ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 13 (5): 253-259, 2014
ISSN 1682-8356

Rapid Detection of *Campylobacter jejuni* in Poultry Products Using Quantum Dots and Nanobeads Based Fluorescent Immunoassay

Hong Wang[1], Yanbin Li[2] and Michael Slavik[1]
[1]Department of Poultry Science, [2]Department of Biological and Agricultural Engineering,
University of Arkansas, Fayetteville, AR-72701, USA

Abstract: *Campylobacter jejuni* causes 2.1 to 2.4 million cases of foodborne illnesses in the United States each year with some of the cases linked to eating undercooked poultry or handling raw poultry and poultry products. Thus, a rapid, specific method is needed to detect *C. jejuni* on poultry and poultry products. The objective of this research was to develop a sensitive immunoassay method for rapid detection of *C. jejuni* by using both magnetic nanobeads to separate and concentrate the target bacteria and quantum dots (QDs) as fluorescent markers. In this research, both streptavidin conjugated QDs 620 (8 nm diameter) and magnetic nanobeads (150 nm diameter) were separately coated with the specific biotin conjugated anti-*C. jejuni* antibody. The conjugated magnetic nanobeads then were mixed with a sample containing *C. jejuni*. After immunomagnetic separation, the magnetic nanobeads-*C. jejuni* conjugates were mixed with the conjugated QDs. Then, unattached conjugated QDs were removed using immunomagnetic separation. A spectrometer was used to measure the fluorescence of the complexes of magnetic beads-*C. jejuni*-QDs. The results showed that this method could detect *C. jejuni* in pure culture, ground turkey, chicken juice or chicken carcass wash solution at concentrations down to 2-3 cells/0.1 mL sample (20-30 cfu/mL). The total detection time was less than 2 h. This study would provide the poultry industry a more effective rapid method for detection of major foodborne pathogens in poultry products to ensure food safety.

Key words: *Campylobacter* detection, magnetic nanobeads, quantum dots, Immunoassay

INTRODUCTION

Campylobacter jejuni is one of the most important bacterial pathogens associated with foodborne diseases reported worldwide. *C. jejuni* is estimated to cause 2.4 million cases of foodborne illness in the United States each year (CDC, 2010, 2012). Many of the previous cases have been linked to eating undercooked poultry or handling raw poultry and poultry products. Studies have found *Campylobacter* contamination of up to 88% of chicken carcasses (Clark, 2013; FDA, 2013). The development of rapid, sensitive and specific methods for detection of food-associated bacterial pathogens remains challenging for ensuring food safety. Immunoassays are one category of rapid methods that have been developed for detection of pathogenic bacteria in food, environmental and clinical samples. Although conventional culture methods hypothetically allow the detection of a single cell of specific pathogens, they are extremely time-consuming, typically requiring at least 24 h and complicated multi-steps to confirm the analysis. Even current, well studied rapid methods such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) can take 10 to 24 h and 4 to 6 h, respectively, with detection limits varying from 10^2 to 10^3 cfu/mL.

Quantum dots (QDs) as colloidal nanocrystalline semiconductor were first studied and reported in 1998 (Bruchez *et al*., 1998; Chan and Nie, 1998). QDs are composed of CdSe/ZnS core/shell nanopaticles. The inorganic fluoropes QDs have advantages over traditional organic dyes and fluorescent proteins due to their high fluorescence quantum yields, their excellent stability against photobleaching and chemical degradation, and their properties of broad absorption spectra, narrow-emission spectra (Jaiswal *et al*., 2003; Kloepfer *et al*., 2005; Shao *et al*., 2011). Hahn *et al*. (2005) reported that under continuous excitation, QDs retain high fluorescence intensities for hours whereas the typical organic dye bleaches within seconds. Water soluble QDs are suitable for biological detection and biomolecular conjugation. Desirable biological molecules can be coupled to QDs and retain their biological activities, which leads to the development of molecular conjugates of QDs that are biocompatible and suitable for use in cell biology and immunoassays. Researchers have investigated the applications of QDs for biological detection of cellular imaging (Chalmers *et al*., 2007; Jaiswal and Simon, 2004) and bacteria (Su and Li, 2004; Yang and Li, 2005, 2006; Xue *et al*., 2009; Wang *et al*., 2011).

Corresponding Author: Hong Wang, Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

Magnetic nanobeads (MNBs) are well-established core-shell nanoparticles with controlled size. The magnetic core consisted of Fe_3O_4 has an external magnetic force for the immunomagnetic separation. Nanobeads exhibit properties of ferrofluids and remain stable colloids (Lekowska-Kochaniak *et al*., 2002; Liu *et al*., 2003). Currently, researchers conjugated MNBs with antibodies, aptamers, antibiotics, probes, proteins and dyes for bacterial separation and detection, protein purification and toxin decorporation (Gu *et al*., 2006; Varshney and Li, 2007; Gao *et al*., 2009; Göransson *et al*., 2010).

In this research, we successfully developed a sensitive immunoassay method using magnetic nanobeads and quantum dots for rapid detection of *C. jejuni* in pure culture, chicken juice, ground turkey or chicken carcass wash solution.

MATERIALS AND METHODS

Pathogenic bacterial cultures and surface plating methods: Stock cultures of *C. jejuni* (ATCC 29428) purchased from American Type Culture Collection (Manassas, VA) were grown for 18-20 h at 42°C in *Campylobacter* enrichment broth (Remel, Lenexa, KS). Serial 10-fold dilutions were made in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) and the viable cell numbers of *C. jejuni* were determined by surface plating on *Campylobacter* enrichment selective agars (Remel, Lenexa, KS). Stock cultures of *S.* Typhimurium (ATCC 14028), *L. monocytogenes* (ATCC 43251) and *E. coli* O157:H7 (ATCC 43888) purchased from American Type Culture Collection (Manassas, VA) were grown for 18-20 h at 37°C in brain heart infusion (BHI) broth (Remel, Lenexa, KS). After serial 10-fold dilutions in PBS, The viable cell numbers of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* were determined by surface plating on selective agars.

Preparation of immunomagnetic nanobeads and Qds: Biotinylated rabbit anti-*C. jejuni* polyclone antibody (4-5 mg/ml) obtained from Thermo Scientific (Rockford, IL). A 1:10 dilution of anti-Campylobacter antibody was prepared with PBS (0.01 M, pH 7.4) for further use. Streptavidin captivate ferrofluid conjugated magnetic nanobeads (MNBs 150 nm diameter) purchased from R&D Systems (Minneapolis, MN) were coated with the anti-*C. jejuni* antibodies. To avoid using extra reagents, the ratio of the streptavidin conjugated magnetic nanobeads and biotinylated antibody were calculated according their binding sets. In this step, 20 µL of immunomagnetic nanobeads were mixed with 20 µL biotin-conjugated rabbit anti-*Campylobacter* antibody (0.5 mg/mL). Streptavidin conjugated quantum dots (QDs) 620 (1 µM, 8 nm diameter) purchased from Ocean Nanotech (San Diego, CA) were coated with the biotin labeled anti-*C. jejuni* by biotin-streptavidin complex. The ratio of the streptavidin conjugated QDs and biotinylated antibodies were tested to minimize the materials used. 20 µL of 1:3 diluted QDs 620 were mixed with 20 µL biotin-conjugated rabbit anti-*Campylobacter* antibody (0.5 mg/ml). Both MNBs-antibody and QDs-antibody mixtures were shaken on a RKVSD 10101 mixer (ART, Inc., Laurel, MD) at a speed of 10 rpm at room temperature for 30 min.

Inoculation of chicken juice, chicken carcass wash and ground turkey wash solutions: For preparation of chicken juice, commercially frozen chickens without giblets were placed in a container and thawed over night at room temperature. Chicken juice was obtained from each chicken and mixed together (Birk *et al*., 2004). The mixed chicken juice was subsequently cleared by centrifugation to eliminate large particles, by sterilized filtration and was then stored at -20°C. Before use, the sterilized chicken juice was thawed at 4°C overnight. Post-chilled chicken carcasses were individually washed with 100 mL of 0.1% buffered peptone water (BPW) by hand shaking for 1 min and the wash solutions were collected. Each of 25 g samples of ground turkey (purchased from local grocery store) was mixed with 225 mL of 0.1% PBW and stomacher washed for 1 min. The wash solutions were collected. All chicken juice, chicken carcass wash and ground turkey wash solutions were divided into two portions. One portion was inoculated with various dilutions of *C. jejuni* broth cultures to obtain final inoculation concentrations of 10^1 to 10^4 cfu/mL wash solution during the testing day. The second portion was not inoculated with the bacterial culture and served as controls. The final inoculation concentrations from 10^1 to 10^3 cfu/mL and controls were confirmed with the tests using bacterial plating methods.

Assay procedure: The assay procedure is outlined in Fig. 1. First, 100 µL of the each diluted 10^1-10^3 cfu/mL cultures was mixed with 40 µL antibody coated magnetic nanobeads (MNBs-Ab) for 30 min. The MNBs-Ab-cell conjugates were separated from the solution by putting the tube on a magnetic separator for 2 min and then removing the liquid with a syringe. After washing twice with PBS, the captured cells were mixed with 40 µL anti-*C. jejuni* antibody coated QDs 620 (QDs-Ab) for another 30 min. After removing excess QDs solution by immunoseparation, the final conjugates MNBs-*C. jejuni*-QDs were ready to be measured by a spectrometer. 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. Fluorescence intensity produced by Qds 620 gave a fluorescent peak at 620

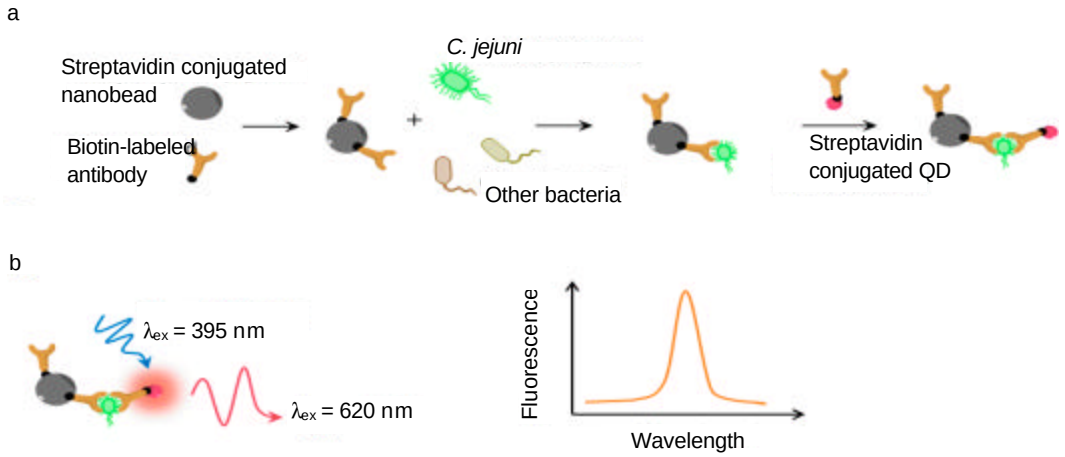

Fig. 1(a-b): Procedure of the immunoassay for detection of *Campylobacter jejuni*: (a) Schematic diagram of the detection procedure. Biotinylated rabbit anti-*C. jejuni* antibody coated streptavidin captivate ferrofluid conjugated MNBs were used for capturing *C. jejuni* cells from the samples. The biotinylated rabbit anti-*C. jejuni* antibody coated streptavidin conjugated QDs were used as fluorescence labels. (B) The *C. jejuni* were detected through the spectrometer measurement of fluorescence intensity produced by bound QDs

nm when QDs were excited at 395 nm wavelength. The fluorescent peaks of interference reaction signals increased with the increasing cell number of target cells. Separation of target bacteria from multiple bacteria in a pure culture was tested for the recovery rate, as well as detection limit. All tests were duplicated.

Test for possible interfering with multiple bacteria: The immunoassay was tested for possible cross-reaction with other foodborne bacteria including *S.* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7. Those bacteria were tested individually and in combination with the target *C. jejuni*.

Statistical analysis: The results of experiments on the detection of *C. jejuni* at different concentrations in pure culture and three different poultry samples were compared individually to those of negative controls. The means and standard deviations of measured florescent intensity in each test were calculated and statistically evaluated by means of one-way analysis of variance and Student's t-test for multiple comparisons among all concentrations of *C. jejuni* using JMP®10 statistical software (SAS Institute, Inc., Cary, NC). In comparison, the means were considered to be significantly different when $p<0.1$. Coefficient of determination R^2 represent the linear relationships of the change in fluorescent intensity vs. *C. jejuni* at concentrations ranging from 0 to 10^2 cells/0.1 mL in all samples were calculated using Excel.

RESULTS AND DISCUSSION

Tests for possible interfering with mixed multiple bacteria: The results of the bacterial interference test are shown in Fig. 2. All *S.* Typhimurim, *L.*

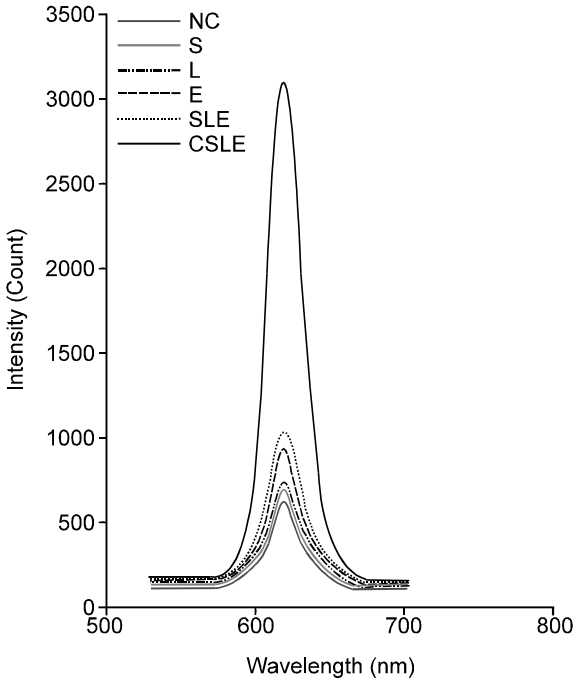

Fig. 2: Representative fluorescence spectra obtained for the mixed bacterial cultures: samples of negative control (NC), *S.* Typhimurium (S) at level of $6x10^2$ cfu/0.1mL, *L. monocytogenes* (L) at levels of $2x10^2$ cfu/0.1 ml, *E. coli* O157:H7 (E) at levels of $3x10^2$ cells/0.1 mL, mixture of *S.* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7 (SLE) at levels of $2x10^2$ cfu/0.1 mL and the mixture of SLE cultures with *C. jejuni* (CSLE) at levels $1x10^2$ cfu/0.1 mL

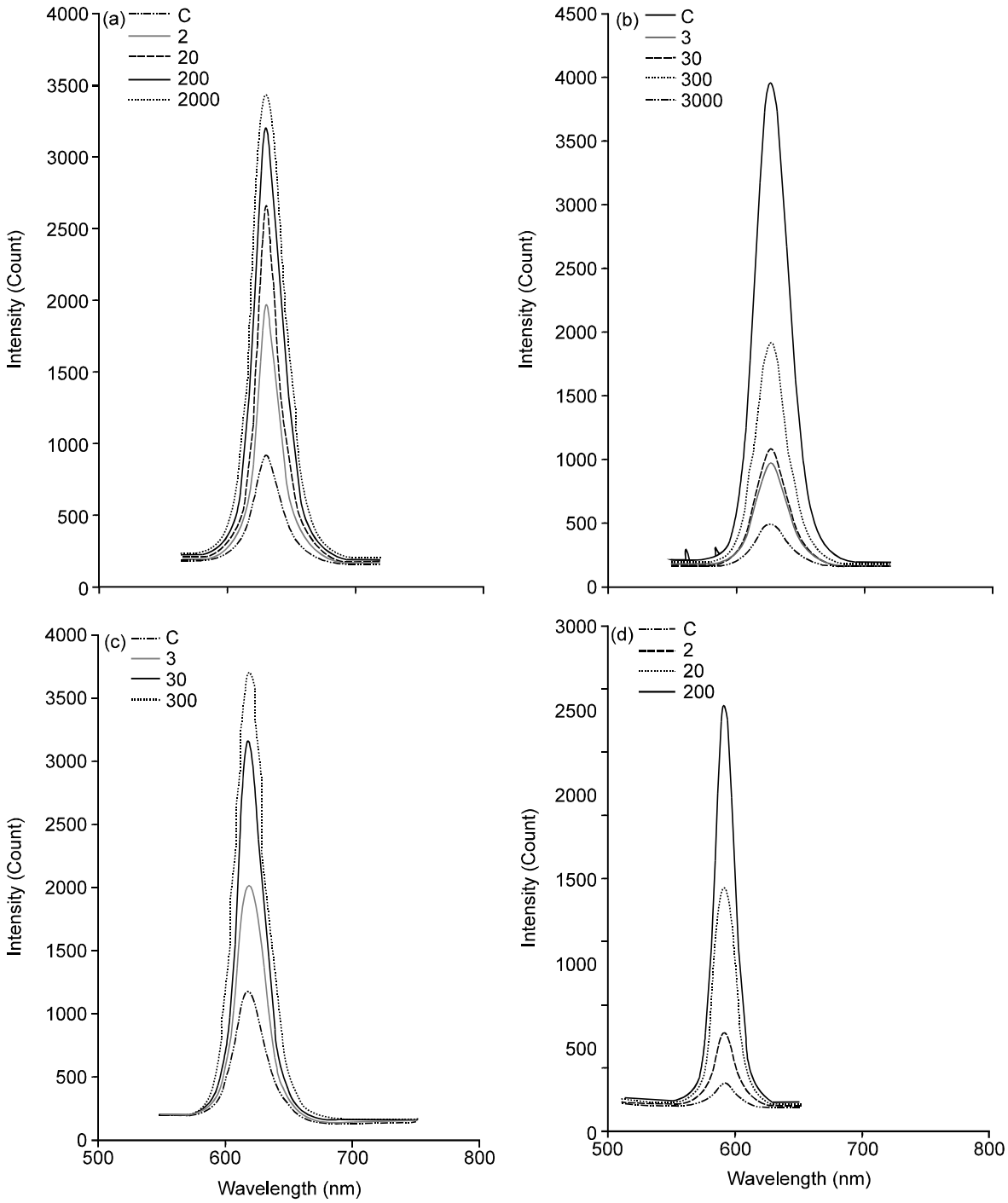

Fig. 3(a-d): (a) Representative fluorescence spectra obtained for samples negative control and different *C. jejuni* in PBS at levels of 2 $x10^0$, $2x10^1$, $2x10^2$ and $2x10^3$ cells/0.1 mL in pure culture; (b) Representative fluorescence spectra obtained for samples containing *C. jejuni* at concentrations of 0 (or negative control), $3x10^0$, $3x10^1$, $3x10^2$ and $3x10^3$ cells/0.1 mL in ground turkey solutions; (c) Representative fluorescence spectra obtained for samples containing *C. jejuni* at concentrations of 0 (or negative control), $3x10^0$, $3x10^1$ and $3x10^2$ cells/0.1 mL in wash chicken carcass solutions; and (d) Representative fluorescence spectra obtained for samples containing *C. jejuni* at concentrations of 0 (or negative control), $2x10^0$, $2x10^1$ and $2x10^2$ cells/0.1 mL in chicken juice solutions

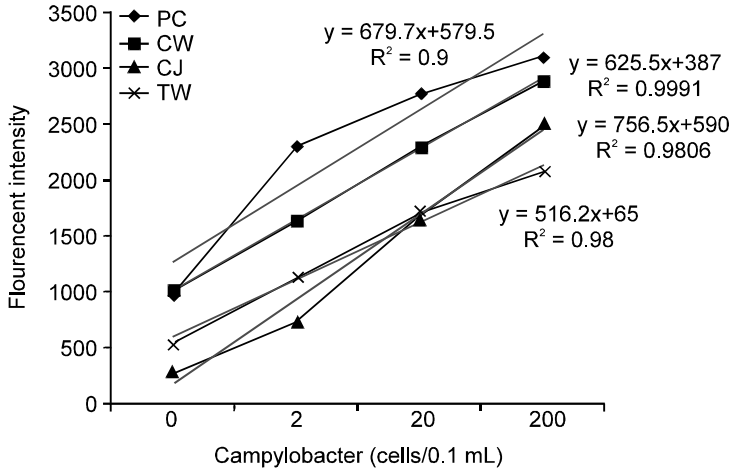

Fig. 4: Means of fluorescent detection data at different concentrations of *C. jejuni* in different samples. Coefficient of determination R^2 = 0.90, 0.99, 0.98 and 0.98 represent the linear relationships of the change in fluorescent intensity vs. *C. jejuni* at concentrations ranging from 0 to 10^2 cells/0.1 mL in pure culture (PC), chicken wash (CW), chicken juice (CJ) and ground turkey wash (TW) solution samples with fluorescent intensities

monocytogenes and *E. coli* individual or mixed cultures show low fluorescent signal similar to the negative control. Only the mixtures with the target *C. jejuni* culture show above 2000 counts fluorescent intensities.

Detection of *C. jejuni* from pure cultures and different poultry samples: Figure 3(a) shows the results of the experiments to enumerate cells of *C. jejuni* in pure culture. The difference of fluorescent intensities for the lowest detection concentrations, 1-2 cells/0.1 mL of *C. jejuni* cells, above their background fluorescent signal at 620 nm are over 1000 counts. The results of the experiments to enumerate cells of *C. jejuni* in ground turkey wash solution are shown in Fig. 3(b). The difference of fluorescent intensities for the lowest detection concentrations, 1-3 cells/0.1 mL of *C. jejuni* cells, above their background fluorescent signal at 620 nm are close to 1000 counts. Figure 3(c) shows the results of the experiments to enumerate cells of *C. jejuni* in chicken carcasses wash solution. The difference of fluorescent intensities for the lowest detection concentrations, 1-3 cells/0.1 mL of *C. jejuni* cells, above their background fluorescent signal at 620 nm are 800 counts. The results of the experiments to enumerate cells of *C. jejuni* in chicken juice are shown in Fig. 3(d). The difference of fluorescent intensities for the lowest detection concentrations, 1-2 cells/0.1 mL of *C. jejuni* cells, above their background fluorescent signal at 00620 nm are 900 counts.

Figure 4 shows the means of fluorescent detection data at different concentrations of *C. jejuni* for each sample. It can be seen that the fluorescence intensity increased with the increasing cell number of *Campylobacters* from 2-3x10^0 to 10^3 cells/0.1 mL. These results demonstrated that the more bacterial cells in the sample, the greater amount of the QDs they could bind and, thus, the stronger fluorescence signal they produced. Linear relationships are found for all of different poultry samples with R^2 = 0.90 to 0.99.

The use of magnetic nanobeads for bacterial separation and concentration in combination with QDs as fluorescent labels provides a method that gives the quantitative detection of *C. jejuni*. Conjugating nanobeads and QDs with desirable antibodies that are specific to target bacteria resulted in a detection method that is both sensitive and specific for *C. jejuni* in pure culture, chicken juice, chicken wash and ground turkey wash solutions at a concentration of 2-3 cells/0.1 mL sample (20-30 cfu/mL). The difference of fluorescent intensities for the lowest detection concentrations, 1-3 cells/0.1 mL of *Campylobacter* cells, above their background fluorescent signal at 620 nm are from 800 to 1000 counts. Possible cross-reactivity against *S.* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7 was tested and no interference spectrum showed up. Fluorescence intensity increased with the increasing cell number of *C. jejuni* and linear relationships were determined with R^2>0.90. Results demonstrated that the more bacterial cells in the sample, the greater amount of the QDs they could bind and, thus, the stronger fluorescent signal they could produce. The detection time from sampling through a result report was within 2 h. The fluorescence intensities for different concentrations of *C. jejuni* in each type of poultry samples are significantly different ($p<0.1$).

Campylobacter jejuni is the most common *Campylobacter* species associated with foodborne illness. Conventional microbiological methods to identify *C. jejuni* are not only tedious and time-consuming, but also prone to misdiagnoses due to the viable but

nonculturable (VBNC) state (Tholozan *et al*., 1999; Ziprin *et al*., 2003; Lin *et al*., 2008; Melero *et al*., 2011). Molecular method based PCR and real-time PCR assays may provide an alternative to culture methods for the detection of *C. jejuni*. However, those assays either need 24 to 48 h enrichment (Sails *et al*., 2003; Oliveira *et al*., 2005; Mayr *et al*., 2010) or only work at high detection limits (LaGier *et al*., 2004; Debretsion *et al*., 2007; Leblanc-Maridor *et al*., 2011). Immunoassays using magnetic nanobeads to capture bacteria from food samples in combination with quantum dots as a fluorescent label have been studied for quantitative detection of *E. coli* O157:H7 (Wang *et al*., 2011b), *Listeria monocytogenes* (Wang *et al*., 2007; 2011a, b), *Salmonella* (Joo *et al*., 2012) and *Staphyloccocus aureus* (Chen and Zhang, 2012). The development of a quantum dots and magnetic nanobeads based immunoassay for rapid and sensitive detection of *C. jejuni* described in this study is the first quantum dots immunoassay used to detect *C. jejuni* from poultry samples.

Conclusion: Our research demonstrates a rapid, specific method to detect *C. jejuni* in poultry samples using the magnetic nanobeads to separate and concentrate the target bacteria and the quantum dots as fluorescent labels. The detection limit of 2-3 cells/0.1 mL achieved within 2 h in this study is comparable with most rapid methods that have been investigated in recent years.

ACKNOWLEDGMENTS

This research was supported in part by Arkansas Biosciences Institute. The authors thank Ocean Nanotech, LLC, for providing QDs used in this research.

REFERENCES

Birk, T., H. Ingmer, M.T. Andersen, K. Jørgensen and L. Brøndsted, 2004. Chicken juice, a food-based model system suitable to study survival of *Campylobacter jejuni*. Lett. Appl. Microbiol., 38: 66-71.

Bruchez, M., M. Moronne, P. Gin, S. Weiss and A.P. Alivisatos, 1998. Semiconductor nanocrystals as fluorescent biological labels. Sci., 281: 2013-2015.

CDC, 2010. *Campylobacter*, General information-NCZVED. http://www.cdc.gov/nczved/ divisions/ dfbmd/diseases/campylobacter.

CDC, 2012. Trends in foodborne illness, 1996-2010. http://www.cdc.gov/ foodsafety.

Chalmers, N.I., R.J. Palmer, L. Du-Thumm, R. Sullivan, W. Shi and P.E. Kolenbrander, 2007. Use of quantum dot luminescent probes to achieve single-cell resolution of human oral bacteria in biofilms. Appl. Environ. Microbiol., 73: 630-636.

Chan, W.C.W. and S. Nie, 1998. Quantum dot biocojugates for ultrasensitive nonisotopic detection. Sci. 281: 2016-2018.

Chen, L. and J. Zheng. 2012. Bioconjugated magnetic nanoparticles for rapid capture of gram-positive bacteria. J. Biosens. Bioelectron, S:11:005.

Clark, M., 2013. *Salmonella*-Foodborne illness. 2005-2013 Out Break, Inc.

Debretsion, A., T. Habtemariam, S. Wilson, D. Nganwa and T. Yehualaeshet. 2007. Real-time PCR assay for rapid detection and quantification of *Campylobacter jejuni* on chicken rinses from poultry processing plant. Molec. Cell. Prob., 21: 177-181.

FDA, 2013. Bad Bug Book: Foodborne pathogenic microorganisms ans natural toxins handbook-*Campylobacter jejuni*. January 18, 2013. http://www.fdc.gov/food/foodsafety/foodborneillness.

Gao, J., H. Gu and B. Xu, 2009. Multifunctional magnetic nanoparticles: design, synthesis and biomedical applications. Accoun. Chem. Res., 42: 1097-1107.

Göransson, J., T.Z.G. De La Torre, M. Strömbery, C. Russell, P. Svedlinedh, M. Strømme and M. Nilsson, 2010. Sensitive detection of bacterial DNA by magnetic nanoparticles. Lett. Anal. Chem., 82: 9138-9140.

Gu, H., K. Xu, C. Xu and B. Xu, 2006. Biofunctional magnetic nanoparticles for protein separation and pathogen detection. Chem. Com., 941-949.

Hahn, M.A., J.S. Tabb and T.D. Krauss, 2005. Detection of single bacterial pathogens with semiconductor quantum dots. Anal. Chem., 77: 4861-4869.

Jaiswal, J.K., H. Mattoussi, J.M. Mauro and S.M. Simon, 2003. Long-term multiple color imaging of live cells using quantum dot bioconjugates. Natu. Biol., 21: 47-51.

Jaiswal, J.K. and S.M. Simon, 2004. Potential and pitfalls of fluorescent quantum dots for biological imaging. TREN. Cell Bio., 14: 497-504.

Joo, J., C. Yim, D. Kwon, J. Lee, H.H. Shin, H.J. Cha and S. Jeon, 2012. A facile and sensitive detection of pathogenic bacteria using magnetic nanoparticles and optical nanocrystal probs. Anal., 137: 3609-3612.

Kloepfer, J.A., R.E. Mielker and J.L. Nadeau, 2005. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. Appl. Environ. Microbiol., 5: 2548-2557.

LaGier, M.L., L.A. Joseph, T.V. Passaretti, K.A. Musser and N.M. Cirino, 2004. A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. Molecul. Cell. Prob., 18: 275-282.

Leblanc-Maridor, M., F. beaudeau, H. Seegers, M. Denis and C. Belloc, 2011. Rapid identification and quantification of *Campylobacter coli* and *Campylobacter jejuni* by real-time PCR in pure cultures and in complex samples. BMC Microbiol., 11: 113.

Lekowska-Kochaniak, A., D. Czajkowska and J. Popowski, 2002. Detection of *Escherichia coli* O157:H7 in raw meat by immunomagnetic separation and multiplex PCR. Acta Microbiol. Pol., 51: 327-337.

Lin, S., X. wang, H. Zhang, Z. Mao and B. Jiang, 2008. Direct detection of *Campylobacter jejuni* in human stool samples by real-time PCR. Can. J. Microbiol., 54: 742-747.

Liu, Y., J. Ye and Y. Li, 2003. Rapid detection of *E. coli* O157:H7 in ground beef, chicken carcass and lettuce samples using an immunomagnetic chemiluminescence fiber optic biosensor. J. Food Prot., 66: 512-517.

Mayr, A.M., S. Lick, J. Bauer, D. Thärigen, U. Busch and I. Huber, 2010. Rapid detection and differentiation of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* in food, using multiplex real-time PCR. J. Food. Prot., 2: 241-250.

Melero, B., L. Cocolin, K. Rantsiou, I. Jaime and J. Rovira, 2011. Comparison between conventional and qPCR methods for enumerating *Campylobacter jejuni* in a poultry processing plant. Food Microbiol., 28: 1353-1358.

Oliveira, T.C.R.M., S. Barbut and M.W. Griffiths, 2005. Detection of *Campylobacter jejuni* in naturally contaminated chicken skin by melting peak analysis of amplicons in real-time PCR. Int. J. Food Microbiol., 104: 105-111.

Sails, A.D., A.J. Fox, F.J. Bolton, D.R.A. Wareing and D.L.A. Greenway, 2003. A real-time PCR assay for detection of *Campylobacter jejuni* in foods after enrichment culture. Appl. Environ. Microbiol., 3: 1383-1390.

Shao, L., Y. Gao and F. Yan, 2011. Semiconductor quantum dots for biomedical application. Sensor, 11: 11736-11751.

Su, X. and Y. Li, 2004. Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. Anal. Chem., 76: 4806-4810.

Tholozan, J.L., J.M. Cappelier, J.P. Tissier, G. Delattre and M. Federighi, 1999. Physiological characterization of viable-but-nonculturable Campylobacter jejuni cells. Appl. Environ. Microbiol. 65: 1110-1116.

Varshney, M. and Y. Li, 2007. Interdigitated array microelectrodes based impedance biosensors coupled with magnetic nanoparticle-antibody conjugates for detection of *Escherichia coli* O157:H7 in food samples. Biosens. Bioelectron, 22: 2408-2414.

Wang, H., Li, Y. and M.F. Slavik, 2007. Rapid detection of *Listeria monocytogenes* using quantum dots and nanobeads based optical biosensor. J. Rapi. Meth. Auto. Microbiol., 15: 67-76.

Wang, H., Li, Y. and M.F. Slavik, 2011a. Rapid detection of *Listeria monocytogenes* in different food samples using magnetic nanobeads and quantum dots based fluorescent immunoassay. Biol. Eng. Trans, 4: 183-194.

Wang, H, Y. Li, A. Wang and M. Slavik, 2011b. Rapid, sensitive and simultaneous detection of three foodborne pathogens using magnetic nanobead immunoseparation and quantum dot based multiplex immunoassay. J. Food Prot., 74: 2039-2047.

Wang, Q., F. Ye, T. Fang, W. Niu, P. Liu, X. Min and X. Li, 2011. Bovine serum albumin-directed synthesis of biocompatible CdSe quantum dots and bacteria labeling. J. Coll. Int. Sci., 355: 9-14.

Xue, X., J. Pan, H. Xie, J. Wang and S. Zhang, 2009. Fluorescence detection of total count of *Escherichia coli* and *Staphylococcus aureus* on water-soluble CdSe quantum dots coupled with bacteria. Talan. 77: 1808-1813.

Yang, L. and Y. Li, 2005. Quantum dots as fluorescent labels for quantitative detection of *Salmonella* Typhimurium in chicken carcass wash water. J. Food Prot., 68: 1241-1245.

Yang, L. and Y. Li, 2006. Quantum dot bioconjugates for simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Anal., 131: 394-401.

Ziprin, R.L., R.E. Droleskey, M.E. Hume and R.B. Harvey, 2003. Failure of viable nonculturable Campylobacter jejuni to colonize the cecum of newly hatched leghorn chicks. Avian Dis., 47: 753-758.