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Plastic-Adherent DNA Aptamer-Magnetic Bead and Quantum Dot Sandwich Assay for *Campylobacter* Detection

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Abstract DNA aptamers were developed against $MgCl_2$-extracted surface proteins from *Campylobacter jejuni*. The two highest affinity aptamers were selected for use in a magnetic bead (MB) and red quantum dot (QD)-based sandwich assay scheme. The assay was evaluated using both heat-killed and live *C. jejuni* and exhibits detection limits as low as an average of 2.5 colony forming unit (cfu) equivalents in buffer and 10–250 cfu in various food matrices. The assay exhibits low cross-reactivity with bacterial species outside the *Campylobacter* genus, but exhibits substantial cross-reactivity with *C. coli* and *C. lari*. The assay was evaluated with a spectrofluorometer and a commercially available handheld fluorometer, which yielded comparable detection limits and ranges. Remarkably, the sandwich assay components adhere to the inside face of polystyrene cuvettes even in food matrices near neutral pH, thereby enabling a rapid homogeneous assay, because fluorescence is concentrated to a small, thin planar area and background fluorescence from the bulk solution is minimized. The plastic cuvette-adherent technology coupled to a sensitive handheld fluorometer may enable rapid (15–20 min), portable detection of foodborne pathogens from "farm-to-fork" by obviating the slow enrichment culture phase used by other food safety tests.

Keywords Aptamer · Foodborne · Magnetic Bead · Quantum Dot · SELEX

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Introduction

In recent years foodborne pathogen outbreaks in beef, poultry, spinach, peanut butter, frozen pizzas, and most recently peppers or tomatoes have led to huge losses in terms of human health and productivity. Economic losses have been enormous as well and include bankruptcy of Topps Meat Co. following the catastrophic recall of 21.7 million pounds of ground beef in the Fall of 2007. Benoit and Donahue [1] have verbalized a fact that many in the food safety industry have long known, namely that even "rapid" food safety testing is too slow to prevent the distribution of contaminated foods and the resultant costly recalls which lead to severe human illness or death and diminish consumer confidence.

Clearly, technologies must be developed to eliminate the hours to days of enrichment culturing prior to actual food safety testing and to increase the frequency of ultrasensitive testing at a reasonable cost. This is a daunting task, however, because the U.S. Centers for Disease Control and Prevention (CDC) place infective doses of the four major foodborne pathogens (*C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium*) in the range of 10 to several hundred bacterial cells (cfu) depending on the pathogen. Even popular PCR-based techniques have difficulty consistently reaching these ultralow detection limits without enrichment culturing. In addition, many food matrices are known to inhibit *Taq* polymerase [2–4] and even real-time PCR can be relatively slow due to processing steps. Finally, PCR is not generally amenable to portable detection, although some companies have developed and attempted to market portable PCR instrumentation.

Other investigators have reported very low detection limits for the major foodborne pathogens by pre-concentrating pathogenic bacteria via immunomagnetic separation and coupling to sensitive detection methods such as electro-chemiluminescence (ECL) [5] or fluorescent beads [6] and recently fluorescent nanoparticles or quantum dots (QDs) [7–12]. Our approach has been to develop and combine the highest affinity and most specific receptors, possibly DNA aptamers [13–17], with the rapid concentrating ability of magnetic bead (MB)-based separation and highly sensitive QD-based detection, to produce ultrasensitive assays potentially capable of obviating enrichment culturing without sacrificing speed and portability.

Aptamers are nucleic acids with antibody-like binding ability because they are developed via iterative cycles of affinity selection and PCR amplification [13–23]. Aptamers hold significant advantages over antibodies in that they do not require a host animal for production because they are produced by entirely *in vitro* combinatorial biochemistry and are therefore less expensive and tedious to produce [15, 16]. Additionally, in head-to-head comparisons with antibodies of similar specificity, aptamers appear to exhibit higher affinity leading to greater assay sensitivity [23]. Aptamers have also demonstrated target specificity which equals or exceeds that of antibodies [14, 17–19, 21]. Hence we sought to couple aptamers to QDs which are extremely quantum efficient and photostable (i.e., do not photobleach) and could eventually be used in simultaneous multicolored or multiplexed tests for various foodborne pathogens [11]. We have recently reported on the use of various components of our aptamer-MB-QD sandwich assays for detection of *Bacillus* endospores [20] and reovirus particles in diluted stool samples [24]. In addition, we briefly described our prototype aptamer-MB plus aptamer-QD *Campylobacter* sandwich assays in a previous report [7] for detection of heat-killed *Campylobacter jejuni*. Here, we further characterize our *Campylobacter* assay with live and dead *C. jejuni* and report a newly discovered advantageous feature which eliminates the need for a wash step to accelerate test results without sacrificing sensitivity even in various food matrices.

This novel feature is adhesion of the assay components to the inner face of polystyrene cuvettes even after the collection magnet is removed, thereby enabling detection of concentrated fluorescence in one plane with low background signal from the bulk solution (Figs. 2, 3, 4). This same basic strategy is used in ECL sensors such as the original Origen® analyzer in which ECL emanating from captured analytes on the MB surface is collected on a magnetized electrode and partitioned away from the bulk solution to facilitate a much greater signal-to-noise ratio without a wash step [5]. Curiously, only DNA aptamer-conjugated MBs appear capable of significant adhesion to polystyrene after magnetic collection which may be attributable to the DNA aptamer coating [25, 26]. In our experience antibody-conjugated MBs adhere poorly or not at all to polystyrene cuvettes. The plastic adherence of the assays is therefore probably due to the DNA aptamers and seems to be related to DNA "combing" which is a technique used in biophysics to pin down and straighten single DNA molecules between lines of polystyrene even at neutral pH [25, 26]. This assay adhesion trait enables very sensitive and rapid (15–20 min) detection with a handheld battery-operated fluorometer (the Turner Biosystems' Picofluor™) [27] and could facilitate presumptive or early detection of pathogens in the field or along the production line from "farm-to-fork."

Experimental

Heat-killed and live bacteria and culture conditions

Heat-killed *C. jejuni*, *E. coli* O157:H7, *Listeria monocytogenes*, and *S. typhimurium* bacteria were obtained from Kirkegaard Perry Laboratories, Inc. (KPL, Gaithersburg, MD; BacTrace® positive controls) and each was rehydrated in 1 ml of 1X aptamer binding buffer (1XBB; O.5 M NaCl, 10 mM Tris-HCl and 1 mM $MgCl_2$, pH 7.5-7.6). Live *C. jejuni* (subspecies jejuni, ATCC 29428) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured on blood agar plates (BAPs) in Becton, Dickinson and Co. (Sparks, MD) microaerophilic GasPak™ EZ Campy pouches at 42 °C for up to 10 days or until visible colonies emerged. *Campylobacter coli* (ATCC BAA-1061) and *Campylobacter lari* (ATCC BAA-1060) were also purchased from ATCC and grown on BAPs in GasPak™ EZ Campy pouches at 37 °C for up to 10 days or until visible colonies formed. *E. coli* (ATCC 8739, Crooks strain), and *Enterococcus faecalis* were obtained from MicroBioLogics, Inc. (St. Cloud, MN) as lyophilized Kwik-Stiks® and cultured for 48 h on BAPs at 37 °C.

Assay components, assembly and lyophilization

Surface proteins from live *C. jejuni* (ATCC No. 29428) were extracted using 1.5 M $MgCl_2$ as a chaotrope overnight at 4 °C [7, 18, 28]. Surface protein immobilization on tosyl-M280 (2.8 micron diameter) MBs, aptamer development, cloning, sequencing, aptamer-MB-QD sandwich assay development, and assay assembly have been described elsewhere [7, 13, 14, 18, 19]. Briefly, the *C. jejuni* surface proteins were immobilized on tosyl-MBs overnight in phosphate buffered saline (PBS, pH 7.2-7.4) followed by 3 washes in 1 ml of 1XBB using a Dynal MPC-S magnetic

separator (Invitrogen Inc., Carlsbad, CA). The protein-conjugated MBs were then used to accomplish 5 rounds of DNA aptamer selection as described by Bruno and Kiel [13]. Round 5 DNA was cloned into chemically competent *E. coli* using a GC™ cloning kit (Lucigen Corp., Middleton, WI). White and blue colonies were sent to SeqWright, Inc. (Houston, TX) for DNA sequencing of plasmid inserts, since aptamers are quite short and can appear even in the plasmids of blue colonies, if they do not interrupt the *lacZ* gene sufficiently to disrupt color formation [14].

The actual aptamer DNA sequences cannot be revealed for proprietary reasons while patents are pending. However, 6 dominant sequences emerged from the round 5 pool and their secondary structures were determined using web-based Vienna RNA software [29, 30] as shown in Fig. 1. Candidate aptamer sequences designated C2 and C3 were previously shown to perform optimally in our sandwich assay [7] and were therefore synthesized with 5′ amino modifications by Integrated DNA Technologies, Inc. (Coralville, IA). Five mg of 5′-amino C2 capture aptamer were conjugated to 1 ml of stock Dynal tosyl-M280 MBs (2 X 10^9 MBs, Invitrogen) for 2 hr at 37 °C in 5 ml of PBS after which the C2 aptamer-MBs were collected using a Dynal MPC-1 magnetic separator and blocked with 2% ethanolamine in PBS for 1 h at 37 °C. Finally, the C2 aptamer-MBs were washed 3 times in 5 ml of 1XBB using the magnetic separator. Five mg of 5′-amino C3 reporter aptamer were conjugated to 240 µl of 8 µM Qdot 655 amino-ITK (polyethylene glycol) QDs (Invitrogen) using 100 µl of 0.1 M BS^3 (bis(sulfosuccinimidyl)suberate) bifunctional linker from Pierce Chemical Co. (Rockford, IL) in 700 µl of 1XBB for 30 min at 25 °C

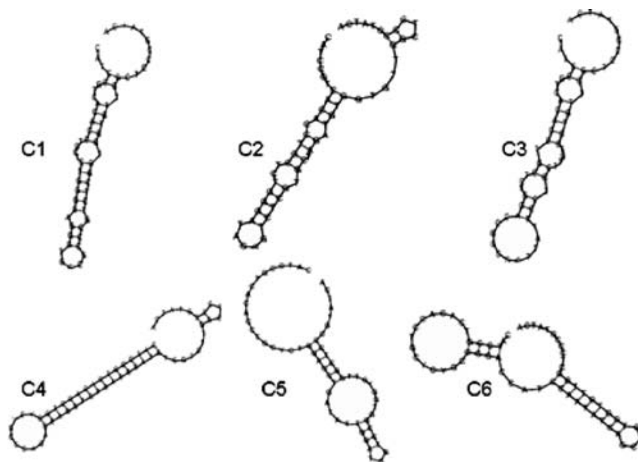

Fig. 1 Dominant candidate DNA aptamer secondary structures developed against *C. jejuni* surface proteins. Web-based Vienna RNA free energy minimization software using DNA parameters and a temperature of 25 °C was used to derive the secondary structures

and purified through Sephadex G-25 that had been equilibrated with 1XBB.

The stock capture (C2 aptamer-MB) and reporter (C3 aptamer-QD) sandwich assay components were diluted to 1 liter in 1XBB. The stock *Campylobacter* sandwich assay materials were then aliquoted at 0.25 ml per 1 cm square polystyrene cuvettes (Perfector Scientific Inc., Atascadero, CA, Cat. No. 9012) which were optically clear from 340–800 nm and lyophilized by Quality BioResources, Inc. (Seguin, TX) for 16 h followed by a nitrogen backflush and capping.

Sample preparation and assay protocol

Heat-killed bacteria from KPL were rehydrated in 1 ml of 1XBB and stored at 4 °C. A loopful of live *C. jejuni*, *C. coli*, *C. lari*, *E. coli* ATCC 8739, *E. coli* O157:H7 or *E. faecalis* were each gathered off of BAPs and resuspended in 5 ml of sterile 1XBB. A single cell suspension was made by extruding clumps of live bacteria through a 22-guage needle and syringe 10 times. Stock suspensions of live bacteria were stored at 4 °C and extruded with the syringe and needle several times prior to use in assay experiments. Live bacteria were quantitated by both hemocytometer counts with phase-contrast microscopy and spread plate counts on BAPs in microaerophilic pouches for 5–10 days. Both methods agreed to within 10% of one another. Both live and heat-killed bacteria were serial 10-fold diluted in microfuge tubes in 1 ml total volumes of 1XBB. These serial dilutions were then transferred to polystyrene cuvettes containing either 0.25 ml of lyophilized assays or 0.25 ml of fresh assay components in 1 ml of 1XBB or 1 ml of various 1XBB-diluted food matrices (20% fat ground beef extract, chicken juice containing visible fat globules, and 2% milk) as indicated in the text and figures. For the ground beef extract, a 25 g sample of ground chuck was added to a 50 ml tube and shaken for 2 min with 1XBB. The beef particulates were allowed to settle and the supernatant fluid was siphoned off to act as the food matrix. The 2 ml volumes of assay and sample mixtures were capped and mixed for 15 min at 25 °C. Next, the cuvettes were transferred to a rack and magnetic separation was accomplished for 5 min using ½ in X 1/8th in X 1/16th in Neodymium magnets (Applied Magnets Inc., Plano, TX) which had been glued to plastic strips and slipped between the rack and cuvette (Fig. 3). The height of the magnet from the bottom of the cuvette was designed to place the collected MBs in the light paths of both the table-top spectrofluorometer and the handheld Picofluor™ fluorometer [27]. When the magnets were removed, the MBs and other assay components adhered to the inner face of the cuvette (Figs. 3 and 4) and their fluorescence was assessed as described below.

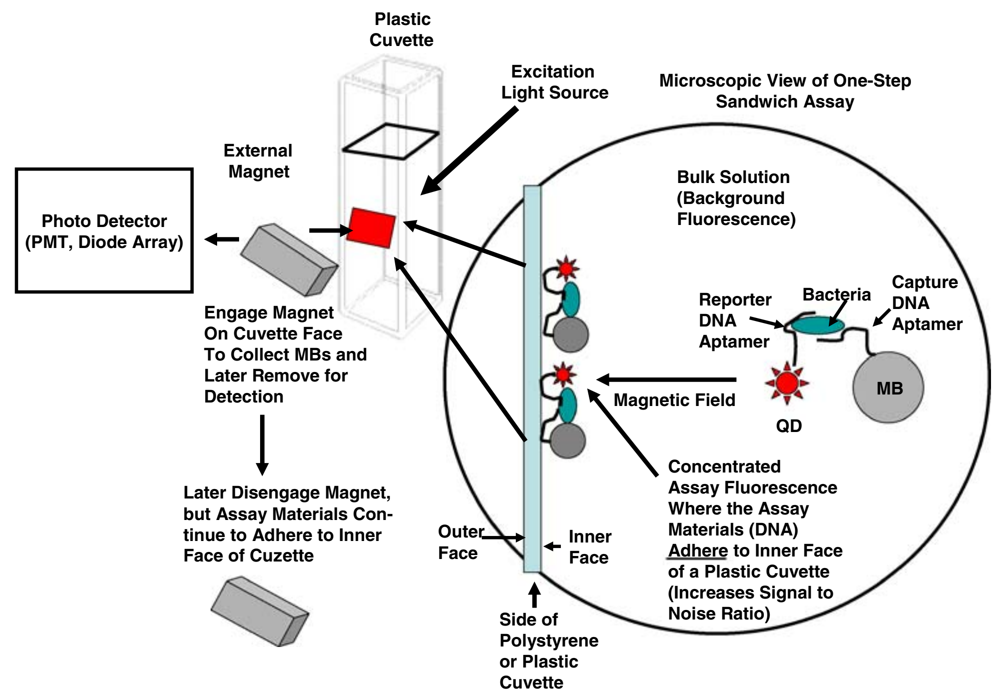

Fig. 2 Conceptual diagram of the self-assembling plastic-adherent aptamer-magnetic bead plus aptamer quantum dot sandwich assay and magnetic collection system

Fluorescence measurements with the picofluor™ and a spectrofluorometer

Adherent MBs and assay components in the plastic cuvettes were turned toward the photodetector in all cases as shown in Fig. 1. Samples were assessed by means of a Turner Designs, Inc. (Sunnyvale, CA) Picofluor™ handheld fluorometer using the green excitation light emitting diode (LED; rhodamine channel; excitation 525±20 nm) and >570 nm emission filter with photodiode detector [27]. The Picofluor™ was calibrated by a two-point procedure using a 2 ml 1XBB blank as the low calibrator and a fresh 2 ml 1:30,000 dilution of Q-dot 655 nanoparticles (Invitrogen) in 1XBB as the high calibrator. The Picofluor™ was hard-wired to a desktop computer and all readings were collected 5 times per sample into an Excel spreadsheet. Samples were

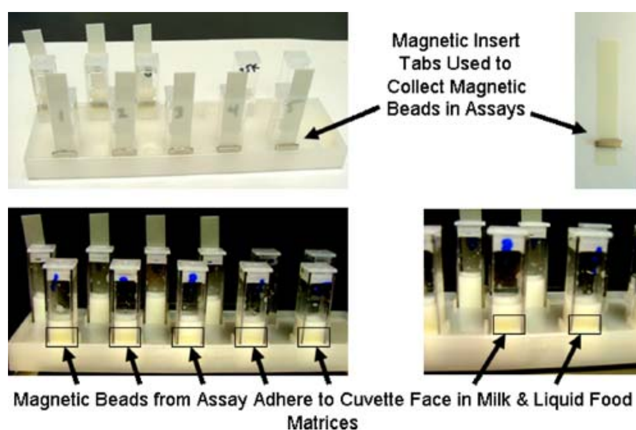

Fig. 3 Actual live Campylobacter plastic-adherent sandwich assays performed in diluted milk. Lyophilized assays were reconstituted using a 1:1 mixture of 2% milk and 1X binding buffer (1XBB). Adherent assay components were collected with the *magnetic insert tabs* as shown. Note the brown bands (*boxed*) that emerge and remain adherent even when the magnetic tabs are removed

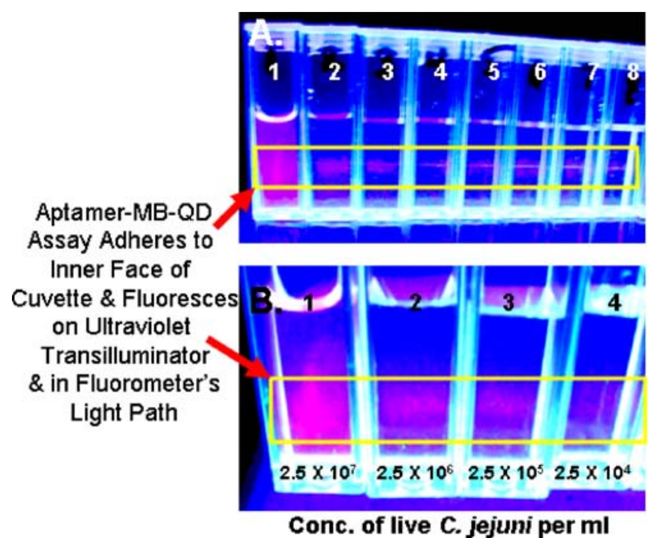

Fig. 4 Plastic-adherent *Campylobacter* sandwich assay processed in buffer and illuminated on an ultraviolet transilluminator. Photographs illustrate pinkish-red fluorescence of adherent assay components (*boxed*) due to the Qdot 655 emissions. A sequence of 10-fold serial diluted live *C. jejuni* beginning with 25 million bacteria/ml in cuvette number 1 were visually detected when placed on a UV transilluminator with peak excitation at 312 nm. Note the generally declining fluorescence across the 10-fold dilutions for cuvettes number 1 through 8 (25 million to 2.5 bacteria/ml)

also assessed with a Varian Inc., Cary Eclipse spectrofluorometer using 380 nm excitation, 5 nm slits and emission scanning from 400–700 nm. Spectra were collected at a medium scan rate and photomultiplier tube (PMT) setting of 900 V. The spectrofluorometer was able to take advantage of higher energy UV excitation and the long Stokes shift of the red emitting QDs, but the Picofluor's optics are currently more limited to accommodate conventional fluorophores. While a UV excitation is available with the Picofluor™, its emission filter passes all light >430 nm and could gather significant autofluorescence background from foods between 400–600 nm.

Results

Aptamer sequencing, secondary structure determinations and pilot assays

Six dominant candidate DNA aptamer sequences emerged from the fifth round of aptamer selection. The exact DNA sequences are proprietary, but their secondary structures are shown in Fig. 1. We previously screened all 6 of these sequences for their affinity and specificity in a preliminary set of aptamer-MB plus aptamer-QD sandwich assay experiments and determined that the C2 aptamer-MB was optimal for capture of *C. jejuni* while the C3 aptamer-QD conjugate was optimal in the role of reporter wherein we achieved an ultrasensitive detection limit of <10 heat-killed *C. jejuni* bacterial equivalents per ml in 1XBB [7]. The specificity of our *Campylobacter* assay was proven against the other three major foodborne pathogen species in our previous report [7] and later verified by a military laboratory [21].

Development of polystyrene-adherent assay

We originally collected the sandwich assays (C2 aptamer-MB-bacteria-C3 aptamer-QD complexes) in a conventional Dynal MPC-S magnetic separator and gently washed the materials several times in 1XBB prior to transferring to plastic cuvettes for fluorescence assessment [7]. In the initial experiments, we only captured a fraction of the available fluorescence in the entire cuvette, because only a small part of the cuvette is ever illuminated by the spectrofluorometer's light path. We also knew that potential end users of the technology wished to eliminate wash steps and that wash steps were not necessary, because the assay materials adhered to the inside face of the polystyrene cuvette (perhaps due to the DNA aptamers [25, 26]), when collected with a strong magnet placed on the outer face of the cuvette as illustrated in Figs. 2, 3, 4. Remarkably, we found that by partitioning and concentrating the assay components away from the bulk solution in the cuvette, we achieved a signal-to-noise ratio that allows elimination of wash steps without sacrificing detection limits even in various food matrices (Figs. 6 and 7). The fluorescence of the adherent assay materials can be seen clearly when a serial 10-fold assay titration of live or heat-killed *C. jejuni* cells is placed on an ultraviolet transilluminator and excited as in Fig. 4 where distinct pink fluorescence from the 655 nm emitting QDs is seen at the same height as the brown bands of MBs collected in 1:1 diluted 2% milk along the cuvettes pictured in Fig. 3.

The assay components remain adherent to the inner face of the cuvette for days or weeks in sterile buffer even at ambient temperatures. In similar experiments with antibody-coated MBs, most of the antibody-MBs fall away from the collection site when the magnetic insert is removed, but a very thin brown film sometimes remains. In our experience, the DNA aptamer-MB and aptamer-QD sandwich assay components can be magnetically concentrated to the inner face of a polystyrene cuvette with much stronger adherence than antibody-MBs. It is well known that proteins such as antibodies can adhere to polystyrene when the pH is elevated as is common in some enzyme-linked immunosorbent assay (ELISA) microplate coating protocols. However, others have reported adherence of DNA to polystyrene as well [25, 26]. Captured bacteria may assist in adherence of assay materials to the plastic [31–34], but bacteria are not required for plastic adherence of the assay, since tests run without added bacteria still exhibit adherence of the aptamer-MBs. Regardless of the mechanism of adherence, the partitioning and concentrating of fluorescence to a small, thin planar area inside the cuvette is advantageous in that it increases the signal-to-noise ratio by minimizing the background fluorescence of the bulk solution in the cuvette.

Preliminary polystyrene-adherent assay characterization

Adherent *Campylobacter* assay materials were scraped from the inner face of the cuvettes and examined by brightfield and fluorescence microscopy. As Fig. 5 illustrates, *C. jejuni* can be found attached to C2 aptamer-MBs from the inner cuvette scrapings and these captured bacteria emit strong red fluorescence when excited with a UV filter cube.

The detection limit in pristine 1XBB buffer can be as low as 2.5 cfu or bacterial equivalents per ml on average for heat-killed or live *C. jejuni* bacteria using either a spectrofluorometer or the handheld and battery-operated Picofluor™ fluorometer as seen in Figs. 6 and 7. The Picofluor™ gives a more linear response to log changes in bacterial concentration (Fig. 7) due to its photodiode detector [27], while the spectrofluorometer produces an

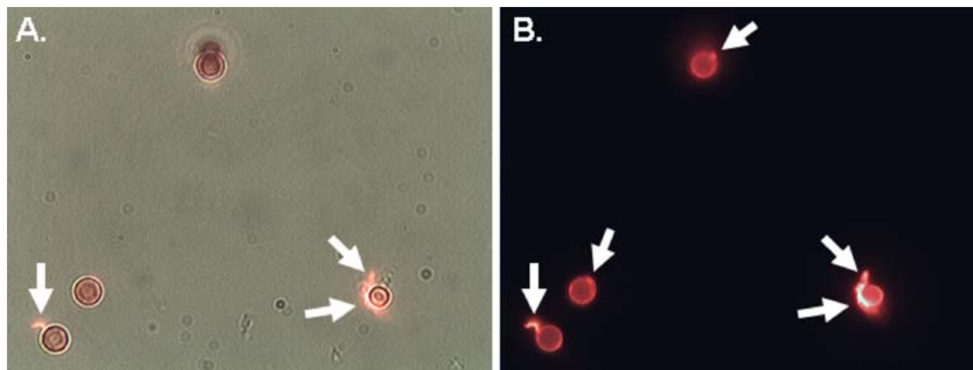

Fig. 5 Microscopic characterization of plastic-adherent assay materials. Panel **a** shows 1,000× combination brightfield and fluorescence microscopy of live *C. jejuni* captured by the C2 aptamer-MBs and detected with C3 aptamer-QDs after being scraped from the inner face of a polystyrene cuvette following magnetic collection. Panel **b** shows the same microscopic field photographed with a UV filter by fluorescence microscopy alone (at 1,000×). *Arrows* indicate probable MB-aptamer-captured *C. jejuni* bacteria which are decorated with red aptamer-QD conjugates and fluoresce brightly

exponential response due to its PMT detector (Fig. 6). The precision of the Picofluor™ is remarkable as attested to by the very small standard deviations (error bars) in Fig. 7, but the response is sometimes not completely linear (Fig. 7a). This anomaly may be partly due to geometric irregularities in the rectangular magnetic capture area of some cuvettes as shown in Fig. 4 and the circular aperture around the Picofluor™'s photodiode detector or random clumping of captured bacteria.

Figures 6 and 7 illustrate that lyophilized assays can be used to detect very low levels of live *C. jejuni* in a variety of diluted food matrices to varying degrees without a wash step. Indeed, in some experiments (data not shown), attempts to remove the food matrices and wash the assay components in place on the inner wall of the cuvette were detrimental to the detection limit, because assay materials were partially dislodged and lost during the wash steps. Detection limits in diluted food matrices were in the range

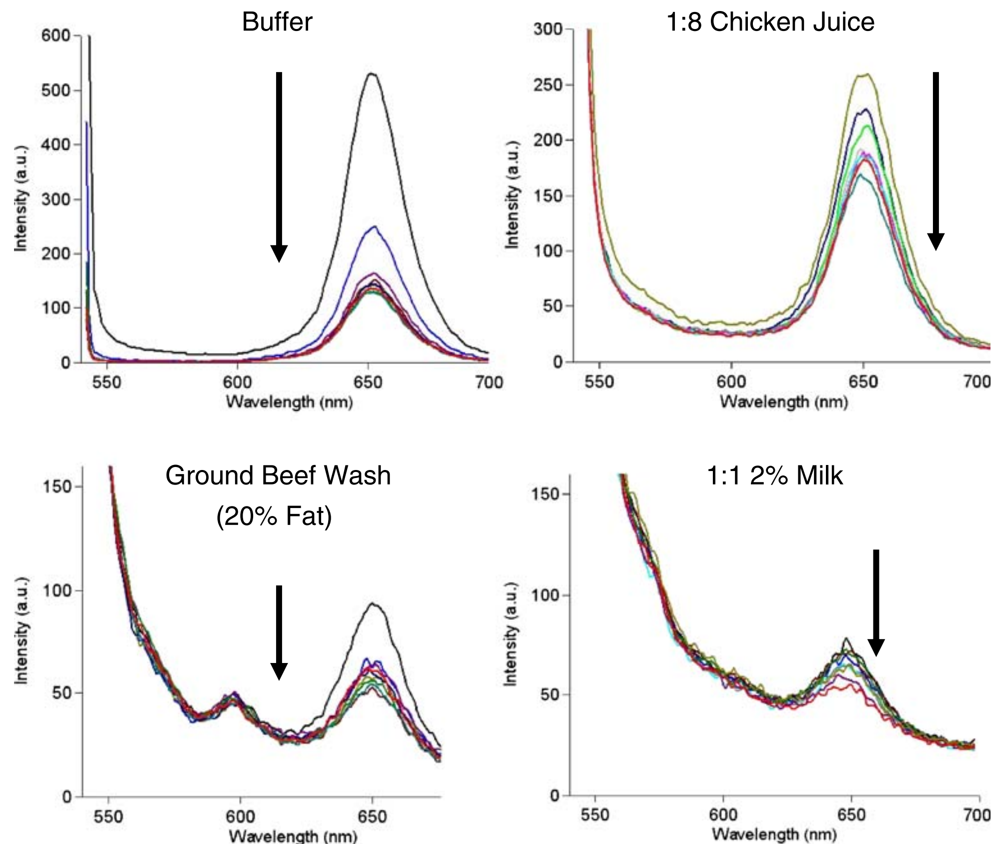

Fig. 6 Spectrofluorometric comparison of adherent *Campylobacter* assays in buffer and various diluted food matrices. Ten-fold serial dilutions of live *C. jejuni* bacteria were added to lyophilized plastic-adherent sandwich assays in 1XBB buffer or various diluted food matrices as shown beginning with 2.5×10^6 cfu/ml and titrating down in the direction of the arrows to the final cuvette with no bacteria added (lowest peak in each case). Assays were conducted for 15 min and were not washed after magnetic collection of MBs on the inner face of the plastic cuvette. Excitation was at 380 nm and emissions were scanned with a PMT setting of 900 V and 5 nm slit widths

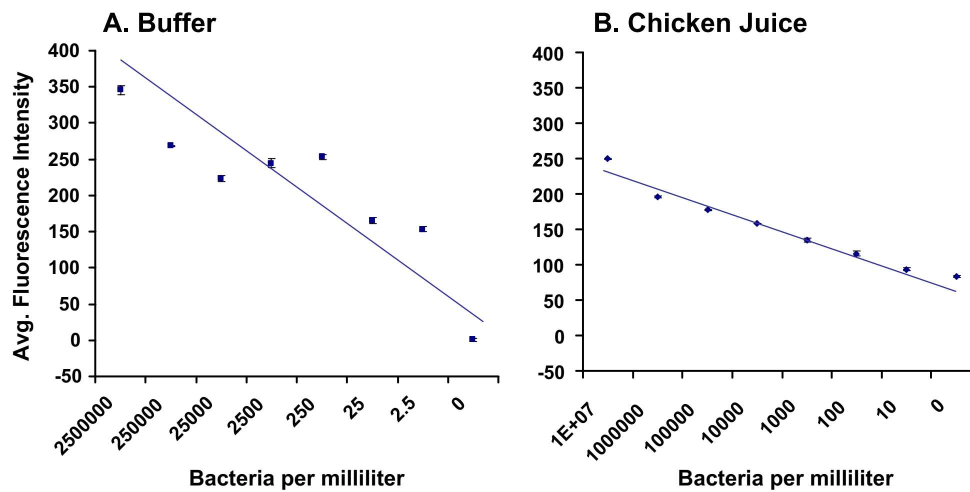

Fig. 7 Assessment of lyophilized plastic-adherent *Campylobacter* assay in buffer using the handheld Picofluor™ fluorometer. Ten-fold serial dilutions of heat-killed *C. jejuni* at the equivalent live cell concentrations shown were made in 1XBB buffer and added to lyophilized cuvette sandwich assays. Panel **a** shows results of bacterial titration in buffer (1XBB). Panel **b** illustrates results of a similar experiment in undiluted chicken juice. For both panels the means and standard deviations (error bars) of five independent readings are shown using the green excitation or "rhodamine" channel of the Picofluor™ (ex. 525±20 nm and emission >570 nm)

of 10–250 cfu/ml for both live and dead *C. jejuni*. Chicken juice collected from a pack of fresh chicken thighs was the most amenable to our assay, but all of the food matrices exhibited detection of live *C. jejuni* in the red region of the spectrum (centered around 655 nm) as shown in Fig. 6. All of the food matrices themselves also exhibited large intrinsic autofluorescence background across the range from 400 to 600 nm, because UV excitation (380 nm) was used to excite QDs directly in the food matrices. The high energy UV excitation provides the most power to penetrate diluted food samples and maximizes energy absorbance of the QDs. We have excited the assays in the visible range via the Picofluor™, but lose much of the fluorescence signal at 655 nm. Still, the integrating power of the Picofluor™ enables excitation at 525±20 nm with detection at >570 nm using the green or "rhodamine" channel without sacrificing detection limits. This fact is poignantly illustrated by detection of 100 live *C. jejuni* per ml in undiluted chicken juice using the Picofluor™ as shown in Fig. 7b. In addition, although a direct comparison was not shown for brevity, lyophilized assays performed nearly as well as fresh assays.

Cross-reactivity studies

We previously reported a general lack of cross-reactivity of our fresh assay with the other 3 major foodborne pathogen species including heat-killed *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* with maximal cross-reactivity of 11% [7]. The affinity and low cross-reactivity of our polyclonal aptamers were later confirmed by McMasters and Stratis-Cullum [21] in capillary electrophoresis studies using *C. jejuni* and other bacterial species as binding targets. In the present work, we have expanded the cross-reactivity studies to examine other live *Campylobacter* pathogens including *C. coli* and *C. lari* as well as other genera of bacteria. As data in Table 1 indicate cross-reactivity was low for all unrelated bacterial species (outside the *Campylobacter* genus), but was as high as 57–70% for *C. coli* and *C. lari*. This is encouraging, of course, because *C. coli* and *C. lari* are also human pathogens in the *Campylobacter* genus [3] and it would appear that our plastic-adherence assay can detect them at useful levels as well.

Discussion

The fresh and lyophilized aptamer-MB plus aptamer-QD sandwich assays have achieved detection levels of approximately 2.5 cfu or their equivalents for live or dead *C. jejuni* in buffer and 10–250 cfu per ml in various

Table 1 Cross-reactivity of the adherent *campylobacter* assay

Species	Sample	% Cross-Reactivity
Campylobacter jejuni	live	100%
Campylobacter coli	live	70%
Campylobacter lari	live	57%
Escherichia coli 8739 (Crooks)	live	4.9%
Escherichia coli 0157:H7	live	14.2%
Enterococcus faecalis	live	4.7%
Listeria monocytogene	heat-killed	7.9%
Salmonella typhimurium	heat-killed	5.3%

$$\%\text{Cross}-\text{reactivity} = \frac{(\text{Peak fluorescence of target bacteria}/A_{600\text{nm}})}{(\text{Peak fluorescence C. } jejuni/A_{600\text{nm}})} \times 100$$

food matrices. These detection limits are well below the infective dose levels of 400–500 reported by the CDC for *C. jejuni* and generally in agreement with the ultralow detection limits reported in immuno-QD bacterial assays by other investigators [8–12]. These plastic-adherent sandwich assays are self-assembling and have several other desirable traits. First, the assays are homogeneous (washless), rapid (15–20 min), and extremely sensitive. We are currently investigating means to improve sensitivity even further in foods such as addition of higher levels of assay components (i.e., more aptamers, MBs and QDs). Second, the assays are rugged for field use and have a long shelf-life once they are lyophilized. Third, the assays and handheld Picofluor™ are highly portable and inexpensive [27]. DNA aptamers are less expensive to develop and produce than comparable antibodies [15, 16] and relatively few MBs and QDs are needed per test to achieve the detection limits reported herein.

We are currently seeking to replace the emission filter in the UV channel (excitation centered at 380 nm) of the Picofluor™ with a ≥620 nm band pass emission filter to better accommodate the long Qdot 655 Stokes shift in our assays and eliminate all autofluorescence from food matrices in the visible region. Still, as Fig. 7 illustrates even the green channel of the Picofluor™ (designed for rhodamine detection with excitation of 525 ± 20 nm and emission >570 nm) detected as few as 2.5 *C. jejuni* bacteria per ml in buffer and 100 or fewer bacteria in undiluted chicken juice.

While we seem to have discovered that DNA aptamers adhere tightly to the inner polystyrene face of cuvettes when held in place for several minutes during magnetic collection [25, 26] and that aptamers can support the weight of 2.8 micron diameter (M280) MBs. Heavier 4.5 micron (M450) DNA aptamer-MBs tend to slough off readily perhaps due to a greater gravitational force. Similarly, in our hands, antibody-coated MBs appear to adhere to polystyrene cuvettes with lesser affinity at neutral pH and have not supported plastic adherence of even M280 MBs to an appreciable extent. We do not rule out specialized proteins from nature or genetically engineered proteins with greater hydrophobicity being able to adhere to polystyrene at neutral pH and physiologic salt concentrations [31–34], but antibody-coated MBs have not adhered as well as DNA aptamer-coated MBs in our experience thus far. These observations are generally in agreement with conditions for DNA "combing" on or between polystyrene boundaries [25, 26] and the well-known fact that most proteins adhere optimally to polystyrene microtiter plates only at elevated pH.

The plastic-adherent assays appear to be approximately as sensitive toward both live and heat-killed *C. jejuni* (≤10 cfu or bacteria per ml) in buffer, suggesting that the aptamers' epitopes are heat-stable. It would be important for personnel conducting real food safety tests to discriminate live from dead *Campylobacter* bacteria, so we are presently exploring addition of a fluorescence viability assay for use on one of the Picofluor™'s two fluorescence channels. In this way, food safety tests might be conducted in which detection of the pathogen at one peak wavelength is followed shortly thereafter by assessment of viability at a second peak wavelength.

As more researchers develop specific high affinity aptamers against bacteria [35–38] and other targets, the homogeneous plastic-adherent assay format may provide an important portable fluorescence assay platform technology. Such rapid and facile, yet sensitive, detection technology could be quite valuable for on-site detection of foodborne and other clinical pathogens [39], biodefense targets, or other important analytes. Clearly, food safety testers are seeking simpler and more rapid means to test for foodborne pathogens from "farm-to-fork" [39, 40] without sacrificing sensitivity. The specificity and sensitivity which results from combining DNA aptamers with MBs and QDs is complemented by the speed of the homogeneous plastic-adherent assay format to yield the potential for on-site handheld fluorescence detection that may help to prevent major foodborne pathogen outbreaks.

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