Food Microbiology 33 (2013) 166–171

Contents lists available at SciVerse ScienceDirect

Food Microbiology

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

# Multiplex fiber optic biosensor for detection of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* from ready-to-eat meat samples

Seung-Ho Ohk [a,1], Arun K. Bhunia [a,b,*]

[a] *Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, IN 47907, USA*
[b] *Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA*

ARTICLE INFO

*Article history:*
Received 25 May 2012
Received in revised form
1 September 2012
Accepted 22 September 2012
Available online 29 September 2012

*Keywords:*
Fiber optic biosensor
*Listeria monocytogenes*
*Escherichia coli* O157:H7
*Salmonella enterica*
RTE meat
Detection

ABSTRACT

*Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* are the most common foodborne bacterial pathogens and are responsible for many outbreaks. Therefore, multiplex detection of these three using a single assay platform is highly desirable. The objective was to develop and optimize a fiber optic sensor for simultaneous detection of these three from food. The streptavidin coated optical waveguides were immobilized with biotinylated polyclonal antibodies and exposed to the bacterial suspensions or enriched food samples for 2 h. Pathogens were detected after reacting with Alexa-Fluor 647-labeled monoclonal antibodies. Ready-to-eat beef, chicken and turkey meats were inoculated with each pathogen (~100 cfu/25 g), enriched in SEL (*Salmonella*, *E. coli*, *Listeria*), a multipathogen selective enrichment broth for 18 h and tested with the biosensor. The biosensor was able to detect each pathogen, individually or in a mixture with very little cross-reactivity. The limit of detection for the sensor was ~$10^3$ cfu/ml for all three pathogens. Furthermore, the biosensor successfully detected each pathogen, grown in a mixture from enriched meat samples under 24 h. The pathogen presence was further verified by PCR and immunofluorescence assay. The multiplex fiber optic sensor shows promise for detection of the three pathogens if present in the same sample eliminating the use of multiple single pathogen detection platforms.



## 1. Introduction

Foodborne pathogens are a major public health concern due to continued outbreaks, emergence of new pathogens and associated fatalities (Scallan et al., 2011). To reduce foodborne infections, high-risk products must be tested before sold for human consumption. Among the foodborne pathogens, *Salmonella enterica*, *Listeria monocytogenes* and Shiga-toxin producing *Escherichia coli* are responsible for the majority of recent outbreaks and hospitalizations (Lynch et al., 2006; Scallan et al., 2011). Therefore, developing methods that can detect the presence of any or all of the three organisms in a single analysis would help ensure safety against the three major foodborne pathogens. At present, the commercially available test kits are designed for a single analyte, thus to test a product for multiple analytes, multiple assay kits must be used. This approach is not cost effective and may discourage food producers from adopting it for routine practice since this would ultimately affect the profit margin. Thus, the array-based multi-pathogen testing approaches are becoming attractive owing to their advantage in addressing not only the routine food safety need but also the food biosecurity demand (Bhunia, 2011; Gehring and Tu, 2011; Lim et al., 2005). For detection of multiple targets in one single experiment, nucleic acid-based microarray (Miller and Tang, 2009), multiplex PCR (Kawasaki et al., 2010; Park et al., 2011) or protein microarray (Rebe Raz and Haasnoot, 2011) have been proposed. Among the biosensor platforms, evanescent wave based detection of multiple analytes on glass slides in an array format (Ligler et al., 2007), surface plasmon resonance sensor (Homola, 2008) and cell-based sensors show promise (Banerjee and Bhunia, 2009). For effective multipathogen analysis on the same platform, the sample preparation step (Brehm-Stecher et al., 2009) must allow recovery of all potential target pathogens, and their concentrations should exceed the detection limit threshold of the sensor platform to avoid false-negative results.

In this study, our goal was to develop an evanescent-based fiber optic sensor for multiplex detection of *S. enterica* serovar Enteritidis, *L. monocytogenes* and *E. coli* O157:H7 from food samples. The fiber-optic sensor utilizes laser excitation to generate evanescent

* Corresponding author. Department of Food Science, Purdue University, West Lafayette, IN 47907, USA. Tel.: +1 765 494 5443; fax: +1 765 494 7953.
*E-mail address:* Bhunia@purdue.edu (A.K. Bhunia).
[1] Present address: Department of Oral Microbiology, 2nd Stage of Brain Korea 21, Chonnam National University, Gwangju 500-757, South Korea.

0740-0020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2012.09.013

wave that corresponds to the quantity of analyte present on the surface of the waveguide in a sandwich format where the fluorophore (Cyanine 5 or Alexa Fluor 647) conjugated antibody serves as a reporter (Bhunia, 2008; Leung et al., 2007; Taitt et al., 2005). The fiber optic sensor using the Analyte 2000 (Research International, Monroe, WA) has been successfully developed for detection of several foodborne or biothreat pathogens individually including *E. coli* O157:H7 (DeMarco et al., 1999; Geng et al., 2006), *Salmonella* (Valadez et al., 2009), *L. monocytogenes* (Geng et al., 2004; Ohk et al., 2010), *Bacillus anthracis* and *Francisella tularensis* (Jung et al., 2003), and staphylococcal enterotoxins (Anderson and Nerurkar, 2002). In this work, we developed a multipathogen assay system using the Analyte 2000 for simultaneous detection of *L. monocytogenes*, *E. coli* O157:H7 and *S.* Enteritidis inoculated into food samples.

## 2. Materials and methods

### 2.1. Bacterial cultures and growth media

*L. monocytogenes* F4244 (serovar 4b), *E. coli* O157:H7 EDL 933 and *S. enterica* serovar Enteritidis PT1 were used as standard reference strains and were maintained on brain heart infusion (BHI) agar plates at 4 °C. For concurrent enrichment of all three pathogens, a multipathogen enrichment broth, SEL (*Salmonella*, *Escherichia*, and *Listeria*), previously developed in our laboratory was used (Kim and Bhunia, 2008). For bacterial enumeration on agar plates, modified Oxford agar (MOX) for *L. monocytogenes*, xylose lysine deoxycholate (XLD) for *Salmonella* and cefixime–tellurite containing sorbitol MacConkey (CT-SMAC) for *E. coli* O157:H7 were used. All media were purchased from Acumedia (Lancing, MI).

### 2.2. Antibodies, labeling and sandwich immunofluorescence assay

Pathogen-specific rabbit polyclonal antibodies were used as capture and mouse monoclonal antibodies were used as reporter in a sandwich configuration on the optical waveguide. For *L. monocytogenes*, PAb-P66 (Chen et al., 2005) and MAb-C11E9 (Bhunia et al., 1991); for *E. coli*, PAb-anti-pET32a (our lab, unpublished) and anti-*E. coli* O157 (MyBiosource), and for *S. enterica*, PAb-anti-*Salmonella* (Valadez et al., 2009) and MAb-2F11 (Jaradat et al., 2004) were used. Capture antibodies were biotinylated with EZ-Link Sulfo-NHS-LC-Biotin kit (Pierce, Rockford, IL) and monoclonal antibodies were labeled with Alexa-Fluor 647 (Molecular Probes) (AF-Reporter antibody) as described (Geng et al., 2006).

Performance of the antibody combinations to detect the target pathogens was optimized by an immunofluorescence assay (IFA) using a streptavidin coated 96-well plate (Thermo Fisher Scientific, Rockford, IL). Each biotinylated capture antibody (0.1 μg/ml) was added at 100 μl/well and incubated at 4 °C 2 h. The plates were subsequently blocked with Superblock (Pierce, Rockford, IL) solution (0.1 ml/well) for 2 h. Target pathogens were added individually ($\sim 1 \times 10^8$ cfu/well) or in a mixture to designated wells, incubated for 2 h, washed three times with 0.1 M PBS (pH 7.2) containing Triton-X 100 (PBS-T) and reacted with Alexa-Fluor 647 labeled reporter antibodies (0.01 mg/ml) for 2 h. Plates were washed and read in a spectrofluorometer at Ex 584 nm/Em 612 nm (Molecular Devices, Sunnyvale, CA).

### 2.3. Fiber optic sensor-based analysis

The polystyrene waveguides (Research International, Monroe, WA) were pre-cleaned and coated with streptavidin (1 mg/ml, Sigma) at 4 °C for 2 h and air-dried for 30 min (Geng et al., 2006). This coating step was repeated three times by recycling the streptavidin solution and then by rinsing the waveguides with distilled sterile water. The waveguides were then immersed in SuperBlock blocking buffer (Pierce) for 1 h followed by exposure to each 100 μl biotinylated capture antibody (100 μg/ml) at 4 °C for 2 h, washed in deionized water three times, and air-dried for 30 min.

To determine the limit of detection for the fiber optic sensor for each pathogen, bacterial cell pellets were harvested from overnight grown test cultures and washed with Tris buffered saline (TBS: 0.1 M, pH 8.0) three times and serially diluted to make desired concentrations. Antibody-coated fibers were immersed in 0.1 ml of bacterial suspensions and incubated at 4 °C for 2 h. After gentle washing, the fibers were again immersed in 0.1 ml of AF-Reporter antibodies at 4 °C for 2 h. Fluorescence intensity for each waveguide was acquired simultaneously in separate channels with the Analyte 2000 Fluorometer (Research International) for 30 s. The schematic of the fiber optic sensor setup for multipathogen analysis is presented in Fig. 1.

To determine the reactivity of each pathogen-specific fiber optic sensor, waveguides were also exposed to pure cultures of *L. monocytogenes*, *E. coli* O157:H7 and *S.* Enteritidis at $1 \times 10^8$ cfu/ml separately and in a mixture of all three for 2 h and the assays were performed as above.

### 2.4. Detection of pathogens from artificially contaminated food samples by fiber optic sensor

Sliced ready-to-eat lunch meats of shredded beef, chicken and turkey breast meats were purchased from local grocery stores in West Lafayette, Indiana. To confirm the absence of target

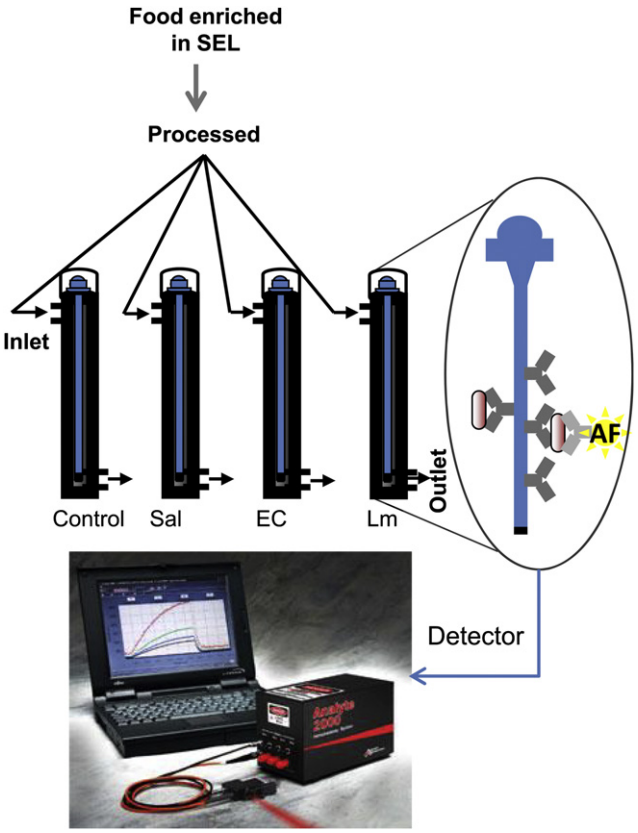


**Fig. 1.** Schematic showing layout of multipathogen detection strategy using fiber optic sensor. SEL (*Salmonella*, *E. coli*, *Listeria*); Sal, *Salmonella*; EC, *E. coli*; Lm, *L. monocytogenes*.

pathogens, the standard pathogen isolation procedures described in the Bacteriological Analytical Manual (FDA, 2001) were carried out before the challenge study.

Twenty five grams of each meat sample in a stomacher bag (Nasco Whirl-Pak® Catalog #B01318, Fort Atkinson, WI) was inoculated with each pathogen (~1 × $10^2$ cfu/ml) separately or a mixture of all three and held for 15 min at room temperature. For enrichment, 225 ml of SEL broth (Kim and Bhunia, 2008) was added in each bag and blended for 2 min using a Stomacher (Stomacher 400, Seward, Norfolk, UK) and the bags were incubated at 37 °C for 18 h. A 0.1 ml aliquot collected from each bag was applied to each fiber-optic sensor and incubated at 4 °C for 2 h. After gentle washing, the waveguides were again immersed in 0.1 ml of AF-Reporter antibodies at 4 °C for 2 h, washed in PBS-T three times and fluorescence intensity was recorded with the Analyte 2000 for 30 s.

Inoculated food samples were also examined by immunofluorescence assays on microtiter plates as described above. Enriched meat samples (0.1 ml/well) were also added and incubated for 2 h, washed and reacted with AF-Reporter antibodies for 2 h at 37 °C. Plates were washed three times with PBS-T and read in a spectrofluorometer (Molecular Devices).

Several aliquots of samples were also collected from each bag, serially diluted in PBS and plated on MOX plates for *L. monocytogenes*, XLD for *Salmonella* and CT-SMAC for *E. coli* O157:H7 enumeration. The presence of each pathogen in enriched samples was also confirmed by PCR using primers specific for *actA* gene of *L. monocytogenes*, *eaeA* of *E. coli* O157:H7 and *sefA* of *Salmonella* as described before (Kim and Bhunia, 2008).

### 2.5. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics 20.0. A Kruskal–Wallis rank test was used to compare the differences in each group. A Mann–Whitney *U*-test was used for multiple mean comparisons. The tests were performed at a significance level of $\alpha = 0.05$.

## 3. Results and discussion

### 3.1. Immunofluorescence assay

The fiber optic sensor utilizes a sandwich format for detection of the target pathogen. Therefore, for initial optimization of the assay parameters including antibody concentrations, assay sensitivity, and reaction to each test pathogen, we used a microtiter plate-based immunofluorescence assay (IFA). Results indicated that IFA could detect a minimum cell concentration of about $10^4$–$10^5$ cfu for all pathogens and the relative fluorescence unit (RFU) increased proportionately with increasing cell concentrations (data not shown). Furthermore, each antibody combination reacted strongly with their corresponding pathogen without showing significant reactions ($P < 0.05$) with other pathogens (Fig. 2). These data indicate that the sandwich assay format with multiple pathogen-specific antibodies may be suitable for biosensor applications.

### 3.2. Selectivity and the limit of detection of fiber optic sensor

For multipathogen sensors to be effective, a pathogen specific molecule i.e., antibody (in this study) within the multiplex sensor configuration should not show cross-reaction with other target analytes. Initial experiments demonstrated that the *L. monocytogenes*-specific fiber optic sensor showed strong reaction (7067 pA) only with the pure culture of *L. monocytogenes* and in the mixture containing all three pathogens (6509 pA) (Fig. 3). As expected, this sensor gave a negligible cross-reaction with *S.* Enteritidis (907 pA) or *E. coli* O157:H7 (1077) (Fig. 3). As reported before, this sensor configuration also did not show signals from other common food associated microorganisms examined (Geng et al., 2004; Nanduri et al., 2006). Similar results were also seen for *E. coli*- and *Salmonella*-specific sensors; however, the *Salmonella*-specific sensor showed some cross-reactions with the other two pathogens, but the values were significantly lower ($P < 0.05$) than the signal for the *Salmonella*. Overall, these data are in agreement with our previous studies where the same combination of antibodies were used on the waveguide for detection of *L. monocytogenes* (Nanduri et al., 2006), *E. coli* O157:H7 (Geng et al., 2006) and *S. enterica* (Valadez et al., 2009) individually.

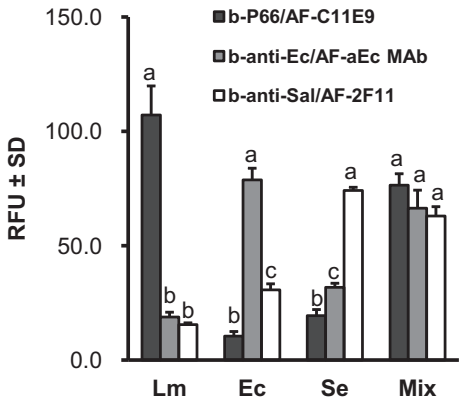


**Fig. 2.** Sandwich immunofluorescence assay using streptavidin coated microtiter plates to detect *L. monocytogenes* (Lm), *E. coli* O157:H7 (Ec), *Salmonella enterica* (Se) using capture and Alexa-Fluor 647-labeled reporter antibodies. Mixture contained equal concentration of *L. monocytogenes* F4244; *E. coli* O157:H7; and *S. enterica* serovar Enteritidis. Data are the average of at least 9 wells from 3 independent experiments. Bars marked with different letters (a, b, c) are statistically different as calculated by Mann–Whitney *U*-test ($P < 0.05$).

Next, we determined the minimum concentration of target pathogens that can be detected by each sensor configuration. Data indicate that all sensors showed a detection limit in the range of 1 × $10^3$ cfu/ml corresponding to signal values from 2000 pA to 4000 pA (Fig. 4), which were at least 2 standard deviation (SD)

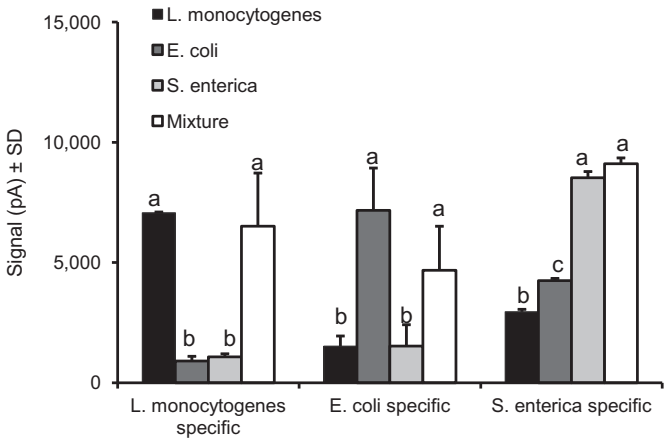


**Fig. 3.** Fiber optic signal response of each pathogen-specific antibody combinations (probes) to *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* Enteritidis suspended in PBS. Each pathogen was used at approx. 1 × $10^8$ cfu/ml individually or in the mixture. Data are the average of three waveguides from 3 independent experiments. Bars marked with different letters (a, b, c) are statistically different ($P < 0.05$) for each pathogen-specific sensor.

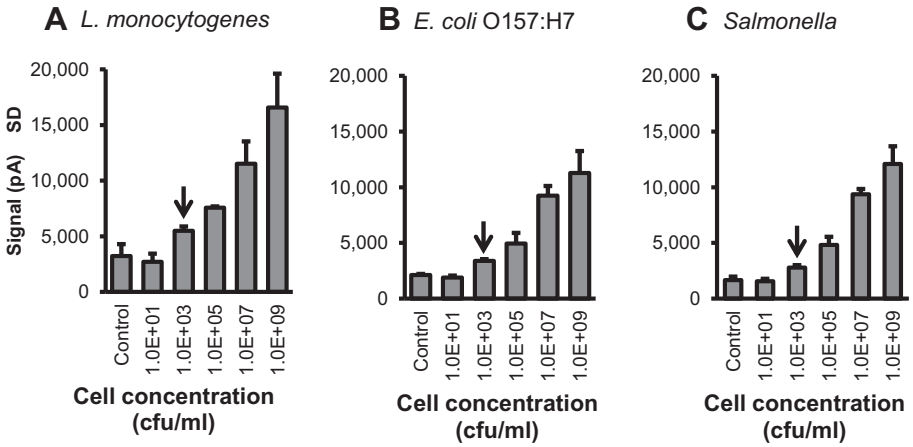


**Fig. 4.** Limit of detection for each pathogen-specific fiber optic sensor tested against different concentrations of bacteria; (A) *L. monocytogenes*, (B) *E. coli* O157:H7, (C) *Salmonella*. Fiber optic signals (pA) are average of three waveguides from 3 independent experiments. Arrow indicates the detection limit ($1 \times 10^3$ cfu/ml) for each pathogen-specific sensor and the signals are significantly higher than the controls (PBS, 10 cfu/ml).

greater than the signals emitted from 10 cfu. The signals also proportionately increased as the concentration of bacteria increased. Based on this result, the detection limit of this sensor was determined to be about $10^3$ cfu/ml. Fiber optic signal for each sensor was also determined for a cell concentration of $10^2$ cfu/ml in one experiment and since there were no significant difference in values among no cell controls (PBS), $10^1$ and $10^2$ cfu/ml, the data for $10^2$ was not included in the graph (Fig. 4). The detection limit for each pathogen-specific fiber optic sensor reported here falls within the general range reported previously for *E. coli* O157:H7 (DeMarco et al., 1999; Geng et al., 2006), *L. monocytogenes* (Geng et al., 2004; Nanduri et al., 2006; Ohk et al., 2010), and *Salmonella* Enteritidis (Valadez et al., 2009). Detection limits for the fiber optic sensor could possibly be improved further by using high affinity capture molecules. In recent years, many different biorecognition molecules including bacteriophage tail proteins, mammalian cell receptors, aptamers, and recombinant antibodies are being evaluated as capture molecule to improve biosensor sensitivity (Chambers et al., 2008; Koo et al., 2009; Lakshmanan et al., 2007; Tolba et al., 2010; Zeng et al., 2011).

### 3.3. Validation of the fiber optic sensor with ready-to-eat food products

The fiber optic biosensor was further evaluated with inoculated ready-to-eat model food systems (beef, chicken and turkey). Inoculated samples were used since the rate of natural contamination in RTE food is very low. Meats were inoculated with about $10^2$ cfu/25 g (~4 cfu/g) and enriched in SEL for 18 h. Actual cell number in inoculated food samples were 256 ± 42 cfu/25 g for *L. monocytogenes*, 175 ± 23 cfu/25 g for *E. coli* O157 and 149 ± 40 cfu/25 g for *Salmonella*. The bacterial cell counts reached to about$10^7$–$10^8$ cfu/ml after 18 h of enrichment (Table 1) as previously observed (Gehring et al., 2012; Kim and Bhunia, 2008) suggesting all three pathogens grew similarly in the co-culture setup. Data from the fiber optic sensor indicate that pathogen-specific sensors were highly selective for their respective target pathogens in each of the meat samples tested without showing any significant ($P < 0.05$) cross reactions with non-target pathogens (Fig. 5). Performance and quality of antibodies used in immunosensor platforms must be thoroughly assessed before use to obtain reproducible results. This is especially critical for multiplex configuration where each antibody combinations are exposed to same microorganisms at equal concentrations after enrichment. Even though the cross reaction of antibodies were statistically insignificant, antigenic homogeneity among test organisms cannot be ruled out as a factor contributing to the response observed (Figs. 3 and 5). Furthermore, the variation in signals obtained for a pathogen in different meat samples could be attributed to the product formulations or preservatives present in the RTE products which may have caused differential antigen expression (Banada and Bhunia, 2008; Lathrop et al., 2008), yielding differential bacterial capture on fibers. The uninoculated meat samples produced a very low background signal (1720–3530 pA), which were subtracted from the pathogen-specific signals. These data indicate that the multiplex fiber optic setup used was suitable for detecting all or any of the three pathogens present in the test samples. Previously this sensor was used primarily for detection of individual foodborne pathogens (Bhunia, 2008; Leung et al., 2007) or in some cases configured to detect multiple pathogens or toxin of bioterrorism significance (Ligler et al., 2007; Lim et al., 2005) but none, in our knowledge, used for detection of *L. monocytogenes*, *E. coli* O157:H7 and *S. enterica* serovar Enteritidis simultaneously.

IFA analysis performed in parallel with the enriched meat samples also produced similar results substantiating the fiber

**Table 1**
Bacterial counts[a] in ready-to-eat lunch meat samples after enrichment in SEL for 18 h.

| | Inoculation cfu/25 g | cfu/ml ± SD | | | |
|---|---|---|---|---|---|
| | | Beef | Chicken | Turkey | Mixture |
| *L. monocytogenes* | 256 ± 42 | $2.2 \pm 0.9 \times 10^8$ | $3.1 \pm 0.21 \times 10^8$ | $2.4 \pm 0.4 \times 10^8$ | $2.1 \pm 0.2 \times 10^8$ |
| *E. coli* O157:H7 | 175 ± 23 | $8.3 \pm 1.1 \times 10^7$ | $6.4 \pm 1.5 \times 10^7$ | $1.0 \pm 0.9 \times 10^7$ | $5.2 \pm 2.7 \times 10^7$ |
| *S. enterica* | 149 ± 40 | $4.0 \pm 0.6 \times 10^7$ | $3.4 \pm 0.8 \times 10^7$ | $2.7 \pm 0.8 \times 10^7$ | $1.5 \pm 0.6 \times 10^7$ |

[a] *L. monocytogenes* counts were determined on MOX, *E. coli* O157:H7 on CT-SMAC and *Salmonella* on XLD.

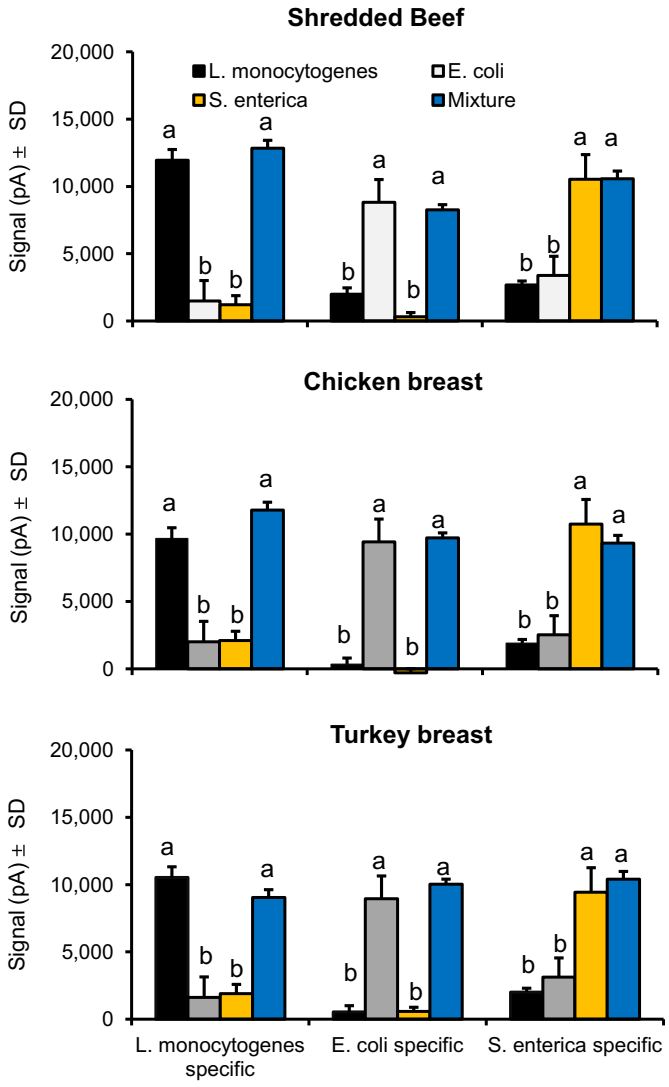


**Fig. 5.** Fiber optic sensor-based detection of three pathogens from inoculated ready-to-eat sliced lunch meat samples. Meats were inoculated with about 100 cfu/25 g, enriched in SEL broth for 18 h and subsequently tested. Fiber optic signals (pA) are the average of three fibers from a representative experiment. Signal for each pathogen-specific sensor (marked with "a") was significantly higher ($P < 0.05$) than the other two pathogens (b) present in the same sample. The uninoculated meat was used as negative controls and the signals originated from these samples (1720–3530 pA) were subtracted from the signals from the inoculated samples.

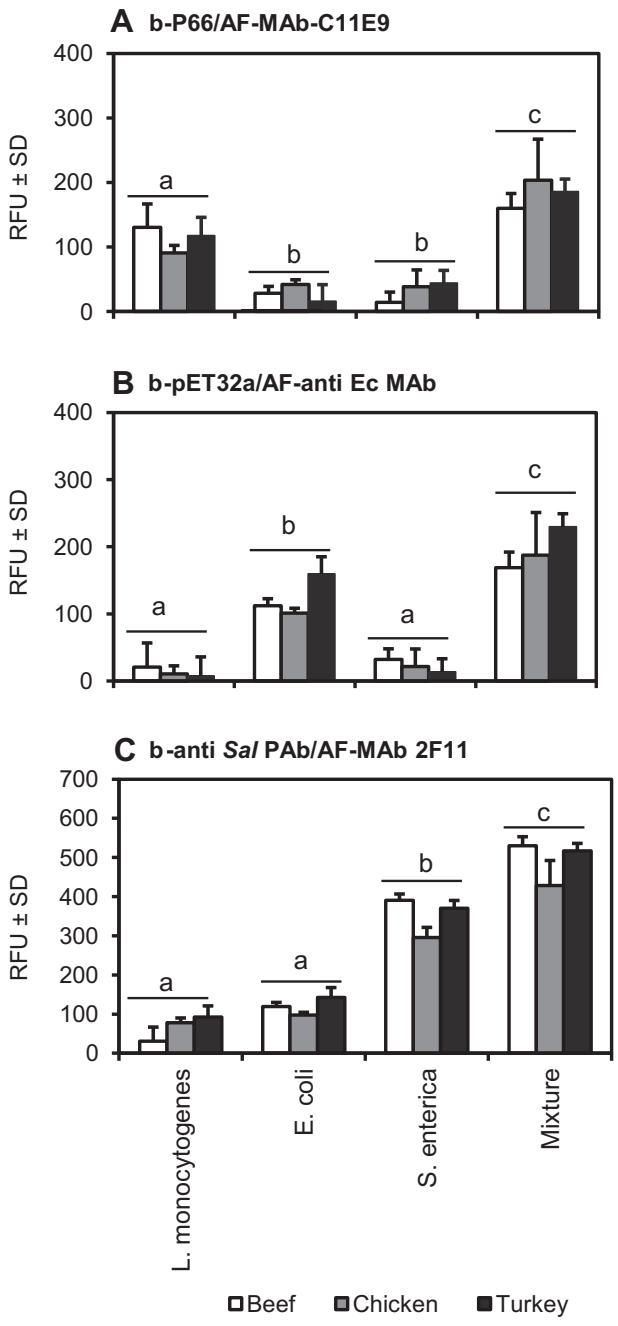


**Fig. 6.** Analysis of food samples employing sandwich immunofluorescence assay in streptavidin coated microtiter plates to detect *L. monocytogenes*, *E. coli* O157:H7, *Salmonella enterica* serovar Enteritidis using capture and Alexa-Fluor 647-labeled reporter antibodies: (A) antibody combinations for *L. monocytogenes*, (B) for *E. coli* O157:H7, and (C) for *Salmonella*. Mixture contained equal concentration of *L. monocytogenes* F4244; *E. coli* O157:H7; and *S. enterica* serovar Enteritidis. Data are presented as relative fluorescence unit (RFU) and are the average of at least 9 wells from three independent experiments and the groups marked with different letters are significantly different at $P < 0.5$.

optic sensor results (Fig. 6). PCR assay targeting pathogen-specific genes also confirmed the presence of each target pathogen in the enriched RTE meat samples (Fig. 7). Nowadays, the multiplex PCR is widely used for pathogen detection with a detection limit of $10^3$–$10^4$ cfu (Settanni and Corsetti, 2007), while most immunoassays can detect $10^5$–$10^7$ cfu (Banada and Bhunia, 2008). Detection limit for the fiber optic sensor reported here ($1 \times 10^3$ cfu/ml) is comparable to the detection limit reported for multiplex PCR (which requires nucleic acid as target), while the fiber optic sensor, in addition to whole cells, can detect toxins, other macromolecular structures or combination of whole cells and toxins in an array format (Leung et al., 2007; Sapsford et al., 2006; Song et al., 2006). Depending on the biosensor platforms used, some are also highly sensitive and can be automated for screening of large numbers of samples rapidly (Bhunia, 2008, 2011; Gehring and Tu, 2011).

In summary, the multipathogen fiber optic sensor described shows promising results for simultaneous detection of all three pathogens from inoculated RTE food samples in less than 24 h. Multiplex detection was facilitated by employing a multipathogen enrichment broth, SEL for simultaneous growth of all three organisms, and the antibody combinations for specific detection of each from a food matrix.

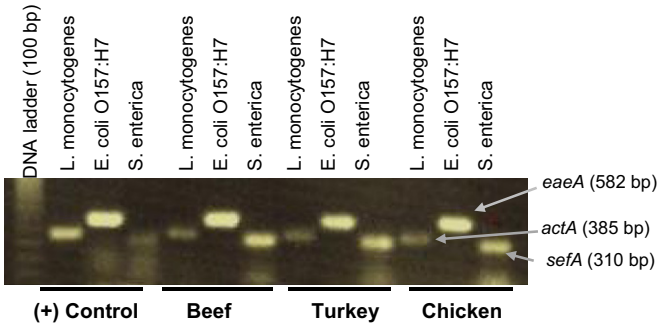


**Fig. 7.** PCR confirmation of *L. monocytogenes*, *E. coli* O157:H7 and *S. enterica* presence in the inoculated beef, turkey and chicken using primers specific for *actA* gene (385 bp) of *L. monocytogenes*, *eaeA* gene (482 bp) of *E. coli* O157:H7, and *sefA* gene (310 bp) of *S. enterica*. Pure culture of each pathogen was used as a positive control.

## Acknowledgments

This research was supported through a cooperative agreement with the Agricultural Research Service of the United States Department of Agriculture project number 1935-42000-072-02G and the Center for Food Safety Engineering at Purdue University. Sincere thanks to Ok Kyung Koo and Amornrat Aroonual for technical assistance and Bruce Applegate for critical reading of the manuscript.

## References

Anderson, G.P., Nerurkar, N.L., 2002. Improved fluoroimmunoassays using the dye Alexa Fluor 647 with the RAPTOR, a fiber optic biosensor. J. Immunol. Methods 271, 17–24.

Banada, P.P., Bhunia, A.K., 2008. Antibodies and immunoassays for detection of bacterial pathogens. In: Zourob, M., Elwary, S., Turner, A. (Eds.), Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems. Cambridge University, Manchester, pp. 567–602.

Banerjee, P., Bhunia, A.K., 2009. Mammalian cell-based biosensors for pathogens and toxins. Trends Biotechnol. 27, 179–188.

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., 2011. Rapid pathogen screening tools for food safety. Food Technol. 65, 38–43.

Bhunia, A.K., Ball, P.H., Fuad, A.T., Kurz, B.W., Emerson, J.W., Johnson, M.G., 1991. Development and characterization of a monoclonal antibody specific for *Listeria monocytogenes* and *Listeria innocua*. Infect. Immun. 59, 3176–3184.

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

Chambers, J.P., Arulanandam, B.P., Matta, L.L., Weis, A., Valdes, J.J., 2008. Biosensor recognition elements. Curr. Issues Mol. Biol. 10, 1–12.

Chen, W.T., Ladisch, M.R., Geng, T., Bhunia, A.K., 2005. Membrane for selective capture of the microbial pathogen *Listeria monocytogenes*. AIChE J. 51, 3305–3308.

DeMarco, D.R., Saaski, E.W., McCrae, D.A., Lim, D.V., 1999. Rapid detection of *Escherichia coli* O157:H7 in ground beef using a fiber-optic biosensor. J. Food Prot. 62, 711–716.

FDA, 2001. Bacteriological Analytical Manual Online, eighth ed. AOAC International, Arlington, VA.

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.

Gehring, A.G., Tu, S.I., 2011. High-throughput biosensors for multiplexed food-borne pathogen detection. Ann. Rev. Anal. Chem. 4, 151–172.

Geng, T., Morgan, M.T., Bhunia, A.K., 2004. Detection of low levels of *Listeria monocytogenes* cells by using a fiber-optic immunosensor. Appl. Environ. Microbiol. 70, 6138–6146.

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

Homola, J., 2008. Surface plasmon resonance sensors for detection of chemical and biological species. Chem. Rev. 108, 462–493.

Jaradat, Z.W., Bzikot, J.H., Zawistowski, J., Bhunia, A.K., 2004. Optimization of a rapid dot-blot immunoassay for detection of *Salmonella enterica* serovar Enteritidis in poultry products and environmental samples. Food Microbiol. 21, 761–769.

Jung, C.C., Saaski, E.W., McCrae, D.A., Lingerfelt, B.M., Anderson, G.P., 2003. RAPTOR: a fluoroimmunoassay-based fiber optic sensor for rapid detection of biological threats. IEEE Sens. J. 3, 352–360.

Kawasaki, S., Fratamico, P.M., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., Kawamoto, S., 2010. Multiplex real-time polymerase chain reaction assay for simultaneous detection and quantification of *Salmonella* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in ground pork samples. Foodborne Pathog. Dis. 7, 549–554.

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.

Lakshmanan, R.S., Guntupalli, R., Hu, J., Kim, D.-J., Petrenko, V.A., Barbaree, J.M., Chin, B.A., 2007. Phage immobilized magnetoelastic sensor for the detection of *Salmonella typhimurium*. J. Microbiol. Methods 71, 55–60.

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.

Leung, A., Shankar, P.M., Mutharasan, R., 2007. A review of fiber-optic biosensors. Sens. Actuat. B Chem. 125, 688–703.

Ligler, F.S., Sapsford, K.E., Golden, J.P., Shriver-Lake, L.C., Taitt, C.R., Dyer, M.A., Barone, S., Myatt, C.J., 2007. The array biosensor: portable, automated systems. Anal. Sci. 23, 5–10.

Lim, D.V., Simpson, J.M., Kearns, E.A., Kramer, M.F., 2005. Current and developing technologies for monitoring agents of bioterrorism and biowarfare. Clin. Microbiol. Rev. 18, 583–607.

Lynch, M., Painter, J., Woodruff, R., Braden, C., 2006. Surveillance for foodborne-disease outbreaks – United States, 1998–2002. MMWR 55, 1–42.

Miller, M.B., Tang, Y.-W., 2009. Basic concepts of microarrays and potential applications in clinical microbiology. Clin. Microbiol. Rev. 22, 611–633.

Nanduri, A., Kim, G., Morgan, M.T., Ess, D., Hahm, B.K., Kothapalli, A., Valadez, A., Geng, T., Bhunia, A.K., 2006. Antibody immobilization on waveguides using a flow-through system shows improved *Listeria monocytogenes* detection in an automated fiber optic biosensor: RAPTOR™. Sensors 6, 808–822.

Ohk, S.H., Koo, O.K., Sen, T., Yamamoto, C.M., Bhunia, A.K., 2010. Antibody-aptamer functionalized fibre-optic biosensor for specific detection of *Listeria monocytogenes* from food. J. Appl. Microbiol. 109, 808–817.

Park, S.H., Hanning, I., Jarquin, R., Moore, P., Donoghue, D.J., Donoghue, A.M., Ricke, S.C., 2011. Multiplex PCR assay for the detection and quantification of *Campylobacter* spp., *Escherichia coli* O157:H7, and *Salmonella* serotypes in water samples. FEMS Microbiol. Lett. 316, 7–15.

Rebe Raz, S., Haasnoot, W., 2011. Multiplex bioanalytical methods for food and environmental monitoring. Trends Anal. Chem. 30, 1526–1537.

Sapsford, K.E., Ngundi, M.M., Moore, M.H., Lassman, M.E., Shriver-Lake, L.C., Taitt, C.R., Ligler, F.S., 2006. Rapid detection of foodborne contaminants using an array biosensor. Sens. Actuat. B Chem. 113, 599–607.

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.

Settanni, L., Corsetti, A., 2007. The use of multiplex PCR to detect and differentiate food- and beverage-associated microorganisms: a review. J. Microbiol. Methods 69, 1–22.

Song, L., Ahn, S., Walt, D.R., 2006. Fiber-optic microsphere-based arrays for multiplexed biological warfare agent detection. Anal. Chem. 78, 1023–1033.

Taitt, C.R., Anderson, G.P., Ligler, F.S., 2005. Evanescent wave fluorescence biosensors. Biosens. Bioelectron. 20, 2470–2487.

Tolba, M., Minikh, O., Brovko, L.Y., Evoy, S., Griffiths, M.W., 2010. Oriented immobilization of bacteriophages for biosensor applications. Appl. Environ. Microbiol. 76, 528–535.

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.

Zeng, X., Shen, Z., Mernaugh, R., 2011. Recombinant antibodies and their use in biosensors. Anal. Bioanal. Chem., 1–12.