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Rapid Detection of *Campylobacter jejuni* in Poultry Products Using QD-FRET Based Fluoroimmunoassay

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Abstract: *Campylobacter jejuni* is one of the leading causes of foodborne human gastrointestinal disease worldwide. Poultry and poultry products have been identified as the major transmission routes to humans for this pathogenic bacterium. The objective of this research was to develop a rapid and sensitive method for detection of *C. jejuni* in chicken carcasses and ground turkey using quantum dots (QDs)-fluorescence resonance energy transfer (FRET) based fluoroimmunoassay. In the experiments, two multicolor QDs with the emission wavelengths of 530 nm (QDs 530) and 580 nm (QDs 580) were conjugated with rabbit anti-*C. jejuni* antibody (primary antibody Ab1) and goat anti-rabbit IgG antibody (secondary antibody Ab2) to serve as energy donors and acceptors, respectively. When the conjugated QDs 530 and QDs 580 were mixed, energy transfer occurred through FRET, causing the detectable fluorescence signal of the acceptor to be increased. When *C. jejuni* was present, donors would bind to the target bacteria and then the acceptors could bind to the donors, resulting in FRET in signal transduction. Poultry samples were spiked with *C. jejuni* at different concentrations and then, the magnetic nanobeads (MNB) coated with anti-*C. jejuni* antibody were added to the samples to capture the target bacteria. After magnetically separating the MNB-*C. jejuni* complexes from food matrices, the complexes were mixed with the QDs 530 and incubated for 30 min. Finally, the QDs 580 were added to bind the QDs 530 for FRET and the fluorescent intensity was measured using a spectrophotometer. The results showed that the fluoroimmunoassay could rapidly detect *C. jejuni* in poultry products with a detection limit of 30-50 cfu/ml and the total detection time of less than 2 hours. This highly adaptive and flexible technique could provide the poultry industry a more rapid and effective method for detection of major foodborne pathogens in poultry products.

Key words: QD-FRET, fluoroimmunoassay, *Campylobacter jejuni*, rapid detection

INTRODUCTION

Campylobacter jejuni is one of the most important bacterial pathogens associated with foodborne diseases reported worldwide (CDC, 2014; FSA, 2014). *Campylobacter*, especially *C. jejuni* is estimated to cause 1.3 million cases of foodborne illness in the United States each year (CDC, 2014). Most cases of human campylobacteriosis are associated with eating undercooked poultry meat or handling raw poultry and poultry products (Boysen *et al*., 2013; CDC, 2014; FSA, 2014; Rosenquist *et al*., 2013). The development of rapid, specific and sensitive methods for detection of *C. jejuni* in chickens and chicken products is therefore vital to ensure food safety. Fluoroimmunoassays due to their specificity and simplicity still represent the mainstream of the biosensor methods reported for detection of foodborne pathogens. Semiconductor nanocrystals, or quantum dots (QDs), are among the most exciting and ubiquitous discoveries that come out of the fluorescent nano-biosensing technique field (Chinnathambi *et al*., 2014; Kirsch *et al*., 2013; Samir *et al*., 2012; Zhang and Wang, 2012). The inorganic fluoropes QDs have advantages over traditional organic fluorophores due to their unique optical properties such as high fluorescence quantum yields, longer fluorescent lifetime, better photostability, as well as broad absorption spectrum and narrow-emission spectrum (Shao *et al*., 2011). 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 (Xue *et al*., 2009; Wang *et al*., 2014a,b). In addition, the more advanced quantum dot fluorescence resonance energy transfer (QD-FRET) sensors may be designed to achieve highly sensitive detection. FRET is an electrodynamic phenomenon that occurs through non-radiative energy transfer process whereby the electronic excitation energy of a donor fluorophore to a suitable

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acceptor molecule through near-field dipole-dipole interactions. The acceptor must absorb energy at the emission wavelength of the donor, but does not necessarily have to remit the energy fluorescently itself (Hussain *et al*., 2015; Sapsford *et al*., 2006). The Förster energy transfer involves neither photon emission nor molecular contact between the donor and acceptor, but is highly dependent on the distance between them. It is generally most efficient when the distance between donors and acceptors is in 10-100A range or an overlap between the emission spectrum of the donor and the absorption spectrum of acceptor. The Förster distance is defined as the distance between the donor and acceptor at which energy transfer efficiency is 50% (Loura and Prieto, 2011). QDs have highly advantageous properties to serve as FRET donors compared to organic fluorophores include their strong emission multiplexing abilities as well as the option to choose the QDs with the emission wavelength more suitable for the available acceptors (Samir *et al*., 2012). Recently, QD-FRET based fluoroimmunoassay has been reported to detect pathogenic bacteria in food, environmental and clinical samples (Ramadurai *et al*., 2008; Boas, 2011; Kattke *et al*., 2011; Saraheimo *et al*., 2013; Shamirian *et al*., 2015; Wang *et al*., 2015). Therefore, the objective of this research was to develop a sensitive QD-FRET based fluoroimmunoassay method by using two different sizes of QDs served as fluorescence energy transfer donor-acceptor pair for rapid detection of *C. jejuni* in pure culture, chicken carcass wash and ground turkey.

MATERIALS AND METHODS

Bacteria culture: Stock cultures of *Campylobacter jejuni* (ATCC 29428) purchased from American Type Culture Collection (Manassas, VA) were grown at 42°C for 20-24 h in *Campylobacter* enrichment broth (Neogen, Lansing, MI) under anaerobic container with a DB GasPak™ EZ gas generating systems (Becton, Dickson and company, Sparks, ML). Serial 10-fold dilutions were made in phosphate-buffered saline (PBS) and the viable cell numbers of *C. jejuni* were determined by surface plating on *Campylobacter* enrichment selective agar (Neogen, Lansing, MI).

Preparation of immunomagnetic nanobeads and QDs: Biotinylated rabbit anti-*C. jejuni* polyclone antibody (4-5 mg/ml) obtained from Thermo Scientific (Rockford, IL) and biotin conjugated goat anti-rabbit IgG antibody (1 mg/ml) obtained from LifeSpan BioSciences (LSBiO, Seattle, WA) were 1:10 diluted with PBS (0.01 M, pH 7.4) for further use. Both streptavidin conjugated magnetic nanobeads (MNBs, 1 mg/ml, 150 nm diameter) and streptavidin conjugated quantum dots (QDs) 530 nm (1 μM), purchased from Ocean Nanotech (San Diego, CA), were separately coated with biotinylated rabbit anti-*C. jejuni* polyclone antibody (Ab1, 0.5 mg/ml). QDs 580 nm (1 μM) purchased from Ocean Nanotech (San Diego, CA) were coated with biotinylated goat anti-rabbit IgG secondary antibody (Ab2, 0.1 mg/ml). To avoid using extra reagents, the ratio of the streptavidin conjugated magnetic nanobeads and biotinylated antibodies were calculated according their binding set to minimize the materials used.

In this step, 30 μl of MNBs were mixed with 20 μl Ab1 and the 20 μl of QDs 530 were coated with the 20 μl Ab1 by biotin-streptavidin complex. The MNBs-antibody conjugates were magnetically separated from the solution by placing the tubes on a magnetic separator and washing to remove un-conjugated antibody. Each of 20 μl QDs 580 was coated with 40 μl of Ab2. All MNBs-Ab1, QDs-Ab1, or QDs-Ab2 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 carcasses wash and ground turkey: Post-chilled chicken carcasses (purchased from a local grocery store) were individually washed with 100 ml of 0.1% buffered pepton water (BPW) and the wash solutions were collected. Each of 25 g samples of ground turkey (purchased from a local grocery store) was mixed with 225 ml of 0.1% BPW and stomached for 1 min. The solutions were collected. Both chicken carcasses wash and ground turkey stomaching solutions were inoculated with *C. jejuni* at different concentrations on the test day. The uninoculated solutions were served as negative controls.

Experimental design of QD-FRET tests: The QD-FRET experimental procedure is outlined in Fig. 1. First, 100 μl of each inoculated *C. jejuni* samples at 0-10^2 CFU/100 μl tubes was mixed with 50 μl of MNBs-Abs solution for 30 min. The MNBs-Ab-cell complexes were collected by a magnetic separator and washed twice to remove un-bound target cells. The MNBs-Ab-cell complexes were then mixed with 40 μl of anti-*C. jejuni* antibody-coated QDs 530 for 30 min. After magnetic separation removing excess QDs solution, the samples were mixed with goat anti-rabbit IgG antibodies coated QDs 580. After magnetic separation, the samples were ready for detection of target *C. jejuni*.

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, Dunedin, FL) and a probe/cuvette holder housed in a dark box. The fluorescence measurement setup is shown in Fig. 2 (a-b). All tests were duplicated.

Statistical analysis: Three types of samples were tested: bacterial cultures, chicken carcasses and ground turkey. For each type of samples, four concentrations of *C. jejuni* at 0, 3-5 x 10^0, 10^1 and 10^2 cfu/0.1 ml were used for inoculation. All tests were repeated 2 times.

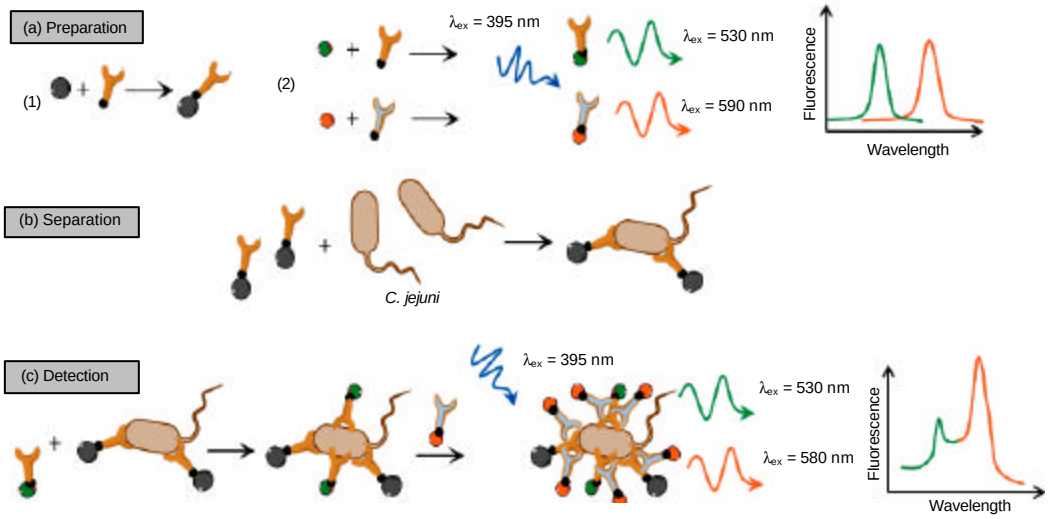

Fig. 1: Procedure of QD-FRET based fluoroimmunoassay for detection of target *C. jejuni*. In the experiment preparation (a), both streptavidin conjugated MNBs and QDs 530 were coated with biotin conjugated anti-*C. jejuni* antibodies (Ab1) and QD 580 was coated with biotin conjugated goat anti-rabbit IgG antibodies (Ab2); The MNBs-Ab1 then mixed with *C. jejuni* in different solutions as shown in (b). After reacting with QDs 530-Ab1 and QDs 580-Ab2, the samples were measured by a spectrometer as shown in (c)

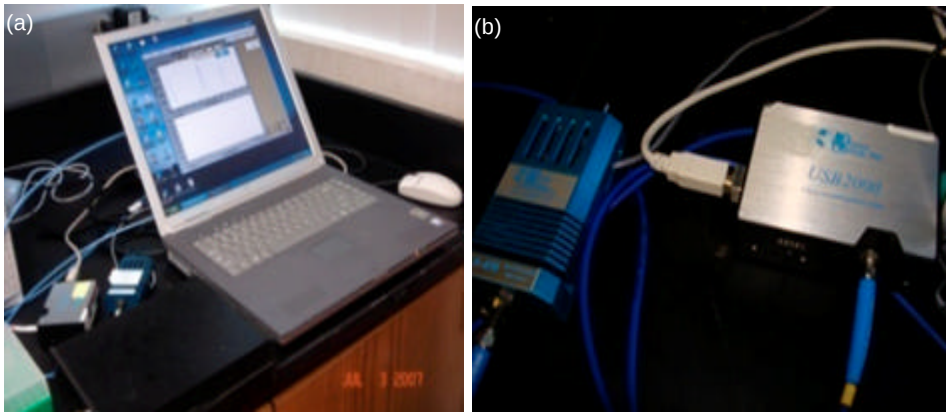

Fig. 2: (a) Setup of the laptop controlled portable system and (b) the light source and spectrometer

The results of experiments on the detection of *C. jejuni* at different concentrations in pure culture and two 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. In comparison, the means were considered to be significantly different when $p<0.05$. 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 Microsoft Excel.

RESULTS AND DISCUSSION

QDs-FRET based fluoroimmunoassay: The emission spectra of unbound QDs 530 and 580 are shown in Fig. 3a and the bound QDs 530 and 580 are shown in Fig. 3b. The unbound QDs 530 and 580 have their individual emission spectrum at wavelengths 530 and 580 nm with fluorescent intensities 1200 and 1400 counts. When both QDs are bound together by the QDs 530 conjugated with antibody and the QDs 580 conjugated with anti-antibody, the QD-FRET occurs by transferring the electronic excitation energy from the donor (QDs 530) to the acceptor (QDs 580). The receptor (QDs 580)

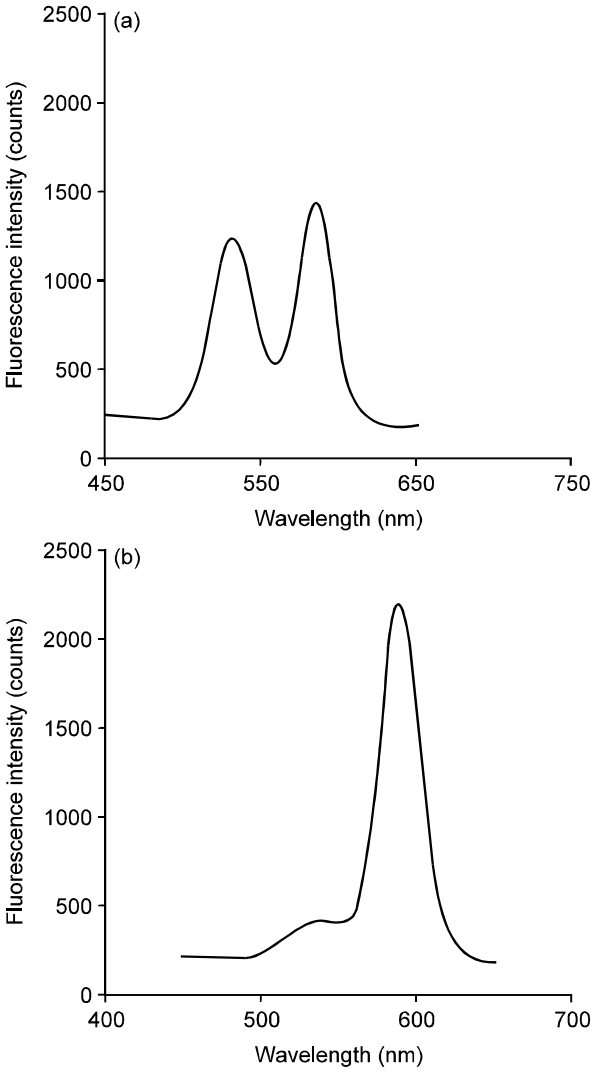

Fig. 3: (a) Spectrum of the unbound QDs 530 and 580 and (b) Spectrum of the bound QDs 530 and 580

shows much higher fluorescent intensity (2200 counts) than the unbound QDs 580.

Detection of *C. jejuni* in cultures, chicken wash and ground turkey: Figure 4 shows the detection results for *C. jejuni* in bacterial culture (4a), chicken wash (4b) and ground turkey (4c). There are significant increases in the fluorescent signal from the QDs when *C. jejuni* are in the test solutions. To detect lower concentrations of target *C. jejuni,* the all samples were performed by exiting each solution containing serial dilution (10^2 down to 10^0 cfu/0.1 ml) of *C. jejuni* at 395 nm and the emission wavelength was measured from 530 to 580 nm with the fluorescent spectra. The lowest concentration of *C. jejuni* that can be detected was 3-5 cfu/0.1 ml (30-50 cfu/ml) with significant difference from the negative controls by

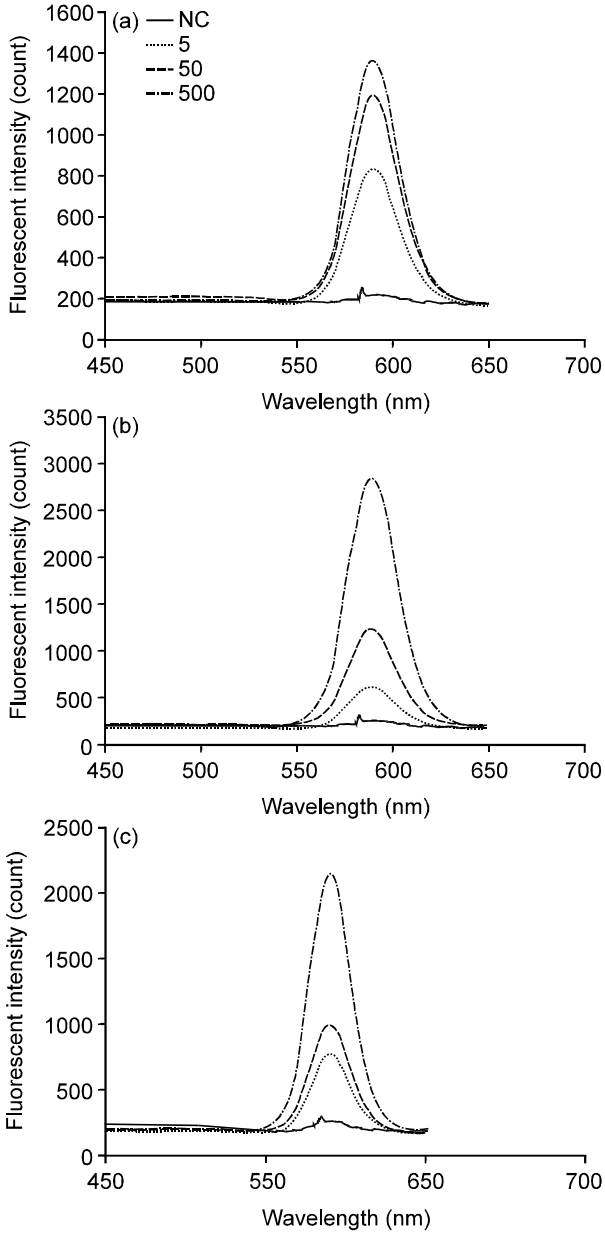

Fig. 4: Representative fluorescence spectra obtained for the samples containing *C. jejuni* at concentrations of 0 (or negative control), 3-5 x 10^0, 10^1 and 10^2 cfu/0.1 ml in bacterial culture (a), chicken carcass wash solutions (b) and ground turkey stomaching solutions (c)

500-600 counts in all bacterial culture, chicken carcass wash and ground turkey samples.

Linear relationships and coefficient of determination: The means and standard deviations of fluorescence intensities measured in each test were calculated and the fluorescence intensity versus the logarithmic value of

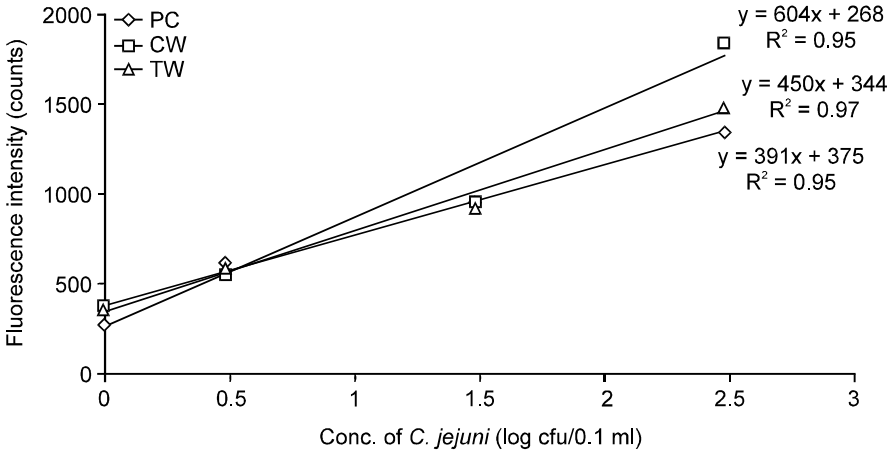

Fig. 5: Linear relationships between fluorescent intensities and different concentrations of *C. jejuni* in pure culture (PC), chicken wash (CW) and ground turkey (TW) samples

target *C. jejuni* in bacterial culture, chicken carcasses wash and ground turkey samples are shown in Fig. 5. The coefficient of determination R^2 = 0.95, 0.97 and 0.95 represent the good linear relationships of the fluorescent intensity vs. *C. jejuni* at concentrations ranging from 0 to 10^2 cells/0.1 ml in pure culture, chicken wash and ground turkey samples.

The detection results confirm that it is possible to detect target pathogens using the FRET-based fluoroimmunoassay by employing different color QDs conjugated with anti-target pathogens antibody and anti-antibody to bind the QDs together. When QDs 580 (Receptor) is added to the solutions containing complexes of target pathogen-QDs 530 (donor), the increase in the fluorescence signal in QDs 580 occurs due to the fluorescence resonance energy transferred from QDs 530. FRET-based immunosensors are more advantageous with fast liquid-phase binding kinetics, long term stability and homogeneous immunoassay (Xu *et al*., 2014). Results demonstrated that the more target *C. jejuni* in the sample solution, the more donor QDs 530 caught to and then the greater amount fluorescence energy transferred to the receptor QDs 580. Our experiments also confirm that if increasing the acceptor to donor ratio, there will be higher FRET efficiencies and then the higher detection sensitivities (Zhang and Wang, 2012). 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 wash and ground turkey solutions at a concentration of 3-5 cells/0.1 ml (30-50 cfu/ml). 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 ($p<0.05$). The fluorescent intensity for the lowest concentration of *Campylobacter* cells 3-5 cells/0.1 ml, are from 500 to 600 counts above their background fluorescent signal at 580 nm. Fluorescence intensity increased with the increasing cell umber of *C. jejuni* and their linear relationships were determined with $R^2>0.95$.

Conclusion: We have demonstrated that the QDs-FRET based fluoroimmunoassay provides a feasible, rapid, sensitive and specific immunoassay for detection of *C. jejuni* in pure culture, chicken carcass wash and ground turkey solutions. The detection time from sampling through result report was within 2 h and the detection limit was 30-50 cfu/ml. This highly adaptive and flexible technique can be extended to detect any other pathogenic bacteria, virus, toxin and contaminants in food, environmental and clinical samples.

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