Journal of Microbiological Methods 117 (2015) 64–73

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Pathogen enrichment device (PED) enables one-step growth, enrichment and separation of pathogen from food matrices for detection using bioanalytical platforms

Byoung-Kwon Hahm [a], Hyochin Kim [a], Atul K. Singh [a], Arun K. Bhunia [a,b,*]

[a] Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, IN, USA
[b] Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA

ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form 16 July 2015
Accepted 17 July 2015
Available online 23 July 2015

Keywords:
Sample preparation
Pathogen enrichment device
Food
Bacterial separation
Detection
Light scattering sensor
qPCR
Lateral flow immunoassay

ABSTRACT

The bottleneck for accurate detection of foodborne pathogens is separation of target analytes from complex food matrices. Currently used sample preparation methods are cumbersome, arduous and lengthy; thus, a user-friendly system is desirable. A hand-held sample preparation system designated pathogen enrichment device (PED) was built that contains a growth chamber, filters, and an ion exchange cartridge to deliver bacteria directly onto the detection platforms. *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* were used as model pathogens. Spinach, ground beef, hotdogs, and eggs were used as model foods to evaluate PED performance, and results were compared with traditional bag enrichment method. Bacterial cells were inoculated at 1, 10, and 100 CFU/g of the sample and enriched in PED using appropriate pathogen-specific selective enrichment broths. The bacterial cell counts in both PED and stomacher-bag were comparable and the pH in PED-recovered cell suspension was close to neutral whereas the pH of cell suspension in the stomacher-bag was slightly acidic. The bacterial recovery from the PED was 79–100% and was directly detected by lateral-flow immunoassay (LFIA), quantitative PCR (qPCR) and light scattering sensor with sample-to-result time of 8–24 h with a detection limit of 1 CFU/g. In qPCR, the amplified PCR products appeared in 4–5 cycles earlier with PED-enriched cultures compared to the cultures enriched in stomacher-bag. The hand-held PED proved to be a one-step procedure for enrichment and recovery of homogenous particle-free bacterial cells for detection using immunological, molecular or biosensor-based platforms.

1. Introduction

Foodborne diseases are one of the most common causes of morbidity and mortality worldwide, and they have significant economic consequences with financial losses accounting to billions of dollars to the food industry (Scharff, 2012). Over the past decade, there has been a noticeable increase in foodborne illnesses due to bacterial contamination of foods including fresh fruits and vegetables (Erickson, 2010; Walsh et al., 2014). Around 48 million illnesses, 128,000 hospitalizations and 3000 deaths occur annually in the US due to foodborne infections (Scallan et al., 2011). More specifically, in recent years a large number of people have been sickened by consumption of spinach, lettuce and beef products contaminated with *Escherichia coli* O157:H7 (CDC, 2015; Olaimat and Holley, 2012) and 3911 cases (47 fatalities) due to *E. coli* O104:H4 contaminated fenugreek sprouts (Scheutz et al., 2011). Thousands of illnesses were reported due to *Salmonella enterica* serovar Tennessee in peanut butter (CDC, 2014), *S. enterica* serovar Saintpaul in pepper (Maki, 2009), and *S. enterica* serovar Enteritidis in eggs (Kuehn, 2010). *Listeria monocytogenes* was responsible for 57 cases (22 fatalities) from consumption of tainted meat products (Annonymous, 2009), 27 cases (8 fatalities) from Quargel sour milk curd cheese (Fretz et al., 2010), 147 cases (33 fatalities) from cantaloupe (CDC, 2011), and most recently in 2015, 32 cases (7 deaths) from caramel apple (http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/) and 10 cases (3 deaths) from ice cream (http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm438104.htm).

Traditional culture-based detection methods involve sample enrichment followed by isolation of target microorganisms on selective or chromogenic differential agar media for presumptive identification, ensued by more confirmatory biochemical, molecular or serological tests. These steps are performed sequentially and routinely extend to 7–10 days or more to obtain preliminary results. To shorten detection time, selective and differential plating step has been replaced by "rapid methods" such as lateral flow immunoassay (LFIA), polymerase chain reaction (PCR), and biosensors providing results in minutes to hours (Bhunia, 2014; Cocolin et al., 2011; Dwivedi and Jaykus, 2011; Fukushima et al., 2010; Velusamy et al., 2010). Though

* Corresponding author at: Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, Indiana, USA.
E-mail addresses: bkhahm@hotmail.com (B.-K. Hahm), kimh0727@gmail.com (H. Kim), aksingh@purdue.edu (A.K. Singh), bhunia@purdue.edu (A.K. Bhunia).

http://dx.doi.org/10.1016/j.mimet.2015.07.016
0167-7012/

these methods can be completed (sample-to-result) in 24–48 h or less, they are highly susceptible to inhibitors, low number of target pathogens and high levels of background microorganisms (Lee and Levin, 2011; Wilson, 1997). Therefore, efficient pathogen separation, concentration, enrichment and recovery strategies are prerequisite for accurate analysis of samples employing antibody, nucleic acid probes or biosensors-based methods (Bhunia, 2008; Brehm-Stecher et al., 2009). Ineffective sample processing and preparation may yield false-negative results rendering consumers to grave consequences. False-positive results may lead to unnecessary product losses with huge economic burdens.

Historically, separation, concentration and recovery of bacteria from samples are achieved by centrifugation (Lindqvist, 1997), filtration (Chen et al., 2005; Hill et al., 2009; Mukhopadhyay et al., 2010), and chromatographic separation (Payne et al., 1992). In recent years, immunomagnetic separation (IMS) (Bhunia, 2008; Stevens and Jaykus, 2004), ultrafiltration (Li et al., 2013; Magana et al., 2014; Murakami, 2012), dielectrophoresis (Brehm-Stecher et al., 2009), and microfluidic chips (Koo et al., 2009; Li et al., 2014) are used. These systems are used directly with beverages or the liquid extract (rinse) from solids or enriched samples after a culturing step. Often, expensive, and specialized bulky equipment are necessary to perform these tasks. An enrichment step, though considered a "bottleneck", is required for all commercially available rapid methods and also recommended for many, if not all, official detection methods (FDA, 2001; USDA-FSIS, 2015). It increases target microorganism counts and thus compensates detection limits of the assay. Since there is a "zero tolerance" policy for many foodborne pathogens, enrichment allows cell number increment even if a sample contains a single viable cell per 25 g of analytical unit. It also reduces the risk of amplifying nucleic acids from dead cells because of higher proportion of live target microbes. Culture enrichment also helps resuscitate stressed or injured cells, allows up-regulation of gene or gene products that serve as targets for detection in the bioanalytical platforms, and helps release (through growth) intimately attached cells from the product surface. Enrichment broth also dilutes the inhibitory substances that may otherwise interfere with the analytical methods. Addition of chelating agent (ex — charcoal) to the enrichment broth can also help remove inhibitors (Brehm-Stecher et al., 2009; Cocolin et al., 2011; Josefsen et al., 2004; Wolffs et al., 2006). Most importantly, an enrichment step enhances the chance of obtaining an accurate result for a sample due to its positive attributes discussed above.

Biosensor platforms have been used for detection of foodborne pathogens, including fiber optic, surface plasmon resonance (SPR), Raman, Fourier transformed infrared (FTIR) spectroscopy, flow cytometry, and impedance-based microfluidic devices (Bae and Bhunia, 2013; Bhunia, 2014; Bisha and Brehm-Stecher, 2009; Cho et al., 2015a; Najafi et al., 2014; Sharma and Mutharasan, 2013; Velusamy et al., 2010). Here we employed a forward light scattering sensor, BARDOT (*bac*terial *r*apid *de*tection using *o*ptical scattering *t*echnology) for detection of colonies of target pathogens. When a red-diode laser (635 nm; 1 mW) impinges on the center of a bacterial colony growing on an agar plate, it generates 2-dimensional luminous scatter fingerprint of colony. During analysis, hundreds of independent parameters and features are extracted from each scatter pattern to build a database of images for a specific microorganism (Bae et al., 2011; Banada et al., 2007). BARDOT has been successfully used for detection of multiple bacterial pathogens including *Bacillus* spp. (Singh et al., 2015), *Campylobacter* spp. (He et al., 2015), Shiga-toxigenic *E. coli* (Tang et al., 2014), *Listeria* spp. (Banada et al., 2009), *Salmonella enterica* (Singh et al., 2014), and *Vibrio* spp. (Huff et al., 2012).

Here, we report the fabrication of a prototype sample enrichment device, PED, for enrichment and delivery of bacterial cells free of any food particulates directly onto a detection platform without intermediate sample manipulation and handling steps. Parts of the preliminary results were reported earlier in a conference (Hahm et al., 2007). PED performance was validated using *E. coli* O157:H7, *S. enterica* serovars Typhimurium and Enteritidis, or *L. monocytogenes* inoculated ground beef, green leafy spinach, boiled egg and ready-to-eat hotdog. Pathogens in the PED-recovered samples were detected using LFIA, qPCR and BARDOT at 1–10 CFU/g after 8–12 h of enrichment.

2. Materials and methods

2.1. Bacterial strains and media

E. coli O157:H7 (EDL933), *S. enterica* serovar Typhimurium (*S.* Typhimurium) and Enteritidis (*S.* Enteritidis), and *L. monocytogenes* V7 strains from our collection were used. Modified EC (mEC) broth containing novobiocin (Novo, 20 mg/ml) (mEC + Novo) and EHEC (enterohemorrhagic *E. coli*) enrichment broth (EEB) were used for *E. coli* O157:H7, Rappaport–Vassiliadis (RV) broth for *S.* Typhimurium and *S.* Enteritidis, and buffered *Listeria* enrichment broth (BLEB) for *L. monocytogenes*. For bacterial enumeration, selective media such as cefixime–tellurite containing Sorbitol-MacConkey (CT-SMAC) agar for *E. coli* O157:H7, Xylose–Lysine–Deoxycholate (XLD) for *S.* Typhimurium and Enteritidis, and modified Oxford (MOX) agar plates for *Listeria* were used. All media were purchased either from Acumedia (Lansing, MI), EMD Chemicals, Inc. (Gibbstown, NJ) or Becton Dickinson (Sparks, MD).

2.2. Description of PED

The prototype unit (Fig. 1) was built with off-the shelf materials in our laboratory, and all parts are made with reusable plastics. It has two major parts: an enrichment chamber (total capacity 30 ml) and a particle separation unit consisting of filtration/ion-exchange cartridge. A cylindrical sample carrier (30-ml syringe barrel) with a perforated (5–10 mm in diameter holes) wall is placed inside the enrichment chamber (60-ml syringe barrel), which holds solid food with a maximum capacity of 15 g solid or pieces of swabs e.g., sponge, cotton, and absorbent paper. For liquid samples, a sample carrier is not required. A polyethylene filter (Parts No. Alltech # 211404) is placed at the bottom of the enrichment chamber to prevent the flow of coarse particles to the filtration/ion exchange cartridge attached to the enrichment chamber. The filtration/ion-exchange cartridge (Alltech® Maxi-Clean™ Cartridges, Fisher Scientific) is filled with Amberlite IRA-96 (Sigma, St. Louis, MO) weak anion exchange resin between two polyethylene filter

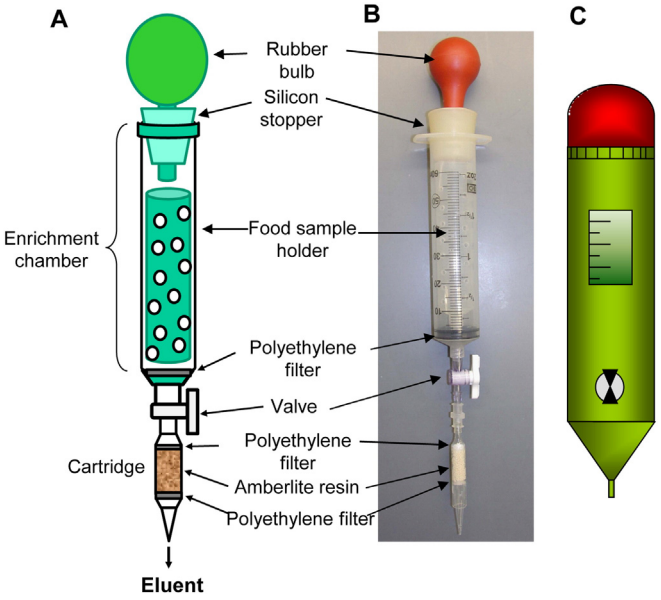

Fig. 1. Pathogen enrichment device (PED); (A) the schematic, (B) prototype, and (C) a futuristic PED.

pads (Parts No. Alltech # 211404 (top) and Alltech # 211406 (bottom)), which allows further removal of particles and ionic molecules, and helps adjustment of the pH of the sample eluent to neutral, and delivers homogenous bacterial suspension. A silicone stopper attached to a pneumatic rubber bulb was attached to the top of the enrichment chamber to aid in sample dispensing onto a sensor platform (Fig. 1). The PED unit, without the pneumatic bulb and the cartridge, was sterilized in autoclave before use. The PED unit was used multiple times, except the cartridge which was disposable.

Food samples (3–15 g) or swabs are placed inside the sample carrier, which is then placed inside the enrichment chamber. Enrichment broth (15–27 ml) is then poured into the chamber and sealed with the silicone stopper. The perforated wall of the sample carrier provides easy access to the enrichment broth and free movement of bacterial cells in and out of the sample matrices. After a period of incubation, a firm squeeze of the pneumatic bulb allows sample flow through the filtration cartridge (cartridge valve should be in open position) and collection tube (1–5 ml) or direct delivery to a detection platform without any requirement for handling the liquid.

2.3. Recovery of bacteria after passing through the cartridge

To determine if there is any loss of cells during filtration through the ion-exchange cartridge, we used *L. monocytogenes* as a model organism. *L. monocytogenes* cells were inoculated into 3 g of hotdog (sliced) at a concentration of about 1, 10, 100 or 1000 CFU/g (based on dilution series) in 27 ml of BLEB and incubated at 37 °C in a shaking incubator set at 150 rpm. The culture broths, before and after passing through the ion-exchange cartridge, were collected at 4-h intervals up to 16 h, and the viable cell counts were enumerated on MOX agar plates.

2.4. Enrichment and separation of pathogen spiked onto food samples

The overall experimental schematic is illustrated in Fig. 2A. Food samples were purchased from local grocery stores (West Lafayette, Indiana, USA) and inoculated with freshly grown cultures of *E. coli* O157:H7, *S.* Typhimurium (or *S.* Enteritidis) and *L. monocytogenes* at 1, 10, or 100 CFU/g into ground beef, boiled-egg and hotdog, respectively. Three grams of each spiked food was placed inside the chamber and 27 ml of the respective selective enrichment broth (mEC + Novo) for *E. coli* O157:H7, RV for *S.* Typhimurium or Enteritidis and BLEB for *Listeria* was added, and the PED was incubated at 37 °C. At the same time, 25 g of each spiked food was mixed with 225 ml of the respective selective enrichment broth in a Whirl-Pak® stomacher filter bag (Nasco, Fort Atkinson, WI) and was incubated at 37 °C in a shaking incubator set at 150 rpm. Bacterial cells were collected from the outlet of the sample delivery port in PED (1–5 ml) and the pH and bacterial counts for each sample were monitored every 2 h for 16 h and then again at 24 h. The pH was measured using a portable pH meter (Fischer Scientific), and bacterial counts were enumerated after plating decimally diluted suspension on appropriate selective plates (CT-SMAC for *E. coli* O157:H7; XLD for *Salmonella*; MOX for *Listeria*). Similarly, bacterial counts and pH of the enriched broth from stomacher bag were determined. PED recovered bacterial suspensions were further tested by LFIA, real-time qPCR, and BARDOT.

2.5. Lateral flow immunoassay (LFIA)

Approximately 100 μl of bacterial suspension after PED enrichment and recovery was directly dispensed into the sample receiving well of the LFIA strip (Reveal; Neogen, Lansing, MI) for detection of either

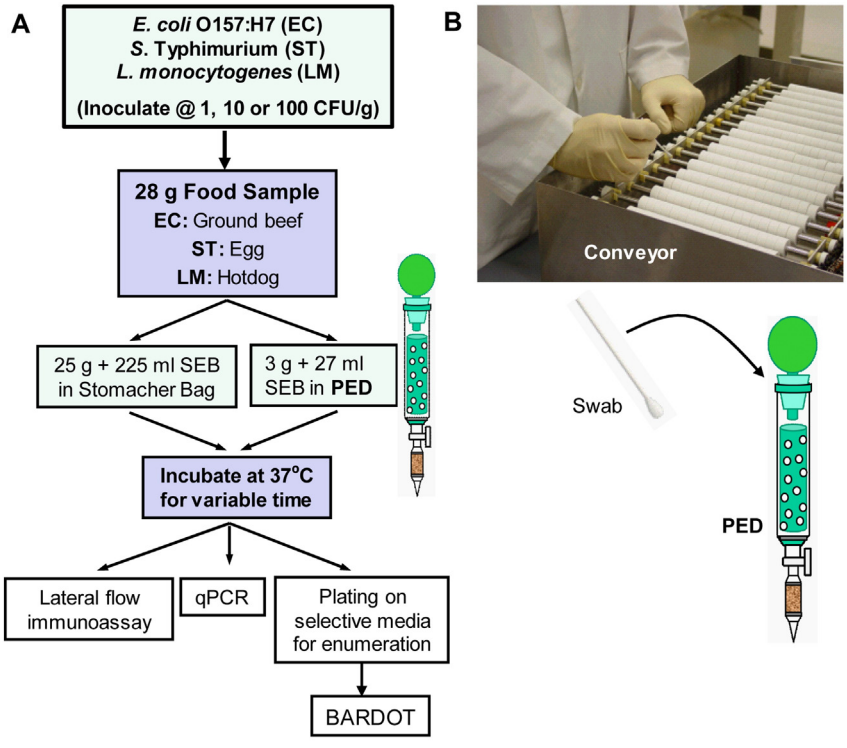

Fig. 2. (A) The flowchart showing overall experimental outline. *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were inoculated into ground beef, boiled egg, and hotdog, respectively, and divided into two parts: 25 g was dispensed into the stomacher bag and 3 g in PED, suspended in the respective pathogen-specific selective enrichment broth (SEB), and incubated at 37 °C. The samples (eluents) were taken at specified time points and were analyzed by plating, lateral flow immunoassay, qPCR and BARDOT. (B) Swab sample collection from various parts of a bench-top conveyor system for pathogen enrichment, separation, and recovery from PED.

E. coli O157:H7, *Salmonella*, or *Listeria* (Fig. 3). In parallel, 100 μl aliquot from the stomacher bag-enriched sample was also dispensed onto the LFIA strip and developed for 15 min or until the appearance of the test bands.

2.6. qPCR

For detection of *E. coli* O157:H7, 2 μl of the enriched broth from both PED and the stomacher bag was directly used for the real-time qPCR in a 25 μl reaction volume using the Taqman® *E. coli* O157:H7 detection kit (Kit # 4307514, Applied Biosystems, Foster City, CA) and amplified using the Applied Biosystems StepOne thermocycler.

For detection of *S.* Typhimurium, 2 μl of the enriched samples was directly used for qPCR. The oligonucleotide primers (Styinva-JHO-2-left, 5′-TCGTCATTCCATTACCTACC-3′; Styinva-JHO-right, 5′-AAACGTTGAA AAACTGAGGA-3′) and a probe (5′-FAM-TCTGGTTGATTTCCTGATCGCA-TAMRA-3′) targeting 119 bp of nucleotide sequence in the *invA* gene were used as described before (Hoorfar et al., 2000).

For *L. monocytogenes* detection, PrepMan™ Ultra sample preparation reagent (Applied Biosystems) was used to extract DNA from enriched samples and 1 μl was used as template in a 25 μl reaction volume containing 100 nM probe and 900 nM of each primer. Amplification conditions include initial denaturation at 94 °C for 10 min followed by 40 cycles consisting of 95 °C for 15 s, 58 °C for 1 min and 72 °C for 30 s. The oligonucleotide primers (LIM2, 5′-CTA AAG CGG GAA TCT CCC TT-3′; LIMRE, 5′-CCA TTG TCT TGC GCG TTA AT-3′) and a probe (LMS2, 5′-FAM — CTT CTG GCG CAC AAT ACG CTA GCA CT — TAMRA-3′) targeting 175 bp of nucleotide sequence in the *iap* gene were used (Hein et al., 2001).

2.7. Light-scattering sensor

To test the applicability of the PED system as a sample preparation/enrichment device for the light-scattering sensor, two different concentrations of *S.* Enteritidis PT21 (10 CFU/g and 100 CFU/g) were artificially inoculated into ground beef samples. Several aliquots of PED enriched cell suspensions (100 μl) collected at different time points during incubation (2–10 h) were spread-plated evenly on the surface of BHI and XLD agar plates at different dilutions and incubated at 37 °C or until the colony size was about 1 mm diameter (Singh et al., 2014). The petri-dish was placed under BARDOT, and the system collected forward-scatter images of illuminated individual semitransparent colonies through a laser (635 nm). The bacterial colony scatter patterns were identified by comparing scattering images with the image library database (Banada et al., 2007; Banada et al., 2009; Singh et al., 2014). The scatter image library of *S.* Enteritidis on BHI and XLD agar consisted of a minimum of 70 scatter patterns of three strains with total of 450 images. The results of the image analysis were represented as percent positive predictive value (PPV) (Ahmed et al., 2013).

2.8. Recovery of bacteria from a model conveyor belt system using swab

A portable bench-top conveyor system driven by an electric powered motor simulating actual roller bed (manufactured by Shuttleworth, LLC, Huntington, IN, USA) was used as a model to collect swab samples (Fig. 2B). Freshly grown *L. monocytogenes* cells were resuspended in 10 ml of 0.1% peptone water to the cell concentration of 10, 100, or 1000 CFU/ml. The cell suspension was sprayed evenly onto the conveyor surface simulating the food contact surface during food manufacturing operation and allowed to sit for 30 min. Sample collection swabs on wooden sticks (VWR) presoaked in 0.1% peptone water were used to wipe different parts of the conveyor and then placed in the PED chamber containing 30 ml of BLEB (Fig. 2B). PED was incubated at 37 °C for 4–12 h with constant agitation at 150 rpm to enrich the cells bound to swab. The culture broth was collected after passing the sample through filter/ion-exchange cartridge at respective sampling time, and the cell recovery was estimated on MOX agar plate.

2.9. Statistical analysis

Statistical differences for Ct values from qPCR were analyzed by all possible pairwise comparisons using two-sample assuming unequal variance *t*-test. Significance was accepted at the level of $P < 0.05$.

3. Results

3.1. PED performance

Initially, to evaluate the ability of PED to enrich and separate pathogen from food matrices, ground beef samples were inoculated with *L. monocytogenes* (1000 CFU/g) in BLEB and incubated for 24 h. PED recovered cells were examined under a phase contrast microscope, and the bacterial cells appeared to be homogeneous, containing only a few micron sized food particles (Fig. 3A). The eluent was also directly dispensed onto *Listeria*-specific LFIA strip which gave positive reaction (line) within 15 min, while the eluent from the un-inoculated ground beef did not show any reaction (Fig. 3B). These data indicate that PED is capable of separating bacteria from food matrices, and the test pathogen can be detected directly without further sample processing.

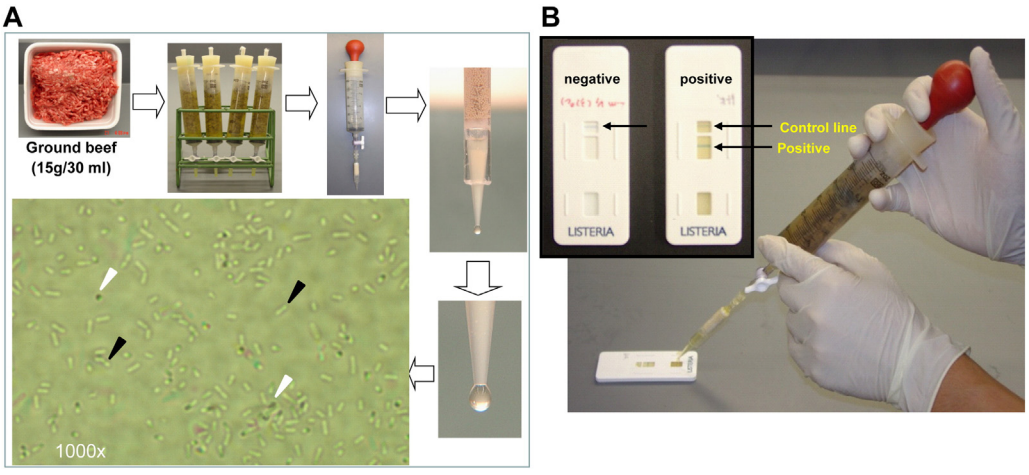

Fig. 3. (A) Phase-contrast microscopic examination of *L. monocytogenes* cells inoculated into ground beef and recovered from PED. (B) Direct testing of PED eluent with *Listeria*-specific lateral flow immunoassay. Dark arrowheads indicate bacterial cells, white arrows are possible micron sized food particles.

3.2. Bacterial growth rate in PED and stomacher bag

Next we determined bacterial growth rate, recovery, and pH of the eluent (Fig. 4). The growth of pathogens (*S.* Typhimurium, *E. coli* O157:H7, *L. monocytogenes*) in PED or stomacher bag with different initial inocula in food samples is shown in Fig. 4A. At 8 h of enrichment, all three bacteria reached the end of logarithmic phase, regardless of growth in the PED or stomacher bag, and growth rate and cell numbers between the PED or stomacher bag methods were similar.

The pH range of the culture taken from the stomacher bag was between 5 and 6, while the pH of the culture eluent from the PED was between 6 and 7.5 (Fig. 4B). These data clearly show that bacterial growth (enrichment) was comparable for both the PED and stomacher bag; however, the lower pH of the enriched samples in stomacher bag may interfere with bioanalytical-based assays if the sample is not further processed. The pH of eluent from the PED was close to neutral, which on the other hand, may enhance accuracy of LFIA and qPCR results (see below) due to the removal of ionic molecules by Amberlite resin.

3.3. Recovery of bacteria after passing through the PED

Percentage of *L. monocytogenes* cell recovery after passing through the filtration/ion exchange cartridge are shown in Table 1. After 8 h of enrichment, samples with different inoculum concentrations, except 1 CFU/g of inoculation, were enumerated by plating, and in most cases, the recovery percentages after passing through the cartridge were over 80. With prolonged enrichment period (up to 16 h), cell masses continue to increase, and the recovery was 100% for the majority of samples (Table 1).

Table 1
Recovery rate of *L. monocytogenes* after passing through the ion-exchange cartridge.

Enrichment time (h)	Percent recovery for inoculated cell numbers after inoculated with			
	1 CFU/g	10 CFU/g	100 CFU/g	1000 CFU/g
4	ND[a]	ND	93.8 ± 19.5	91.6 ± 2.8
8	ND	83.6 ± 18.6	97.3 ± 17.3	126.9 ± 34.6
12	79.1 ± 28.3	116.1 ± 13.6	86.9 ± 21.6	81.1 ± 16.9
16	79.9 ± 23.1	101.7 ± 14.6	101.2 ± 28.4	105.3 ± 12.2

[a] ND: not detected.

3.4. Recovery of bacteria from model conveyor belt system

The recovery rate of *L. monocytogenes* cells from the conveyor belt system after enrichment in the PED is presented in Table 2. After 4-h of enrichment, the conveyor system inoculated with spray-mist containing 1000 CFU/ml under a biosafety cabinet, showed detectable growth on MOX plates. While after 12 h of incubation, all samples from the conveyor system receiving spray-mist containing 10, 100 or 1000 CFU/ml were positive and the cell counts are presented in Table 2.

3.5. Lateral flow immunoassay (LIFA) test for the detection of enriched pathogen spiked into food samples

In the lateral flow immunoassay, PED or stomacher bag-enriched samples inoculated with 1, 10, or 100 CFU/g, tested every 2 h gave positive results only after 6–8 h of enrichment. Food samples inoculated with 1–10 CFU/g gave results after 8 h of enrichment, while food

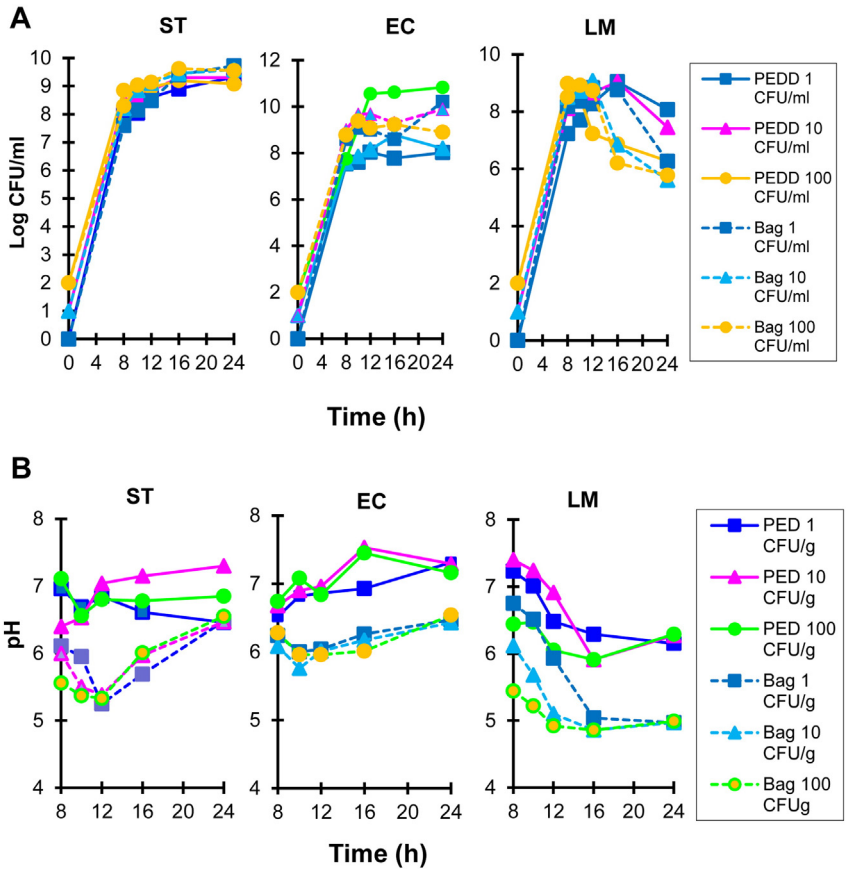

Fig. 4. Comparison of (A) growth and (B) pH of culture broth of pathogens spiked onto food samples and enriched in PED or traditional stomacher bag at different inoculation levels in respective enrichment broths, *S.* Typhimurium (ST) in RV; *E. coli* O157:H7 (EC) in mEC + Novo; *L. monocytogenes* (LM) in BLEB.

Table 2
Enrichment and recovery of *L. monocytogenes* from conveyor belt by PED.

Incubation time (h)	Enriched bacterial counts (CFU/ml) after inoculated with		
	10 CFU/ml	100 CFU/ml	1000 CFU/ml
4 h	ND[a]	ND	$1.2 \times 10^3 \pm 0.06 \times 10^3$
8 h	ND	$7.0 \times 10^2 \pm 0.27 \times 10^2$	$8.9 \times 10^3 \pm 0.13 \times 10^3$
12 h	$3.2 \times 10^2 \pm 0.23 \times 10^2$	$1.8 \times 10^3 \pm 0.02 \times 10^3$	$5.6 \times 10^4 \pm 0.04 \times 10^4$

[a] ND, Not detected.

inoculated with 100 CFU/g gave results after 6 h of enrichment regardless of the enrichment system (Fig. 5).

3.6. Real-time qPCR of respective pathogen spiked onto food samples

The real-time qPCR was performed directly with bacterial cells from both PED- and bag-enriched food samples after 5 and 7 h of enrichment. For *E. coli* O157:H7 in spinach, the amplification started 4–5 cycles earlier when the sample from the PED was used compared to the sample from the stomacher bag (Fig. 6A). Likewise, for *S.* Typhimurium, PED-enriched samples showed amplification 4 cycles earlier than that of bag enriched samples even though the amplification started late compared to that of *E. coli* O157:H7 (Fig. 6B). For *L. monocytogenes*, PCR was performed with DNA extracted from cells obtained from the PED and bag. There were no noticeable differences in Ct values between PED and stomacher bag enriched samples, regardless of the enrichment time, and the ΔRn values were relatively low compared to those from the amplification of *E. coli* O157:H7 or *S.* Typhimurium (Fig. 6C and Table 3).

The PCR amplification experiment was performed three times and the calculated Ct values are presented for each (Table 3). As seen in the amplification plots (Fig. 6), the Ct values between the PED and bag methods were significantly different ($P < 0.05$) for *E. coli* O157:H7 and *S.* Typhimurium, but the Ct values obtained from the amplification of *L. monocytogenes* showed no significant differences between the PED and stomacher bag.

3.7. Light scattering sensor-based detection of pathogens from enriched samples

The PED-enriched cultures of *S.* Enteritidis inoculated at 100 CFU/g of ground beef provided around 40 colonies on BHI and XLD after just 2 h of enrichment, whereas the samples inoculated with 10 CFU/g of ground beef exhibited an average of 30 colonies on BHI and XLD after 4 h of enrichment (Fig. 7). Colony scatter patterns captured with BARDOT for the enriched samples on BHI and XLD agar media were remarkably similar to the scatter images of *S.* Enteritidis in the library (Fig. 7). Image analysis of the scatter pattern of PED-enriched *S.* Enteritidis colonies revealed >90% PPV on both BHI and XLD agar media after matching with the *S.* Enteritidis library generated on BHI and XLD agar.

4. Discussion

The bottleneck for any successful rapid and sensitive detection of microorganisms is sample preparation (Brehm-Stecher et al., 2009). In most cases, pathogens are present in very low proportions compared to the background natural microbiota in a test sample from complex food matrices. The accuracy of a PCR-based or antibody-based test depends on the removal of interfering molecules from food matrices, which consist of lipids, carbohydrates, proteins, ionic molecules, chemical preservatives, salts and natural background microorganisms (Josefsen et al., 2004; Lofstrom et al., 2004). Interference from food

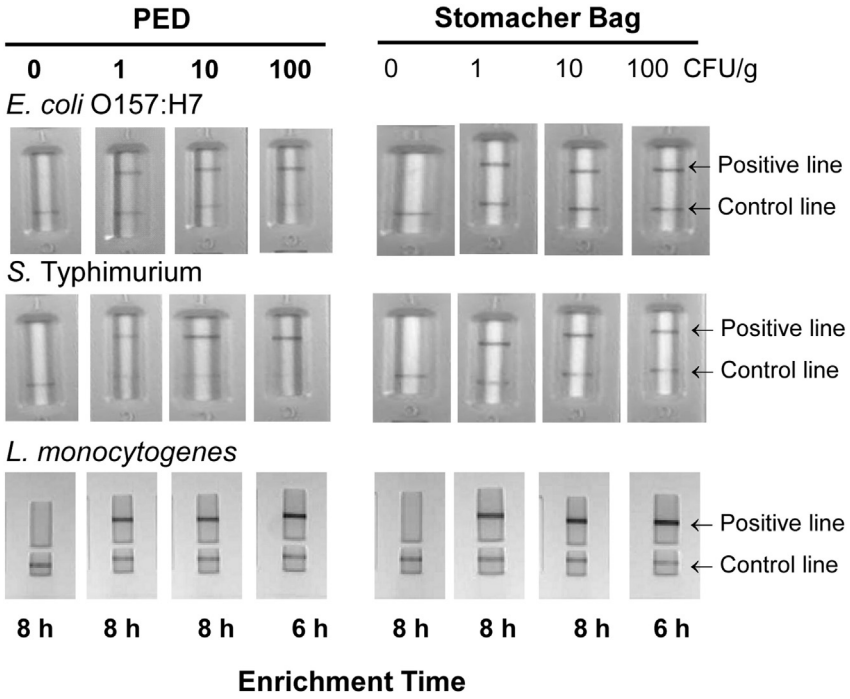

Fig. 5. Detection of the pathogens enriched in PED or stomacher bag using the lateral flow immunoassay (LFIA). Each bacterium was spiked onto the corresponding food sample and enriched for 8 h in the respective enrichment broths. The enriched samples (100 μl) were directly applied to the LIFA for 15–20 min.

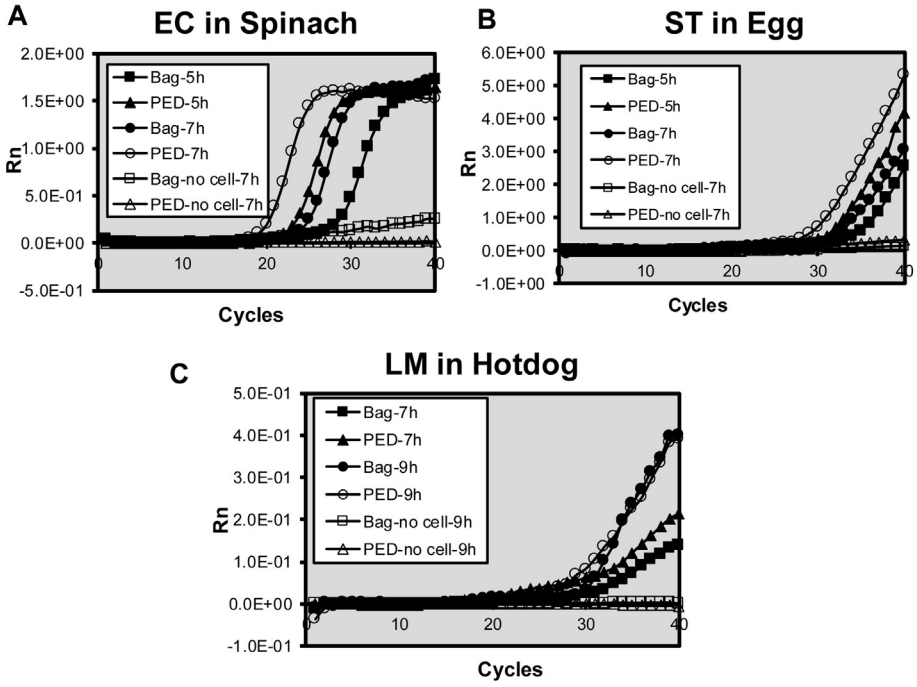

Fig. 6. Real-time qPCR for the detection of pathogens from inoculated food samples after enrichment in PED and the stomacher bag. Two microliters of enriched samples were directly used as the template for (A) *E. coli* O157:H7 (EC) from spinach and (B) *S.* Typhimurium (ST) from boiled egg. For (C) *L. monocytogenes* (LM) from hotdog, DNA was extracted prior to the amplification step.

matrices or natural microorganisms is more evident for highly sensitive assay platforms such as biosensors where the detection limit or assay sensitivity can be severely affected (Dailey et al., 2014; Geng et al., 2006b; Sapsford et al., 2004; Valadez et al., 2009; Varshney and Li, 2007).

PED is a self-contained pathogen enrichment device designed to remove interfering particles and to recover a small volume (1–5 ml) of pH-adjusted homogeneous bacterial cell suspension for wide range of antibody or nucleic acid-based assay platforms such as PCR, ELISA, fiber-optic immunosensor, microfluidic biochip, biosensor, lateral flow strips, and fluorescence microscopy. This device also could provide enrichment of a sample using appropriate selective enrichment broth for a desirable time to suppress natural background bacteria and allow resuscitation and growth of stressed or injured cells to a level that could be detected by a bioanalytical detection system (Biao and Yuexia, 2013; Mondani et al., 2014; Zhou et al., 2011). This is particularly useful because most test organisms routinely encounter some level of stress during processing or storage of foods or food processing environments (Hahm and Bhunia, 2006; Lathrop et al., 2008). Without appropriate resuscitation, the chance for a false-negative result can be very high, which is unacceptable in many situations (Geng et al., 2006a; Mondani et al., 2014).

In PED, several filters and ion exchange resins are strategically placed in the filtration cartridge to remove particles and ionic molecules from the sample so that the homogeneous microorganisms could be delivered to an appropriate detection platform. The PED is comprised of two or more filters or ion exchange cartridges in series. This series of filters are designed to exclude particles based on size. Furthermore, ion exchange resin cartridge-filter is designed to remove ionic molecules likely to be present in a sample. Such size-exclusion and ion exchange components may be optimized to allow increased recovery of microorganisms from a particular sample type. Also activated charcoal or chelators can be used to remove inhibitors (Lee and Levin, 2011). Once isolated, the microorganism may be detected using standard procedures such as spread plating on selective or differential indicator media, real-time PCR, immunoassays, fiber optic sensor, flow cytometry, or microfluidic biochips (Bhunia, 2014).

In theory, enzymatic nucleic acid amplification methods such as PCR could amplify one copy of specific nucleotide sequence, such as DNA or RNA, up to a million-fold, and this means single bacterial cell could be identified by this method in a few hours (Erlich et al., 1991). However, milliliter scale volume of food sample compared to microliter scale volume of amplification reaction, possible reaction inhibitors present in food matrix, and comparably low numbers of the target microorganisms to background microorganisms are the limiting factors for the theoretical detection limits for such nucleic acid-based assays. In fact, 10^2–10^3 CFU of target pathogenic bacterial cells per gram of food sample is the minimum numbers one could detect by enzymatic nucleotide amplification methods and these levels

Table 3
Comparison of Ct values from respective amplification reaction.

	Threshold Ct values from three experimental replicates			Average Ct value	Statistical significance (*P* value)
	1st	2nd	3rd		
E. coli O157:H7					
Bag-5h[a]	25.79	27.12	24.85	25.92 ± 1.14	0.005
PED-5h[b]	21.56	22.45	19.98	21.33 ± 1.25	
Bag-7 h	24.33	23.98	ND	24.16 ± 0.24	0.002
PED-7 h	18.86	19.38	ND	19.12 ± 0.36	
S. Typhimurium					
Bag-5 h	28.54	27.51	29.24	28.43 ± 0.87	0.027
PED-5 h	26.51	26.63	27.12	26.75 ± 0.32	
Bag-7 h	29.29	27.73	ND	28.51 ± 1.10	0.026
PED-7 h	24.91	23.71	ND	24.31 ± 0.84	
L. monocytogenes					
Bag-7 h	31.11	33.29	31.86	32.09 ± 1.10	0.474
PED-7 h	32.24	32.64	31.22	32.03 ± 0.73	
Bag-9 h	28.88	31.91	ND	30.40 ± 2.14	0.387
PED-9 h	28.95	30.69	ND	29.82 ± 1.23	

Significant variation at $P < 0.05$ was calculated for the threshold Ct value obtained for the bacteria enriched in bag and PED system for defined time; ND, not done.
[a] Bag, enrichment in stomacher bag.
[b] PED, enrichment in pathogen enrichment device.

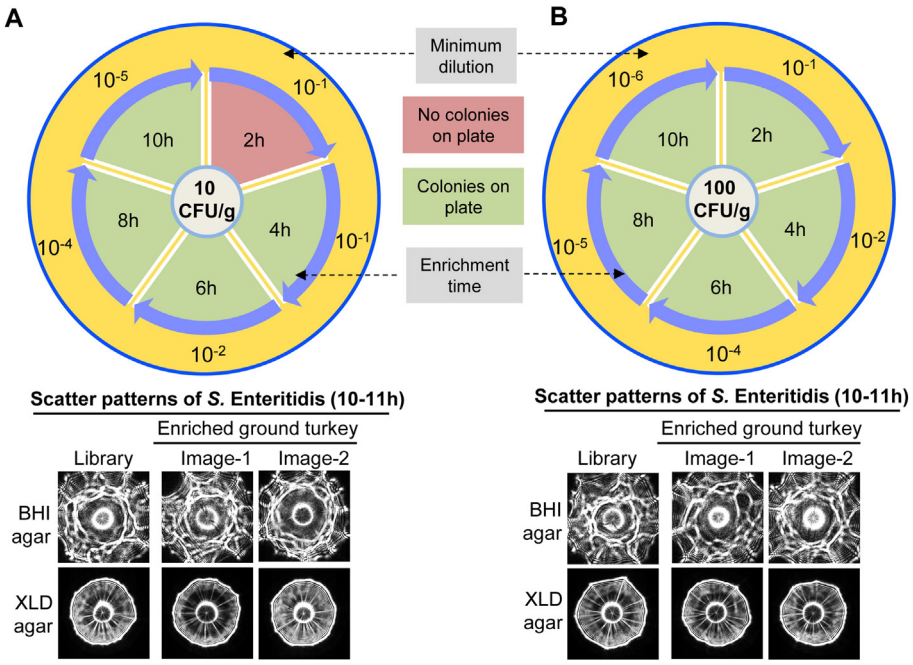

Fig. 7. Enrichment of *S.* Enteritidis PT21 cells inoculated in ground beef sample at different levels with the PED and validation of colonies with light-scattering sensor based on scatter patterns. Scatter patterns were captured when colony diameter reached to ~1 mm. (A) Ground beef samples inoculated with 10 CFU/g of inoculum to capture the scatter pattern on BHI and XLD agar after enrichment. (B) Ground beef samples inoculated with 100 CFU/g of inoculum to capture the scatter pattern on BHI and XLD agar after enrichment. Minimum dilutions, dilutions of enriched sample at different time that resulted in minimum 5–10 colonies on agar plate.

are slightly lower than those of ELISA or microarray method (Bhunia, 2014; Stevens and Jaykus, 2004). In our study, 10 CFU of tested microorganisms per gram of food sample was inoculated, and after 5–7 h enrichment, 1–2 × 10^3 cells could be used for 25 μl volume of real-time PCR reaction. For the antibody-based lateral flow strip test, we had to incubate up to 6–8 h for the detectable positive band on the strip, and the cell numbers were estimated to be between 10^5–10^6 CFU/ml. Use of more sensitive LIFA with a detection limit of 10–100 CFU/ml (Cho et al., 2015b; Hossain et al., 2012) could substantially shorten the sample-to-result detection time.

As shown in Fig. 6, the real-time PCR plot of *E. coli* O157:H7 enriched by PED and traditional bag method showed clear differences in amplification start point (Ct value) between cells, which were used directly as PCR template. This improved detection could be attributed to the removal of ionic inhibitors (pH adjustment) by PED, which was not accomplished in the bag method. Likewise, in *S.* Typhimurium, amplified products from the enriched cells in PED were detected much earlier than the samples taken from stomacher bag, but much of the amplification was noticed much later than *E. coli*. In fact, late amplification could be caused by carry over contamination (Killgore et al., 2000); however, according to a previous report, trace amounts of DNA can be detected and appeared even after 34 cycles on the amplification plot, and the late amplified product was confirmed by sequencing (Mothershed et al., 2002). Samples were also analyzed and confirmed by agarose gel electrophoresis to confirm whether the amplified plot was due to positive amplification of our target sequence or just false amplification, and we found the amplified product of our target sequence with the expected size product in the gel (data not shown). The reason for late amplification of target gene (*invA*) in *S.* Typhimurium is speculated by the fact that in the food sample, the cells were spiked in boiled egg. Substantial egg debris was found in the enrichment broth, even after filtering through the cartridge attached to PED, and this could have interfered with the amplification. Moreover, the yolk contains relatively high amounts of fats and this could trap the bacteria at the fat interface, preventing bacterial separation (Payne et al., 1992). In the case of *L. monocytogenes* in hotdog, there were no differences in Ct values between the PED-enriched and bag-enriched methods. This is because, we had to extract the DNA from the enriched cells using a DNA extraction kit before PCR amplification. Unlike Gram-negative bacteria such as *E. coli* or *Salmonella* cells, *L. monocytogenes* is Gram-positive with cell wall consisting of thick peptidoglycan. Therefore, additional steps were needed for extraction of the template DNA, and it is speculated that this harmonized the amplification plot between the samples from the PED and bag method by eliminating PCR-interfering materials in the broth during the DNA extraction step.

Additionally, light-scattering sensor has also validated and detected *S.* Enteritidis inoculated into ground beef based on optical scatter pattern. In our previous study, this biosensor successfully detected the target bacteria in the inoculated and naturally contaminated chicken and produce samples (Singh et al., 2014; Tang et al., 2014). PED also provides an opportunity for simultaneous enrichment of multiple pathogens in a single device using a multipathogen enrichment broth such as SEL (Biao and Yuexia, 2013; Gehring et al., 2012; Kim and Bhunia, 2008).

In conclusion, apart from the enrichment and separation of the target bacteria, PED-based sample processing by passing through the filters and ion-exchange cartridge prior to a detection step have tremendously improved PCR-based method for pathogen in the food sample. Such an enrichment device is helpful to remove inhibitors, improve the recovery of pathogens, and the stability of reaction for the detection on bioanalytical platforms. The device design shows proof-of concept for one-step enrichment and delivery of samples to assay platform for direct detection with minimal sample manipulation and handling. This study also presents data for future modifications and automation of the device to handle larger sample volumes.

Author's contributions

A.K.B and B.H. conceived the idea of this study. B.H., A.K.S. and A.K.B. analyzed the results. B.H. and HK performed the experiments. B.H., A.K.S., and A.K.B. wrote the manuscript.

Acknowledgments

This research was supported through a cooperative agreement with the Agricultural Research Service of the US Department of Agriculture project number 1935-42000-072-02G and the Center for Food Safety Engineering at Purdue University. The authors acknowledge Taylor Bailey for critical reading of the manuscript. The authors declare no conflict of interest.

References

Ahmed, W.M., Bayraktar, B., Bhunia, A.K., Hirleman, E.D., Robinson, J.P., Rajwa, B., 2013. Classification of bacterial contamination using image processing and distributed computing. IEEE J. Biomed. Health Inform. 17, 232–239.

Annonymous, 2009. 2008 Listeriosis Outbreak in Ontario Epidemiologic Summary (Accessed on July 26, 2011).

Bae, E., Bhunia, A.K., 2013. Nano optical sensors for food safety and security. In: Cusano, A., Arregui, F.J., Giordano, M., Cutolo, A. (Eds.), Optochemical Nanosensors. CRC Press, Taylor and Francis Group, Boca Raton, FL, pp. 497–512.

Bae, E., Aroonnual, A., Bhunia, A.K., Hirleman, E.D., 2011. On the sensitivity of forward scattering patterns from bacterial colonies to media composition. J. Biophotonics 4, 236–243.

Banada, P.P., Guo, S., Bayraktar, B., Bae, E., Rajwa, B., Robinson, J.P., Hirleman, E.D., Bhunia, A.K., 2007. Optical forward-scattering for detection of *Listeria monocytogenes* and other *Listeria* species. Biosens. Bioelectron. 22, 1664–1671.

Banada, P.P., Huff, K., Bae, E., Rajwa, B., Aroonnual, A., Bayraktar, B., Adil, A., Robinson, J.P., Hirleman, E.D., Bhunia, A.K., 2009. Label-free detection of multiple bacterial pathogens using light-scattering sensor. Biosens. Bioelectron. 24, 1685–1692.

Bhunia, A.K., 2008. Biosensors and bio-based methods for the separation and detection of foodborne pathogens. Adv. Food Nutr. Res. 54, 1–44.

Bhunia, A.K., 2014. One day to one hour: how quickly can foodborne pathogens be detected? Future Microbiol 9, 935–946.

Biao, S., Yuexia, W., 2013. Evaluation of a multiplex selective enrichment broth SEL for simultaneous detection of injured *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Braz. J. Microbiol. 44, 737–742.

Bisha, B., Brehm-Stecher, B.F., 2009. Flow-through imaging cytometry for characterization of *Salmonella* subpopulations in alfalfa sprouts, a complex food system. Biotechnol. J. 4, 880–887.

Brehm-Stecher, B., Young, C., Jaykus, L.-A., Tortorello, M.L., 2009. Sample preparation: the forgotten beginning. J. Food Prot. 72, 1774–1789.

CDC, 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. MMWR Morb. Mortal. Wkly Rep. 60, 1357–1358.

CDC, 2014. Reports of Selected Salmonella Outbreak Investigations. CDC (http://www.cdc.gov/salmonella/outbreaks.html).

CDC, 2015. Reports of selected *E. coli* outbreak investigations Vol. http://www.cdc.gov/ecoli/outbreaks.html CDC.

Chen, W.T., Hendrickson, R.L., Huang, C.P., Sherman, D., Geng, T., Bhunia, A.K., Ladisch, M.R., 2005. Mechanistic study of membrane concentration and recovery of *Listeria monocytogenes*. Biotechnol. Bioeng. 89, 263–273.

Cho, I.-H., Bhandari, P., Patel, P., Irudayaraj, J., 2015a. Membrane filter-assisted surface enhanced Raman spectroscopy for the rapid detection of *E. coli* O157:H7 in ground beef. Biosens. Bioelectron. 64, 171–176.

Cho, I.-H., Bhunia, A., Irudayaraj, J., 2015b. Rapid pathogen detection by lateral-flow immunochromatographic assay with gold nanoparticle-assisted enzyme signal amplification. Int. J. Food Microbiol. 206, 60–66.

Cocolin, L., Rajkovic, A., Rantsiou, K., Uyttendaele, M., 2011. The challenge of merging food safety diagnostic needs with quantitative PCR platforms. Trends Food Sci. Technol. 22 (Supplement 1), S30–S38.

Dailey, R.C., Martin, K.G., Smiley, R.D., 2014. The effects of competition from non-pathogenic foodborne bacteria during the selective enrichment of *Listeria monocytogenes* using buffered *Listeria* enrichment broth. Food Microbiol. 44, 173–179.

Dwivedi, H.P., Jaykus, L.-A., 2011. Detection of pathogens in foods: the current state-of-the-art and future directions. Crit. Rev. Microbiol. 37, 40–63.

Erickson, M.C., 2010. Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. Compr. Rev. Food Sci. Food Saf. 9, 602–619.

Erlich, H.A., Gelfand, D., Sninsky, J.J., 1991. Recent advances in the polymerase chain reaction. Science 252, 1643–1651.

FDA, 2001. Bacteriological Analytical Manual Online. 8th ed. AOAC International, Arlington, VA ([http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm]; Date accessed, January 16, 2014).

Fretz, R., Pichler, J., Sagel, U., Much, P., Ruppitsch, W., Pietzka, A.T., Stöger, A., Huhulescu, S., Heuberger, S., Appl, G., Werber, D., Stark, K., Prager, R., Flieger, A., Karpíšková, R., Pfaff, G., Allerberger, F., 2010. Update: multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different *L. monocytogenes* serotype 1/2a strains, 2009–2010. Euro Surveill. 15, 1–2.

Fukushima, H., Kawase, J., Etoh, Y., Sugama, K., Yashiro, S., Iida, N., Yamaguchi, K., 2010. Simultaneous screening of 24 target genes of foodborne pathogens in 35 foodborne outbreaks using multiplex real-time SYBR green PCR analysis. Int. J. Microbiol. 2010, 18.

Gehring, A.G., Albin, D.M., Bhunia, A.K., Kim, H., Reed, S.A., Tu, S.-I., 2012. Mixed culture enrichment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Yersinia enterocolitica*. Food Control 26, 269–273.

Geng, T., Hahm, B.K., Bhunia, A.K., 2006a. Selective enrichment media affect the antibody-based detection of stress-exposed *Listeria monocytogenes* due to differential expression of antibody-reactive antigens identified by protein sequencing. J. Food Prot. 69, 1879–1886.

Geng, T., Uknalis, J., Tu, S.I., Bhunia, A.K., 2006b. Fiber-optic biosensor employing AlexaFluor conjugated antibody for detection of *Escherichia coli* O157:H7 from ground beef in four hours. Sensors 6, 796–807.

Hahm, B.K., Bhunia, A.K., 2006. Effect of environmental stresses on antibody-based detection of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Enteritidis and *Listeria monocytogenes*. J. Appl. Microbiol. 100, 1017–1027.

Hahm, B.K., Kim, H., Bhunia, A.K., 2007. Enrichment and detection of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* using a sample preparation device, PEDD (Pathogen Enrichment and Detection Device). American Society for Microbiology General Meeting. ASM, Toronto, ON, Canada (pp. Abstr. P-118).

He, Y., Reed, S., Bhunia, A.K., Gehring, A., Nguyen, L.-H., Irwin, P.L., 2015. Rapid identification and classification of *Campylobacter* spp. using laser optical scattering technology. Food Microbiol. 47, 28–35.

Hein, I., Klein, D., Lehner, A., Bubert, A., Brandl, E., Wagner, M., 2001. Detection and quantification of the *iap* gene of *Listeria monocytogenes* and *Listeria innocua* by a new real-time quantitative PCR assay. Res. Microbiol. 152, 37–46.

Hill, V.R., Polaczyk, A.L., Kahler, A.M., Cromeans, T.L., Hahn, D., Amburgey, J.E., 2009. Comparison of hollow-fiber ultrafiltration to the USEPA VIRADEL technique and USEPA method 1623. J. Environ. Qual. 38, 822–825.

Hoorfar, J., Ahrens, P., Radstrom, P., 2000. Automated 5′ nuclease PCR assay for identification of *Salmonella enterica*. J. Clin. Microbiol. 38, 3429–3435.

Hossain, S.M.Z., Ozimok, C., Sicard, C., Aguirre, S.D., Ali, M.M., Yingfu, L., Brennan, J.D., 2012. Multiplexed paper test strip for quantitative bacterial detection. Anal. Bioanal. Chem. 403, 1567–1576.

Huff, K., Aroonnual, A., Littlejohn, A.E.F., Rajwa, B., Bae, E., Banada, P.P., Patsekin, V., Hirleman, E.D., Robinson, J.P., Richards, G.P., Bhunia, A.K., 2012. Light-scattering sensor for real-time identification of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* colonies on solid agar plate. Microb. Biotechnol. 5, 607–620.

Josefsen, M.H., Lubeck, P.S., Hansen, F., Hoorfar, J., 2004. Towards an international standard for PCR-based detection of foodborne thermotolerant campylobacters: interaction of enrichment media and pre-PCR treatment on carcass rinse samples. J. Microbiol. Methods 58, 39–48.

Killgore, G.E., Holloway, B., Tenover, F.C., 2000. A 5′ nuclease PCR (TaqMan) high-throughput assay for detection of the *mecA* gene in staphylococci. J. Clin. Microbiol. 38, 2516–2519.

Kim, H., Bhunia, A.K., 2008. SEL, a selective enrichment broth for simultaneous growth of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Appl. Environ. Microbiol. 74, 4853–4866.

Koo, O.K., Liu, Y., Shuaib, S., Bhattacharya, S., Ladisch, M.R., Bashir, R., Bhunia, A.K., 2009. Targeted capture of pathogenic bacteria using a mammalian cell receptor coupled with dielectrophoresis on a biochip. Anal. Chem. 81, 3094–3101.

Kuehn, B.M., 2010. *Salmonella* cases traced to egg producers. JAMA, J. Am. Med. Assoc. 304, 1316.

Lathrop, A.A., Banada, P.P., Bhunia, A.K., 2008. Differential expression of InlB and ActA in *Listeria monocytogenes* in selective and nonselective enrichment broths. J. Appl. Microbiol. 104, 627–639.

Lee, J.-L., Levin, R.E., 2011. Detection of 5 CFU/g of *Escherichia coli* O157:H7 on lettuce using activated charcoal and real-time PCR without enrichment. Food Microbiol. 28, 562–567.

Li, X., Ximenes, E., Amalaradjou, M.A.R., Vibbert, H.B., Foster, K., Jones, J., Liu, X., Bhunia, A.K., Ladisch, M.R., 2013. Rapid sample processing for detection of food-borne pathogens via cross-flow microfiltration. Appl. Environ. Microbiol. 79, 7048–7054.

Li, Y., Yan, X., Feng, X., Wang, J., Du, W., Wang, Y., Chen, P., Xiong, L., Liu, B.-F., 2014. Agarose-based microfluidic device for point-of-care concentration and detection of pathogen. Anal. Chem. 86, 10653–10659.

Lindqvist, R., 1997. Preparation of PCR samples from food by a rapid and simple centrifugation technique evaluated by detection of *Escherichia coli* O157:H7. Int. J. Food Microbiol. 37, 73–82.

Lofstrom, C., Knutsson, R., Axelsson, C.E., Radstrom, P., 2004. Rapid and specific detection of *Salmonella* spp. in animal feed samples by PCR after culture enrichment. Appl. Environ. Microbiol. 70, 69–75.

Magana, S., Schlemmer, S.M., Davidson, G.R., Ryser, E.T., Lim, D.V., 2014. Laboratory and pilot-scale dead-end ultrafiltration concentration of sanitizer-free and chlorinated lettuce wash water for improved detection of *Escherichia coli* O157:H7. J. Food Prot. 77, 1260–1268.

Maki, D.G., 2009. Coming to grips with foodborne infection with peanut butter, peppers, and nationwide *Salmonella* Outbreaks. New Engl. J. Med. 360, 949–953.

Mondani, L., Roupioz, Y., Delannoy, S., Fach, P., Livache, T., 2014. Simultaneous enrichment and optical detection of low levels of stressed *Escherichia coli* O157:H7 in food matrices. J. Appl. Microbiol. 117, 537–546.

Mothershed, E.A., Cassiday, P.K., Pierson, K., Mayer, L.W., Popovic, T., 2002. Development of a real-time fluorescence PCR assay for rapid detection of the diphtheria toxin gene. J. Clin. Microbiol. 40, 4713–4719.

Mukhopadhyay, S., Tomasula, P.M., Luchansky, J.B., Porto-Fett, A., Call, J.E., 2010. Removal of *Salmonella* Enteritidis from commercial unpasteurized liquid egg white using pilot scale cross flow tangential microfiltration. Int. J. Food Microbiol. 142, 309–317.

Murakami, T., 2012. Filter-based pathogen enrichment technology for detection of multiple viable foodborne pathogens in 1 day. J. Food Prot. 75, 1603–1610.

Najafi, R., Mukherjee, S., Hudson Jr., J., Sharma, A., Banerjee, P., 2014. Development of a rapid capture-cum-detection method for *Escherichia coli* O157 from apple juice comprising nano-immunomagnetic separation in tandem with surface enhanced Raman scattering. Int. J. Food Microbiol. 189, 89–97.

Olaimat, A.N., Holley, R.A., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32, 1–19.

Payne, M.J., Campbell, S., Patchett, R.A., Kroll, R.G., 1992. The use of immobilized lectins in the separation of *Staphylococcus aureus*, *Escherichia coli*, *Listeria* and *Salmonella* spp. from pure cultures and foods. J. Appl. Microbiol. 73, 41–52.

Sapsford, K.E., Rasooly, A., Taitt, C.R., Ligler, F.S., 2004. Detection of *Campylobacter* and *Shigella* species in food samples using an array biosensor. Anal. Chem. 76, 433–440.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17, 7–15.

Scharff, R., 2012. Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. 75, 123–131.

Scheutz, F., Møller Nielsen, E., Frimodt-Møller, J., Boisen, N., M.S, Tozzoli, R., Nataro, J., Caprioli, A., 2011. Characteristics of the enteroaggregative Shiga toxin/verotoxinproducing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 6, 19889.

Sharma, H., Mutharasan, R., 2013. Review of biosensors for foodborne pathogens and toxins. Sensors Actuators B Chem. 183, 535–549.

Singh, A.K., Bettasso, A.M., Bae, E., Rajwa, B., Dundar, M.M., Forster, M.D., Liu, L., Barrett, B., Lovchik, J., Robinson, J.P., Hirleman, E.D., Bhunia, A.K., 2014. Laser optical sensor, a label-free on-plate *Salmonella enterica* colony detection tool. mBio 5, e01019.

Singh, A.K., Sun, X., Bai, X., Kim, H., Abdalhaseib, M.U., Bae, E., Bhunia, A.K., 2015. Label-free, non-invasive light scattering sensor for rapid screening of *Bacillus* colonies. J. Microbiol. Methods 109, 56–66.

Stevens, K.A., Jaykus, L.A., 2004. Bacterial separation and concentration from complex sample matrices: a review. Crit. Rev. Microbiol. 30, 7–24.

Tang, Y., Kim, H., Singh, A.K., Aroonnual, A., Bae, E., Rajwa, B., Fratamico, P.M., Bhunia, A.K., 2014. Light scattering sensor for direct identification of colonies of *Escherichia coli* serogroups O26, O45, O103, O111, O121, O145 and O157. PLoS One 9 e105272.

USDA-FSIS, 2015. Microbiology Laboratory Guidebook. http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook (Date Accessed June 1, 2015).

Valadez, A., Lana, C., Tu, S.-I., Morgan, M., Bhunia, A., 2009. Evanescent wave fiber optic biosensor for *Salmonella* detection in food. Sensors 9, 5810–5824.

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

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., Adley, C., 2010. An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol. Adv. 28, 232–254.

Walsh, K.A., Bennett, S.D., Mahovic, M., Gould, L.H., 2014. Outbreaks associated with cantaloupe, watermelon, and honeydew in the United States, 1973–2011. Foodborne Pathog. Dis. 11, 945–952.

Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63, 3741–3751.

Wolffs, P.F.G., Glencross, K., Thibaudeau, R., Griffiths, M.W., 2006. Direct quantitation and detection of salmonellae in biological samples without enrichment, using two-step filtration and real-time PCR. Appl. Environ. Microbiol. 72, 3896–3900.

Zhou, P., Hussain, S.K., Liles, M.R., Arias, C.R., Backert, S., Kieninger, J., Oyarzabal, O.A., 2011. A simplified and cost-effective enrichment protocol for the isolation of *Campylobacter* spp. from retail broiler meat without microaerobic incubation. BMC Microbiol. 11, 175.