Original Article

Journal of Biosystems Engineering

J. of Biosystems Eng. 38(4):335-340. (2013. 12)
http://dx.doi.org/10.5307/JBE.2013.38.4.335

eISSN : 2234-1862
pISSN : 1738-1266

Image Analysis of a Lateral Flow Strip Sensor for the Detection of *Escherichia coli* O157:H7

Giyoung Kim*, Ji-Hea Moon, Saet Byeol Park, Youn-Jung Jang, Jongguk Lim, Changyeun Mo

National Academy of Agricultural Science, 249 Seodun-dong, Suwon, Korea

Received: October 5th, 2013; Revised: November 17th, 2013; Accepted: November 20th, 2013

Abstract

Purpose: This study was performed to develop a lateral flow strip sensor for the detection of pathogenic *Escherichia coli* O157:H7 in various samples. Also, feasibility of using an image analysis method to improve the interpretation of the strip sensor was evaluated. *Methods:* The lateral flow strip sensor has been fabricated based on nitrocellulose lateral-flow membrane. Colloidal gold and *E. coli* O157:H7 antibodies were used as a tag and a receptor, respectively. Manually spotted *E. coli* O157:H7 antibody and anti-mouse antibody on nitrocellulose membrane were used as test and control dots, respectively. Feasibility of the lateral flow strip sensor to detect *E. coli* O157:H7 were evaluated with serially diluted *E. coli* O157:H7 cells in PBS or food samples. Test results of the lateral flow strip sensor were measured with an image analysis method. **Results:** The intensity of the test dot started to increase with higher concentration of the cells were introduced. The sensitivities of the sensor were both 10^4 CFU/mL *Escherichia coli* O157:H7 spiked in PBS and in chicken meat extract, respectively. **Conclusions:** The lateral flow strip sensor and image analysis method could detect *E. coli* O157:H7 in 20 min, which is significantly quicker than conventional plate counting method.

Keywords: *E. coli* O157:H7, Food safety, Image analysis, Lateral flow, Strip sensor

Introduction

Escherichia coli serotype O157:H7 is particular concern because of the severe consequences of infection. *E. coli* O157:H7 is a Gram-negative rod-shaped bacterium, and may produce shiga-like toxins, and is an enterohemorrhagic strain of *Escherichia coli* (Karch et al., 2005). A person infected with this pathogen shows symptoms of severe, acute hemorrhagic diarrhea (although nonhemorrhagic diarrhea is also possible), abdominal cramps, and kidneys fail in some people, particularly children under five years of age and the elderly. The symptoms may resolves in five to 10 days. Transmission of this pathogen occurred by consuming a contaminated or oral contact with contaminated surfaces. This pathogen is usually associated with undercooked ground beef or ground pork; other sources include consumption of unpasteurized milk and juice, raw produce and salami, and contact with infected live animals. Waterborne transmission occurs through swimming in contaminated lakes, pools, or drinking inadequately treated water. *E. coli* O157:H7 outbreaks continue to occur and *E. coli* O157:H7 related outbreaks from various food sources have increased public awareness of this pathogen.

Conventional methods for *E. coli* O157:H7 diagnosis involve prolonged multiple enrichment steps that is time and labor consuming. Swifter assays are possible using Enzyme-linked immunoassay (ELISA) and polymerase chain reaction (PCR). However, they still require enrichment, several lengthy steps, expensive laboratory instruments, and experienced operators (Hart et al., 2011; Hossain et al., 2012). Recently, newer technologies using immunoreactions including biosensors and lateral flow strip sensors have shown great potential for rapid detection

***Corresponding author:** Giyoung Kim
Tel: +82-31-290-1899; **Fax:** +82-31-290-1900
E-mail: giyoung@korea.kr

Copyright ⓒ 2013 by The Korean Society for Agricultural Machinery
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

of foodborne pathogens. Radke and Alocilja (2005) detected *E. coli* O157:H7 with an impedance biosensor that was fabricated by immobilizing antibodies on the surface of high density microelectrode array. Varshney and Li (2007) incorporated magnetic nanoparticle-antibody conjugates to the interdigitated array microelectrode to improve the detection of *E. coli* O157:H7 in food samples. Kim et al. (2013) applied multivariate analysis technique to the impedance spectra data acquired from an impedance biosensor for the detection of *Salmonella* typhimurium. Cho et al. (2010) used potable surface plasmon resonance (SPR) biosensor to detect *Salmonella* Enteritidis. Many groups have reported the application of nanotechnologies for pathogen detection. Fluorescent nanoparticles or quantum dots that have several advantages over conventional organic dyes including high quantum yield and brightness, photostability, and resistance to chemical degradation were used to detect *E. coli* O157:H7 (Su and Li, 2004; Wang et al., 2012; Zhu et al., 2012), *Salmonella* (Kim et al., 2010; Kuang et al., 2013), and *Listeria monocytogenes* (Wang et al., 2007).

Even though biosensors were feasible to detect various pathogens faster than conventional methods, they need skilled persons to prepare and use. In this context, lateral flow strip sensors showed great potential for the detection of food borne pathogens. The lateral flow strip sensors have the many advantages, such as the low-cost, rapid and sensitive detection, user-friendly operation, easy storage, and on-site detection. The strip sensor is based on the specific interaction between antigen and antibody and the colored particles such as gold nanoparticle, carbon, silica, polymer, liposome, quantum dot, magnetic bead, and etc (Posthuma-Trumpie et al., 2009). The strip sensors were used for the detection of *Salmonella* typhimurium (Kim et al., 2011), *Vibrio cholera* (Chus et al., 2011), *B. anthracis* spores (Wang et al., 2013).

The purpose of this study was to develop a lateral flow strip sensor for the detection of pathogenic *Escherichia coli* O157:H7 in various samples. Also, feasibility of using an image analysis method to improve the interpretation of the strip sensor was evaluated.

Materials and Methods

Bacteria and media

Heated killed 10^9 CFU/mL of *Escherichia coli* O157:H7 were bought from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA). *Salmonella* typhimurium KCTC 12401 is obtained from Korean Collection for Type Culture in the Korea Research Institute of Bioscience and Biotechnology. Brain Heart Infusion (BHI) and xylose lysine desoxycholate (XLD) agar were obtained from Difco (Detroit, MI, USA). The *E. coli* O157:H7 cells were diluted (10^3–10^7 CFU/mL) with phosphate-buffered saline (PBS, pH 7.2). PBS buffer alone was used for the negative control.

Selectivity of the lateral flow strip sensor for *E. coli* O157:H7 was evaluated with *S.* typhimurium cells prepared as follows: fresh cultures of *S.* typhimurium were prepared by incubation in BHI broth at 37°C for 14 h. Cell-containing buffer was replaced with 20 mM PBS. Enumeration of the enriched *S.* typhimurium was performed using the standard plate count (SPC) method. A sample containing 10^7 CFU/mL of *S.* typhimurium cells was used for the selectivity test.

Food samples were prepared by placing chicken breast in sterile stomacher bags; adding 200 mL of PBS buffer; vigorously shaking it; and inoculating with 100 μL of bacterial suspension. The cells were also diluted to 10^3–10^7 CFU /mL with chicken extract. Bacteria-free food sample was used as a negative control. Packages of chicken breast were purchased from a local grocery store for food sample preparation.

Reagents and Antibodies

BacTrace affinity-purified anti-*E. coli* O157:H7 polyclonal antibody raised in goat was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA) and mouse anti- *E. coli* O157:H7 monoclonal antibody was purchased from Abcam (Cambridge, UK). BacTrace affinity-purified anti-mouse IgG was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA). Bovine serum albumin (BSA), sucrose, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gold-in-a-Box kit with 40 nm gold nanoparticle was purchased from BioAssay Works (Ijamsville, MD, USA) to label detection antibodies. For lateral flow immunoassay kit, sample pad, conjugation pad, nitrocellulose membrane, and absorbent pad were obtained from Merck Milipore (Billerica, MA, USA).

Lateral flow strip sensor

A lateral flow strip is constructed with four different pads; a sample application pad, a conjugate release pad, a nitrocellulose membrane, and an absorption pad. The detection is based on the retention and formation of visual spots of color-labeled antibodies in sensing zones on a membrane during sample flow through. The sample is driven up the strip sensor by capillary forces. Bacteria cells in the sample are combined to the color-labeled antibodies in the conjugate release pad, and continuously flow to the nitrocellulose membrane. And then, the cell-colored antibody conjugates are reacted with immobilized antibody in the sensing spot to generate signals. Remaining sample is flow though up to the absorption pad. Figure 1 shows the schematic description of the lateral flow strip sensor.

To prepare color-labeled detection antibodies, gold nanoparticles were conjugated to anti- *E. coli* O157:H7 monoclonal antibodies. Since, reaction of the gold nanoparticles and the antibodies are affected by pH, effective pH values were identified. First, solutions containing gold nanoparticles were adjusted to pH 5.4–10.1. The same volume of NaCl solution (1.0 M) was then added and the color of the obtained solutions was observed after 10 min. Because high salt concentrations induce gold nanoparticle aggregation, an insufficient amount of antibodies are adsorbed on the surface of the nanoparticles. At this point, the aggregation could be visually detected changing from red color to blue-grey. A color change from red to red-purple occurred below pH 7.3 or above 8.4. Therefore, pH 8.4 of colloidal gold was considered to be suitable to conjugate with anti- *E. coli* O157:H7 monoclonal antibodies.

Performance of the lateral flow strip sensor is affected by the treatment of the metrocellulose membrane. Nitrocellulose membranes were blocked with PBS and dried at 37 ℃ for 1 h. 1 mg/mL polyclonal *E. coli* O157:H7 antibody as test zone and 1 mg/mL anti-mouse IgG antibody as control zone were manually spotted on the nitrocellulose membrane. Conjugate pads were blocked with PBS buffer with 2% BSA and 10% sucrose and dried at 37 ℃ for 4h. After monoclonal *E. coli* O157:H7 antibody-gold nanoparticle conjugate was applied on the conjugate pad and dried 37 ℃ for 1 h. The sample pad was assembled to the conjugate pad with 3 mm overlap. The conjugate and absorbent pads were attached to the both ends of the nitrocellulose membrane with 3 mm overlap.

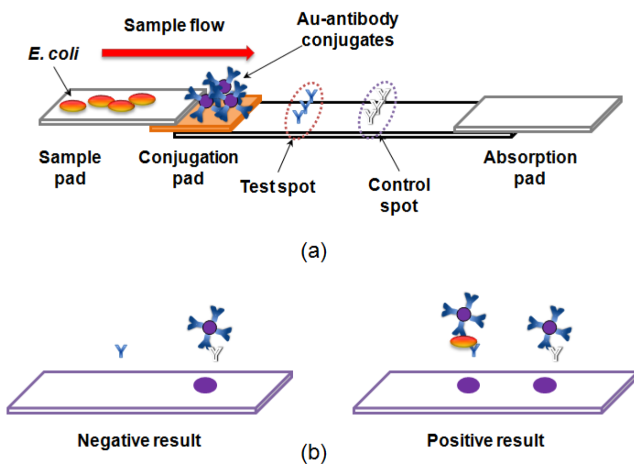

Figure 1. (a) The schematic description of the lateral flow strip sensor. (b) Illustrations of immunochromatographic test results.

Image analysis system

The image analysis system for the lateral flow strip sensor was composed of 12 bit compact size CCD camera (CYLOD Inc., BC, Canada), micro lens with 10 mm focal length (MENGEL ENGINEERING, Virum, Denmark), white LED light (LVS, Inchon, Korea), LED controller, CCD camera stand, and computer as shown Figure 2. ImgaeJ software (ver. 1.47, HIH, MD, USA) was used to analyze the image and to measure the maximum peak area (A_t and A_c) values of the test and control zone on the detection area of the lateral flow strip sensor. The peak area was calculated by adding the intensity values under the intensity profile of the test spot or the control spot. Figure 2 shows the schematic description of the image analysis system.

The image analysis system acquired the image from CCD camera connected with the USB 2.0 port on the computer and this image was saved on the hard drive. Then, the image was analyzed using ImageJ software. The intensity of the detection area was measured and the

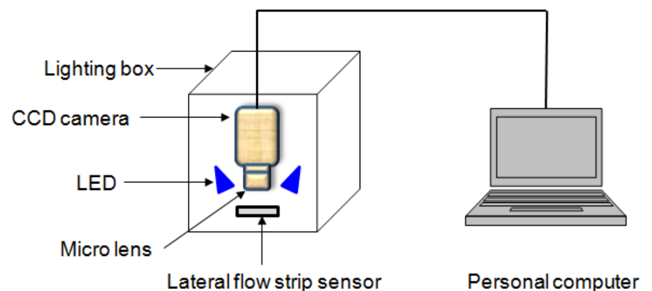

Figure 2. The schematic description of the image analysis system for the lateral flow strip sensor.

peak area values of the test and control zone were calculated from the intensity result. The intensity of the control zone was used as an appropriate normalizing factor. The experimental results were expressed as the peak area of the test zone divided by the peak area of the control zone.

Results and Discussion

Detection of *E. coli* O157:H7 in PBS sample

The efficacy of *E. coli* O157:H7 detection using the lateral flow strip sensor was investigated with serially diluted (10^3–10^7 CFU/mL) cells with PBS buffer. Detection with the lateral flow strip sensor started with an injection of 100 μL of the prepared sample onto the sample pad. The liquid sample flowed to the conjugation pad by capillary force and rehydrated dried anti-*E. coli* O157:H7 antibody-gold nanoparticle conjugates. *E. coli* O157:H7 cells in the sample bound to the conjugates by immunoreactions. And then, *E. coli*-antibody-gold particle conjugates continuously flowed to the nitrocellulose membrane. These conjugates reached at the test spot and bound to the capture antibodies in the test spot. Captured *E. coli*-antibody-gold particle conjugates formed red dot and remaining samples flowed to the control spot. Unreacted antibody-gold particle conjugates bound to anti-mouse antibodies in the control spot and formed another red dot. Finally, rest sample liquid flowed to the absorption pad. As shown in Figure 3, intensity of test spot increased with the increased concentration of *E. coli* O157:H7 cells in the sample. Detection limit of the lateral flow strip sensor was 10^5 CFU/mL by visual reading. Lateral flow strip sensor selectivity was evaluated with 10^7 CFU/mL of *S.* typhimurium cells. A sample containing *S.* typhimurium cells formed very light red color at the test spot (Figure 3), suggesting the lateral flow strip sensor selectivity detect *E. coli* O157:H7 cells.

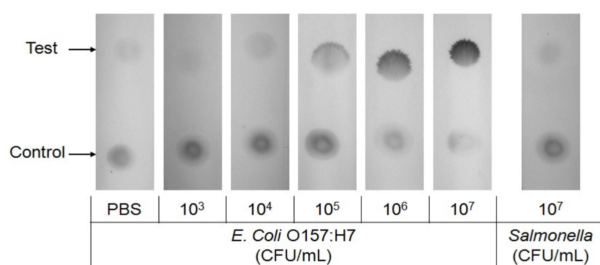

Figure 3. Detection results of *E. coli* O157:H7 cells in PBS buffer with the lateral flow strip sensor. Concentrations of *E. coli* O157:H7 cells were 0-10^7 CFU/mL and concentration of *Salmonella* typhimurium cells were 10^7 CFU/mL.

Detection of *E. coli* O157:H7 in food sample

The feasibility of *E. coli* O157:H7 detection using the lateral flow strip sensor for food sample was investigated with serially diluted (10^3–10^7 CFU/mL) cells with chicken extract buffer. Detection results of *E. coli* O157:H7 in food samples showed similar trends with those of PBS samples. Intensity of test spot increased with the increased concentration of *E. coli* O157:H7 cells in the sample and the darkest color appeared at 10^7 CFU/mL *E. coli* O157:H7 cells (Figure 4). Even though food sample usually contains many interference materials, detection performance wasn't degraded. Detection limit of the lateral flow strip sensor for food sample was also 10^5 CFU/mL by visual reading. Selectivity test with the *S.* typhimurium containing sample showed no significant non-specific binding either (Figure 4).

Image analysis

To improve the lateral flow strip sensor reading, image analysis was performed with images acquired

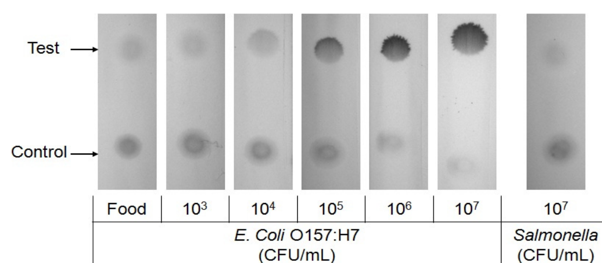

Figure 4. Detection results of *E. coli* O157:H7 cells in chicken extract buffer with the lateral flow strip sensor. Concentrations of *E. coli* O157:H7 cells were 0-10^7 CFU/mL and concentration of *Salmonella* typhimurium cells were 10^7 CFU/mL.

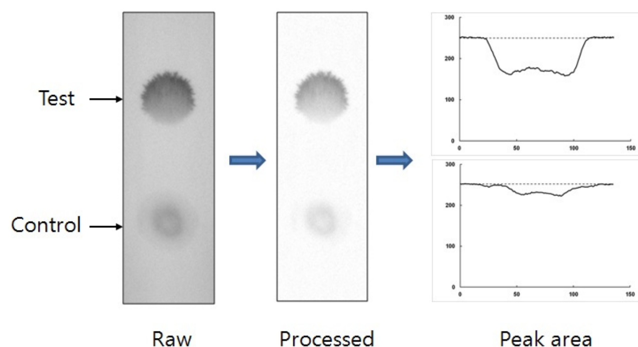

Figure 5. Image analysis procedure and result of a PBS sample (10^6 CFU/mL *E. coli* O157:H7 cells).

from the strip sensors after the end of above experiments. Image analysis procedure and result of a PBS sample (10^6 CFU/mL *E. coli* O157:H7 cells) is shown in Figure 5. As shown in Figure 5, raw images contained uneven illuminated background. To correct for uneven illuminated background, background was subtracted by using a "rolling ball" algorithm with rolling ball radius of 50. The algorithm determined a local background value for every pixel by averaging over a large ball around the pixel. This value was hereafter subtracted from the original image to remove large spatial variations of the background intensities. After the background subtraction, the intensity peak area value of the test spot (A_t) on the detection area and the control spot (A_c) were measured. The peak areas are shown in Figure 5 as the area enclosed between the peak (solid line) and the background baseline (dashed line). The peak area for the test spot was increased and the value for the control spot was decreased as the cell numbers increase. To normalize the intensity value of the test spot and reduce variability, the intensity of test spot was divided by the intensity of the control zone.

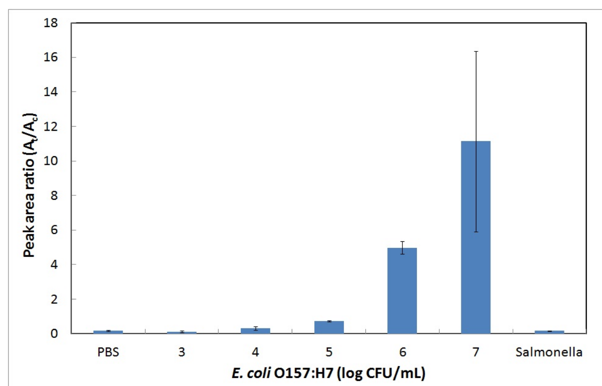

Figure 6. Image analysis results of PBS samples.

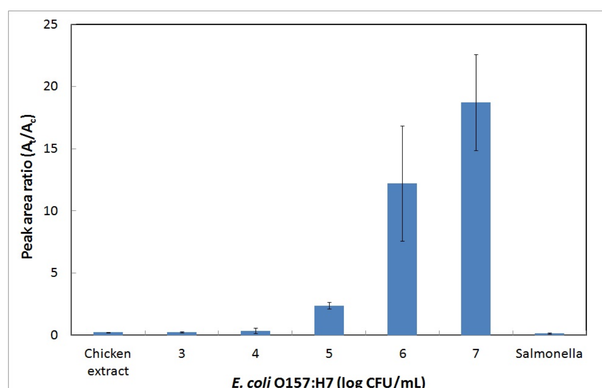

Figure 7. Image analysis results of food samples.

Image analysis results were expressed as (A_t/A_c) versus the number of *E. coli* O157:H7 cells. Figure 6 shows image analysis results of PBS samples. The peak area ratio grows with increasing cell numbers. As the concentration of *E. coli* O157:H7 increased from 10^3 to 10^7 CFU/mL, the peak area ratio increased from 0.12 to 11.13. The limit of detection of this image analysis method was improved to 10^4 CFU/mL. The limit of detection was reported as the cell numbers that produce peak area ratio values higher than 3 times the standard deviation plus the mean of the negative control sample that contained buffer only.

The feasibility of *E. coli* O157:H7 detection with lateral flow strip sensor was also tested on complex food samples. Chicken extracts used as a sample matrix were inoculated with *E. coli* O157:H7. The peak area ratio increased with increasing cell numbers, similar to the results of the test with the PBS sample (Figure 7). As the number of *E. coli* O157:H7 cells increased from 10^3 to 10^7 CFU/mL, the peak area ratio increased from 0.24 to 18.72. The detection limit in food was 10^4 CFU/mL *E. coli* O157:H7.

The lateral flow strip sensor also showed good selectivity. As shown in Figure 6 and 7, the peak area ratios of the *Salmonella* samples in PBS and food buffer were not increased significantly compare to the negative control samples. Detection performance of the lateral flow strip sensor with image analysis method didn't meet the current too strict food safety regulation, which is zero tolerance policy, but it was comparable or better than other methods. The limit of detections of impedimetric biosensors were 8×10^5 CFU/mL in beef sample (Varshney and Li, 2007) and 10^4 CFU/mL (Radke and Alocilja, 2005) in lettuce sample. These biosensors require prolonged sensor preparation and expensive measurement devices. The strip sensor developed in this study has several merits over other methods. The strip sensor is simple, low-cost, and fast while it has comparable sensitivity.

Conclusions

This study was conducted to develop a lateral flow strip sensor and an image analysis method for the detection of pathogenic *Escherichia coli* O157:H7 in various samples. The lateral flow strip sensor has been fabricated based on nitrocellulose lateral-flow membrane. Colloidal gold and *E. coli* O157:H7 antibodies were used as a tag

and a receptor, respectively. Manually spotted *E. coli* O157:H7 antibody and anti-mouse antibody on nitrocellulose membrane were used as test and control lines, respectively. Feasibility of the lateral flow strip sensor to detect *E. coli* O157:H7 were evaluated with serially diluted *E. coli* O157:H7 cells in PBS or food samples. The lateral flow strip sensor with image analysis method showed comparable or better than other methods. The sensitivities of the sensor were both 10^4 CFU/mL *Escherichia coli* O157:H7 spiked in PBS and in chicken meat extract, respectively. Considering simple operation, low-cost, comparable sensitivity, and fast analysis time of the lateral flow strip sensor, this method is a promising candidate as a rapid detection tool for food safety diagnositics.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

This work was supported by a grant from the Agenda Program (PJ008440), Rural Development Administration, Republic of Korea.

References

Cho, H. K., G. Y. Kim, W. H. Kim and M. S. Sung. 2010. Detection of Pathogenic Salmonella Using a Surface Plasmon Resonance Biosensor. J. of Biosystems Eng. 35(2):116-123.

Chua, A., C. Y. Yean, M. Ravichandran, B. Lim and P. Lalitha. 2011. A rapid DNA biosensor for the molecular diagnosis of infectious disease. Biosensors and Bioelectronics 26(9):3825-3831.

Hart, R. W., M. G. Mauk, C. Liu, X. Qiu, J. A. Thompson, D. Chen, D. Malamud, W. R. Abrams and H. H. Bau. 2011. Point-of-care oral-based diagnostics. Oral Diseases 17:745-752.

Hossain, S. M. Z., C. Ozimok, C. Sicard, S. D. Aguirre, M. M. Ali, Y. Li and J. D. Brennan. 2012. Multiplexed paper test strip for quantitative bacterial detection. Anal. Bioanal. Chem. 403:1567-1576.

Karch, H., P. Tarr and M. Bielaszewska. 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. Int. J. Med. Microbiol. 295(6-7):405-18.

Kim, G., G. M. Yang, Y. H. Kim, C. Y. Mo and S. B. Park. 2010. Detection of Pathogenic Salmonella with a Composite Quantum Dot. J. of Biosystems Eng. 35(6):458-463.

Kim, G., G. Yang, S. B. Park, Y. H. Kim, K. J. Lee, J. Y. Son, H. J. Kim and S. R. Lee. 2011. Rapid Detection Kit for Salmonella typhimurium. J. of Biosystems Eng. 36(2): 140-146.

Kim, G., J. H. Moon and M. Morgan. 2013. Multivariate data analysis of impedimetric biosensor responses from Salmonella typhimurium. Analytical Methods. 5: 4074-4080.

Kuang, H., G. Cui, X. Chen, H. Yin, Q. Yong, L. Xu, C. Peng, L. Wang and C. Xu. 2013. A one-step homogeneous sandwich immunosensor for *Salmonella* detection based on magnetic nanoparticles (MNPs) and quantum dots (QDs). Int. J. Mol. Sci. 14:8603-8610.

Posthuma-Trumpie, G. A., J. Korf and A. Amerongen. 2009. Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey. Anal Bioanal. Chem. 393:569-582.

Radke, S. A. and E. C. Alocilja. 2005. A high density microelectrode array biosensor for detection of *E. coli* O157:H7. Biosens. Bioelectron. 20:1662-1667.

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

Varshney, M. and Y. B. Li. 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.

Wang, D. B., B. Tian, Z. P. Zhang, J. Y. Deng, Z. Q. Cui, R. F. Yang, X. Y. Wang, H. P. Wei and X. E. Zhang. 2013. Rapid detection of *Bacillus anthracis* spores using a super-paramagnetic lateral-flow immunological detection system. Biosens Bioelectron. 42:661-667.

Wang, H., Y. Li and M. Slavik. 2007. Rapid detection of *Listeria monocytogenes* using quantum dots and nanobeads-based optical biosensor. Journal of Rapid Methods & Automation in Microbiology 15:67-76.

Zhu, H., U. Sikora and A. Ozcan. 2012. Quantum dot enabled detection of *Escherichia coli* using a cell-phone. Analyst 137:2541-2544.