easy to wash and enrich[10].

Quantum dots, a new class of luminescent inorganic fluorophores, have recently arisen as novel and promising fluorescent labels for biological detection[11,12]. In comparison with conventional fluorophores, QDs have novel optical properties, including high-quantum yield, resistance to photodegradation, narrow emission and broad excitation spectra for multi-target[13,14]. Moreover, the QDs with different sizes and core materials can produce various color emission light; hence, upon above traits of QDs, simultaneous multianalyte detection can be achieved by utilizing multi-size QDs[15,16]. Desirable biological molecules can be coupled to QDs and retain their biological activities. At present, most researchers used QDs for biological detection of cellular imaging and staining. Bruchez et al.[13] demonstrated the use of QDs as fluorescent labels in a dual-emission, single-excitation labeling test on mouse fibroblasts, indicating that QDs are complementary, and in some cases, might be superior to existing fluorophores. Dubertret et al.[17] and Larson et al.[18] reported the use of QDs in vivo imaging of tissues. Meanwhile, many researchers focused on the application of QDs for microorganism detection. Hu et al.[19] researched a dual color imaging of two different QDs as labels for two species of bacteria, Cryptosporidium parvum and Giardia lamblia. Some researchers have demonstrated the use of immunomagnetic nanobeads for separation of target bacteria and QDs as fluorescent labels for detection of *E. coli* O157:H7[20,21], *Salmonella Typhimurium*[22], *L. monocytogenes*[23,24]. Also, simultaneous detection of multi-target pathogenic bacteria has been investigated[25-27]. However, there is almost no report on detection of *S. aureus* using QDs as labels.

In this study, IMBs were used as solid phase carrier for separation and QDs were used as a fluorescent label in immunoassay for quantitative detection of *S. aureus*, one of the most common foodborne pathogenic bacteria, to establish a quantitative immune fluorescence detection method for *S. aureus*.

2 Materials and methods

2.1 Chemicals and biochemicals

Biotinylated anti-*S. aureus* rabbit polyclonal antibody (4-5 mg/mL) was obtained from Thermo Scientific (Rockford, USA). Streptavidin conjugated magnetic nanobeads with diameters of 180 nm were purchased from Aibit Biotech (Jiangyin, Jiangsu Province, China). Qdot 620 streptavidin conjugates (8 μM) were purchased from Ocean NanoTech (Springdale, AR). Phosphate-buffered saline (PBS, 0.01 M, pH 7.4) was purchased from Sigma-Aldrich (Shanghai, China).

2.2 Bacterial cultures and surface plating methods

Stock culture of *S. aureus* (ATCC 27660) was obtained from Prajna Biology (Shanghai, China). Stock culture of *Shigella flexneri* (ATCC 12022) was obtained from American Type Culture Collection (Manassas, USA). Cultures were grown for 24 h at 37°C in LB broth (Land Bridge Tech., Beijing, China). Serial 10-fold dilutions were made in phosphate-buffered saline. The viable cell numbers of *S. aureus* and *Shigella flexneri* were determined by surface plating 0.1 mL of the appropriate dilutions onto Baird-Parker agar and Maconkey agar, respectively. Colonies were counted after incubation at 37°C for 24-48 h.

2.3 Preparation of immuno-MNBs (IMBs)

Streptavidin-bound MNBs (180 nm, 2 mg/mL) were coated with bioth-labeled anti-*S. aureus* antibody via biotin-streptavidin binding. One millilitre MNBs was

washed with 1 mL PBS (0.01 M, pH7.4) for three times. After magnetic separation, the MNBs were mixed with 30 μL of biotin-conjugated anti-*S. aureus* antibody (4-5 mg/mL) with a rotating mixer (Kylin-Bell Lab Instruments, Haimen, Jiangsu Province, China) at 15 r/min for 30 min at room temperature. A magnetic field (0.4 T) was applied to separation of IMBs for 1.5 min, and the beads were washed three times with 1 mL PBS to remove unattached antibody. The IMBs were then sealed up by using 1% BSA, after rotating at 15 r/min for 1 h and washing. The IMBs were resuspended in 1 mL PBS.

2.4 Preparation of antibody-coated QDs

Streptavidin-conjugated QDs with emission wavelength of 620 nm were coated with anti-*S. aureus* antibody. In this step, 20 μL of QDs (8 μM, 1:5 dilution) were mixed with 30 μL of biotin-conjugated antibody (4-5 mg/mL) for 30 min. The mixture was shaken on the mixer at 15 r/min at room temperature. The QDs-antibody conjugates were mixed and held for testing.

2.5 Individual separation of *S. aureus*

Serial dilutions of the pure cultures of *S. aureus* were prepared in 0.01 M PBS. A 80-μL aliquot of the prepared IMBs was mixed with 500 μL of each concentration culture from 10^3 to 10^6 CFU (Colony Forming Unit)/mL on the mixer rotating at 15 r/min for 45 min at room temperature. The IMB-cell complexes were then collected by a magnetic separator (0.4T) for 1.5 min and resuspended in 1 mL PBS. A 100-μL aliquot of the sample was plated on the Baird-Parker plating to obtain population of captured bacteria. The same level for all the original cultures was used as a positive control. The *CE* (capture efficiency) was calculated with the equation: $CE\ (\%) = N_c/N_0 \times 100$, where N_c (CFU) is the number of captured cells and N_0 (CFU) is the number of original cells.

2.6 Assay procedure of detection

The entire assay procedure is outlined in Figure 1. Firstly, *S. aureus* cells were captured by magnetic nanobeads coated with rabbit anti-*S. aureus* antibody and separated from the solution. In this step, 80 μL of IMBs prepared were mixed with 500 μL different 10-fold dilutions of *S. aureus* in microcentrifuge tubes. The mixture was shaken on the mixer at a speed of 15 r/min for 45 min at room temperature. The bead-cell conjugates were separated from the solution by putting the tube on the magnetic separator for 1.5 min and then removing the liquid. The conjugates were resuspended with 1 mL PBS after they were washed with 1 mL PBS twice. A 100-μL aliquot of capture liquid was plated on the selective plating to obtain population of captured bacteria. The same level of the original bacteria was used as a positive control. The CE was calculated as described for individual separation. Secondly, 100 μL bead-cell conjugates were mixed with 20 μL anti-*S. aureus* antibody-coated QDs with emission wavelength of 620 nm for 30 min at 15 r/min. The QDs were attached to the bacterial cells through the immunoreaction between the antibodies on the QDs and the antigens of bacterial cells, and the IMB-cell-QD complexes were formed. After removing excess QD solution, the final complexes were washed with PBS and resuspended with 100 μL PBS. Finally, the fluorescence intensity produced by these QDs was measured.

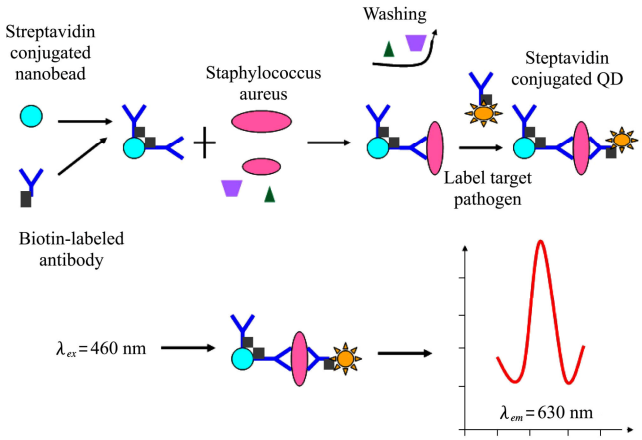

Figure 1 Schematic diagram of the detection procedure

2.7 Fluorescence measurement

The fluorescence measurement was performed by using a laptop-controlled portable system which included a USB4000 miniature fiber-optic spectrometer, an LS-450 light emitting diode (LED) light source module, an R400-7 UV-vis optical probe (Ocean Optics Inc., Dunedin, FL, USA) and the probe in a dark box. The light source with excitation wavelength of 460 nm excited QDs with emission wavelength of 620 nm. The SpectraSuite sofeware (Ocean Optics, Inc., Dunedin, FL,

USA) was used to show the optical spectrum.

2.8 Lamb wash water sample

Lambs were obtained from a supermarket. 25 g lamb pieces were weighed accurately and then put into a triangular flask containing 225 mL PBS (0.1%), and shaken for 1 min. Each of 9 mL wash water was put into aseptic tube, and each tube was inoculated with 1 mL *S. aureus* at different concentrations. The concentration of 10^5 and 10^6 CFU/mL *S. aureus* in lamb wash water was chosen to test. The following steps were the same as the pure culture in PBS.

A 80-μL aliquot of the prepared IMBs was mixed with 500 μL of 10^5 and 10^6 CFU/mL *S. aureus* lamb wash water on the mixer rotating at 15 r/min for 45 min at room temperature. Then separated the *S. aureus* in the sample and calculated the CE. According to the established immunofluorescence method, the concentration of 10^6 CFU/mL *S. aureus* lamb wash water was labeled by QDs and detected the fluorescence intensity with excitation wavelength of 460 nm.

3 Results and discussion

3.1 Capture efficiency of magnetic nanobeads for *S. aureus*

To assess the CE of the IMBs to target bacteria, samples with different cell numbers of *S. aureus* were tested. Table 1 shows the CE of IMBs for separation of viable *S. aureus* in pure and mixed culture with *Shigella flexneri*. The results of samples 1 to 4 indicated that the CE of IMBs for the separation of *S. aureus* in pure culture was between 33.5% and 40.2% in *S. aureus* cell numbers of 10^3 to 10^6 CFU/mL.

Table 1 Capture efficiency of IMB to different numbers of *S. aureus* in pure and mixed culture

Sample	*S. aureus* cell number/CFU·mL^{-1}	Capture efficiency/%
1 pure culture	1.60×10^6	40.17±2.27
2 pure culture	1.16×10^5	38.99±1.27
3 pure culture	1.09×10^4	33.50±6.63
4 pure culture	1.09×10^3	34.84±6.84
5 mixed culture	4.91×10^3 *S. aureus* With 6.46×10^3 *Shigella flexneri*	33.02±4.59 (To *S. aureus*)

Note: Cell numbers were the average of colony numbers from three plates.

The CE of anti-*S. aureus* antibody-coated immunomagnetic nanobeads for sample 5, the mixed culture of 4.91×10^3 CFU/mL *S. aureus* with 6.46×10^3 CFU/mL *Shigella flexneri* was 33.0%, which was close to the CE for pure *S. aureus* culture. This result shows that *Shigella flexneri* cells did not interfere with the capture of *S. aureus* cells by anti-*S. aureus* antibody-coated immunomagnetic nanobeads, suggesting this magnetic separation can be used for capture of *S. aureus* from a sample containing multiple bacterial species.

The CE in this study (33.0% to 40.0%) was low, but it was stable for different concentrations of bacterial cells. This stable CE is critical to obtain a linear correlation of cell number with fluorescence intensity. In this study, CE was calculated on the basis of cell-bead complexes that could result in clumps of cells that grow as a single CFU on the plate.

3.2 Detection of *S. aureus* in pure culture

Figure 2 shows the fluorescence spectra of different numbers of QDs labeled *S. aureus* in pure culture. The background produced a fluorescent peak of an intensity of 50.13 counts at 620 nm. With 2.40×10^3 CFU/mL *S. aureus* cells, the sample produced a fluorescent peak of an intensity of 63.43. It can also be seen that the fluorescence intensity increased with the increasing cell number of *S. aureus* from 2.40×10^3 CFU/mL to 2.37×10^6 CFU/mL. This result demonstrated that the more bacterial cells in the sample, the higher amount of QDs they could bind, and thus the stronger fluorescence they could produce.

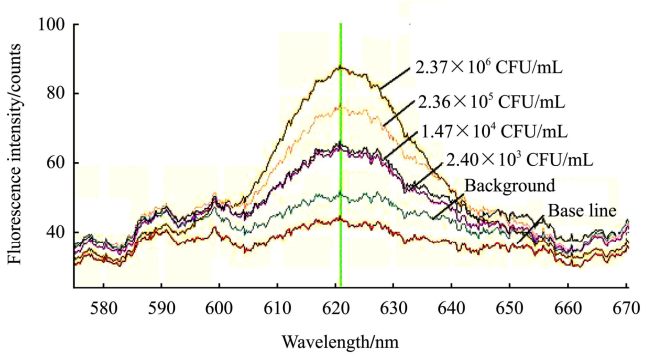

Figure 2 Fluorescence spectra obtained for samples with different *S. aureus* numbers in PBS

Figure 3 shows a linear relationship between *S. aureus* cell number (X) in the samples and the

fluorescence intensity (Y) found for cell concentrations ranging from 10^3 to 10^6 CFU/mL. The regression model can be expressed as: $Y = 7.68X + 35.06$, with $R^2 = 0.94$. The detection limit was 10^3 CFU/mL.

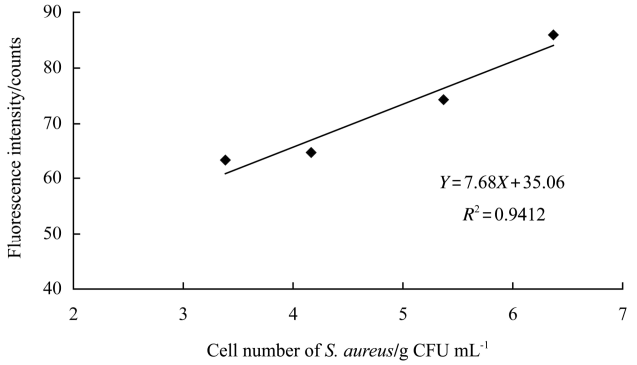

Figure 3 Linear relationship between the logarithmic values of *S. aureus* cell numbers in Phosphate-buffered saline and the fluorescence intensity

In this study, the fluorescence intensity of the highest concentration of cells (10^6 CFU/mL) was 85.92. The fluorescence intensity of bacterial cells labeled by QDs was lower compared with other studies[20,21,25], in which the fluorescence intensity could reach several hundreds even thousands. The reason is probably that the QDs placed too long and was close to its storage life, so it resulted in the fluorescence quantum yield decreasing. To solve the problem, QDs had better to be used as early as possible when they are prepared or purchased, to keep high fluorescence quantum yield and biological activity of marker. Moreover, QDs should be stored in a dark and sealing condition.

3.3 Detection of *S. aureus* in lamb wash water

The CE of 10^5 and 10^6 CFU/mL *S. aureus* in lamb wash water was (19.35±3.38)% and (17.74±2.92)% respectively. The blank sample had no *S. aureus*. The CE was lower than that of *S. aureus* in PBS. The reason probably was the nonspecific adsorption between antibodies and some protein in food sample, so it disturbed the specific capture between antibodies and target bacteria.

Figure 4 shows the fluorescence spectra of 10^6 CFU/mL *S. aureus* in lamb wash water sample. The fluorescence peak intensity was 59.53, which was not obvious, and it was close to the blank count. One reason was that the CE of IMBs in lamb wash water was lower, another was the fluorescence quantum yield decreased. Hence, if the method were effectively applied to the detection of food sample, some simple and effective pretreatment should have been researched to reduce the interference of food substrate.

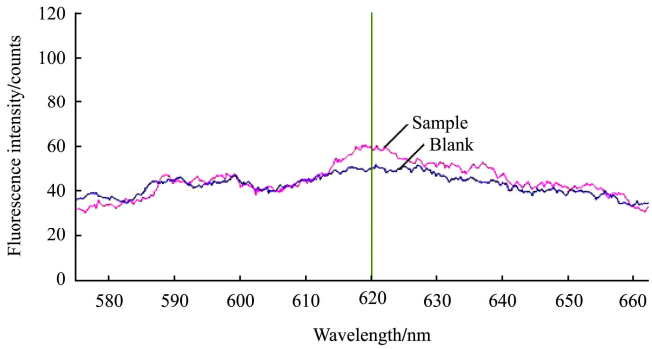

Figure 4 Fluorescence spectra obtained for samples with 10^6 CFU/mL *S. aureus* in lamb wash water

Comparing to the traditional plate count method, the fluorescent detection method saved cultivating time. The use of QDs as labels simplified the detection procedure by avoiding the use of substrate and the enzymatic reaction compared with the enzyme-linked method. As previously mentioned, QDs have several advantages to traditional synthesized fluorescent dyes, so the results could be more stable and accuracy.

There have been several reports on detection of foodborne pathogenic bacteria by conjugating nanobeads and QDs with desirable antibodies, such as *L. monocutogenes*, *S. Typhimurium*, *E. coli* O157: H7. However, little is reported on the use of *S. aureus*. The advances in this fluorescent detection method could provide quantitative detection of more foodborne pathogenic bacteria. Moreover, QDs have the properties that QDs of different sizes can be excited with a single light source, resulting in different emission peaks that can be detected simultaneously. Hence, detecting multiple bacteria in one sample by using different QDs as fluorescent labels can be realized for the future research.

4 Conclusions

The research developed an assay method based on immunomagnetic nanobeads and quantum dots as fluorescent labels for detection of *S. aureus*. The methods adopted commercially available quantum dots

with emission in the 620 nm. The total detection time can be shortened comparing with traditional plate count method and the quantitative detection model has high precision. The detection limit of the assay was 10^3 CFU/mL in PBS. The results showed that QD-based fluorescent immunoassay is a relatively rapid and selective analytical method. The detection of *S. aureus* in food sample was explored initially. The lower CE and fluorescence intensity are probably caused by the nonspecific adsorption between antibodies and some protein in food sample, so it was disturbed by the specific capture between antibodies and target bacteria. If the method were effectively applied to detect food samples, some simple and effective pretreatment should be researched to reduce the interference of food substrate. It took about 3 h to complete the assay without pretreatment and enrichment step of actual sample. In consideration of the pretreatment work, the total detection time would cost several hours probably. The further study will focus on the application of the method for detection of *S. aureus* in food sample. In addition, the multiplexed immunoassay using different QDs conjugated with different antibodies for simultaneous detecting multiple bacteria will be tried.

Acknowledgments

This research was financially supported by the Fundamental Research Funds for the Central Universities of China (No. QN2011144) and the Yangling Modern Agriculture International Institute (No. A213021005).

[References]

[1] Gracias K S, Mckillip J L. A review of conventional detection and enumeration methods for pathogenic bacteria in food. Canadian Journal of Microbiology, 2004; 50: 883-890.

[2] Zhang Y Q, Liu J X, Luo D L, Li P Y, Xu B C, Zhong X F, et al. Research advance on detection methods of *Staphylococcus aureus* in food. Academic Periodical of Farm Products Processing, 2009; 9: 84-86.

[3] Mansfield L P, Forsythe S J. The detection of *Salmonella* using a combined immunomagneticseparation and ELISA end-detection procedure. Letter Applied Microbiology, 2000; 31: 279-283.

[4] Varshney M, Li Y, Nanapanneni R, Johnson M, Griffis C L. A chemiluminescence biosensor coupled with immunomagnetic separation for rapid detection of *Salmonella* Typhimurium. Journal of Rapid Methods and Automation in Microbiology, 2003; 11: 111-131.

[5] Cloak O M, Duffy G, Sheridan J J, Mcdowell D A, Blair I S. Development of a surface adhesion immunofluorescent technique for rapid detection of *Salmonella* spp. from meat and poultry. Journal of Applied Microbiology, 1999; 86: 583.

[6] Tu S, Golden M, Andreotti P, Yu L S L, Irwin P. Applications of time-resolved fluoroimmunoassay to detect magnetic bead captured *Escherichia coli* O157: H7. Journal of Rapid Methods and Automation in Microbiology, 2001; 9: 71-84.

[7] Jaiswal J K, Simon S M. Potential and pitfalls of fluorescent quantum dots for biological imaging. Trends in Cell Biology, 2004; 14(9): 497-504.

[8] Soukka T, Harma H, Paukkunen J, Lovgren T. Utilization of kinetically enhanced monovalent binding affinity by immunoassays based on multivalent nanoparticles-antibody bioconjugation. Analytical Chemistry, 2001; 73: 2254-2260.

[9] Varshney M, Yang L, Su X, Li Y. Magnetic nanoparticle-antibody conjugates for the separation of *Escherichia coli* O157:H7 in ground beef. Journal of Food Protection, 2005; 68: 1804-1811.

[10] Bushon R N, Likirdopulos C A, Brady A M G. Comparison of immunomagnetic separation /adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and *enterococci* enumeration in wastewater. Water Research, 2009; 43: 4940-4946.

[11] Zhang H, Yee D, Wang C. Quantum dots for cancer diagnosis and therapy: Biological and clinical perspectives. Nanomedicine, 2008; 3: 83-91.

[12] Alivisatos P. The use of nanocrystals in biological detection. Nature Biotechnology, 2004; 22: 47-51.

[13] Chan W C W, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science, 1998; 281: 2016-2018.

[14] Kerman K, Endo T, Tsukamoto M, Chikae M, Takamura Y, Tamiya E. Quantum dot-based immunosensor for the detection of prostate-specific antigen using fluorescence microscopy. Talanta, 2007; 71: 1494-1499.

[15] Goldman E R, Clapp A R, Anderson G P, Uyeda H T, Mauro J M, Medintz I L, et al. Multiplexed toxin analysis using four colors of quantum dot fluororeagents. Analytical Chemistry, 2004; 76: 684-688.

[16] Akhtar R S, Latham C B, Siniscalco D, Fuccio C, Roth K A. Immunohistochemical detection with quantum dots. Methods in Molecular Biology, 2007; 374: 11-28.

[17] Dubertret B, Skourides P, Norris D J, Noireaux V, Brivanlou A H, Libchaber A. In vivo imaging of quantum dots enscapsulated in phospholipid micelles. Science, 2002; 298: 1759-1762.

[18] Larson D R, Zipfel W R, Williams R M, Clark S W, Bruchez M P, Wise F W, et al. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science, 2003; 300: 1434-1436.

[19] Zhu L, Ang S, Liu W. Quantum dots as a novel immunofluorescent detection system for *Cryptosporidium parvum* and *Giardia lamblia*. Applied & Environmental Microbiology, 2004; 70: 597-598.

[20] Su X L, Li Y B. Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. Analytical Chemistry, 2004; 76(16): 4806-4810.

[21] Dudak F C, Boyaci I H. Enumeration of immunomagnetically captured *Escherichia coli* in water samples using quantum dot-labeled antibodies. Journal of Rapid Methods and Automation in Microbiology, 2008; 16: 122-131.

[22] Yang L J, Li Y B. Quantum dots as fluorescent labels for quantitative detection of *Salmonella Typhimurium* in chicken carcass wash water. Journal of Food Protection, 2005; 68(6): 1241-1245.

[23] Wang H, Li Y B, Slavik M F. Rapid detection of *Listeria monocytogenes* using quantum dots and nanobeads-based optical biosensor. Journal of Rapid Methods and Automation in Microbiology, 2007; 15: 67-76.

[24] Wang H, Li Y B, Slavik M F. Rapid detection of *Listeria monocytogenes* in different food samples using magnetic nanobeads and a quantum dots based fluorescent immunosensor method. Biological Engineering, 2011; 4(4): 183-194.

[25] Yang L J, Li Y B. Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* using quantum dots as fluorescence labels. Analyst, 2006; 131: 394-401.

[26] Zhao Y, Ye M, Chao Q, Jia N, Ge Y, Shen H. Simultaneous detection of multifood-borne pathogenic bacteria based on functionalized quantum dots coupled with immunomagnetic separation in food samples. Journal of Agricultural and Food Chemistry, 2009; 57: 517-524.

[27] Wang H, Li Y B, Wang A, Slavik M F. Rapid, sensitive, and simultaneous detection of three foodborne pathogens using magnetic nanobead-based immunoseparation and quantum dot-based multiplex immunoassay. Journal of Food Protection, 2011; 74(12): 2039-2047.