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# An ELISA-on-a-Chip Biosensor System for Early Screening of *Listeria monocytogenes* in Contaminated Food Products

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An enzyme-linked immunosorbent assay (ELISA)-on-a-chip (EOC) biosensor combined with cell concentration technology based on immuno-magnetic separation (IMS) was investigated for use as a potential tool for early screening of *Listeria monocytogenes* (*L. monocytogenes*) in food products. The target analyte is a well-known pathogenic food-borne microorganism and outbreaks of the food poisoning typically occur due to contamination of normal food products. Thus, the aim of this study was to develop a rapid and reliable sensor that could be utilized on a daily basis to test food products for the presence of this pathogenic microorganism. The sensor was optimized to provide a high detection capability (e.g., $5.9 \times 10^3$ cells/mL) and, to eventually minimize cultivation time. The cell density was condensed using IMS prior to analysis. Since the concentration rate of IMS was greater than 100-fold, this combination resulted in a detection limit of 54 cells/mL. The EOC-IMS coupled analytical system was then applied to a real sample test of fish intestines. The system was able to detect *L. monocytogenes* at a concentration of 2.4 CFU/g after pre-enrichment for 6 h from the onset of cell cultivation. This may allow us to monitor the target analyte at a concentration less than 1 CFU/g within a 9 h-cultivation provided a doubling time of 40 min is typically maintained. Based on this estimation, the EOC-IMS system can screen and detect the presence of this microorganism in food products almost within working hours.

**Key Words**: Food-borne microorganism, Early detection, Quantitative analysis, High sensitivity, Immuno-magnetic separation

## Introduction

The genus *Listeria* is a Gram-positive, non-spore-forming rod-shaped pathogenic bacterium.[1] A well known bacterial infection, referred to as listeriosis, is typically caused by *Listeria monocytogenes* (*L. monocytogenes*) and occurs primarily in newborn infants, elderly patients, pregnant women, and patients who are immuno-compromised.[2] The symptoms of listeriosis include vomiting, nausea, stomach cramps, diarrhea, severe headache, constipation, persistent fever, and convulsions. The microbe can grow over a wide range of temperatures (1 to 45 °C), which makes it dangerous, particularly, for refrigerated food products that require long-term maintenance.[3-5] Since the microorganism is widespread in nature, it is present in not only food products, but also in natural environments such as water, air, soil, water plants, and waste water.[5] Therefore, rapid, accurate, and simple analytical methods are needed to reliably identify *L. monocytogenes* in food products and natural environments encountered by humans.

Three methods are applicable to microorganism detection: colony culture, which is the conventional method,[6-8] genetic approaches that are based on the polymerase chain reaction,[9-11] and immunological assays[12-15] such as enzyme-linked immunosorbent assay (ELISA). Although the conventional method is the most reliable method for detecting the existence of microbes in contaminated food, completion of the analysis is laborious, complicated, and time-consuming (e.g., 5 days).[16] Such drawbacks can be reduced if the genetic or immunological approach is relatively rapid, sensitive and reproducible, and easy-to-use protocols are employed. Nevertheless, the analytical methods still require multiple reaction steps, long reaction time, well-equipped facilities, and skilled technician in addition to the need for a pre-enrichment process through cultivation.[17] Therefore, the currently available techniques are inadequate screening methods, particularly, for food products that require a fast distribution time such as frozen foods, marine foods, and vegetables.

For rapid tests, an immuno-chromatographic assay based on lateral flow by means of the capillary phenomenon through membrane strips was developed.[18] This method has the advantage of reduced analytical time (within 20 min), easy-to-handle, and one-step analysis. The device is usually formulated as a qualitative kit, offering yes-or-no results and uses colloidal gold as the tracer, which is perceivable by the naked eyes.[19-21] However, this chromatographic assay has not been able to achieve a low detection limit (e.g., $10^5$ cells/mL) of the target analyte in samples compared to ELISA, which is the traditional immunoassay.[22] Due to this inferior performance, this technique may not be able to detect the target microorganism at the concentration levels it exists in certain food products. Moreover, the law demands that food products must be screened at a level where even a single cell of the food-poisoning bacterium must

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be detected. Thus, it is necessary to develop a rapid analytical system with high sensitivity that can be used for the early detection of a low number of bacterium after pre-enrichment by cultivation.

In this study, we examined the potential of using the ELISA-on-a-chip (EOC) biosensor to detect *L. monocytogenes* based on the concept of cross-flow chromatography.[23,24] This method would simultaneously provide a high analytical performance, high sensitivity and rapidity, all of which are required for early detection. To further enhance the detection capability, this biosensor technology was combined with immuno-magnetic separation (IMS)[25] to concentrate the cells prior to analysis using the EOC sensor. This would allow us to measure a minimal number of the food-poisoning agent in a shortened time period after the onset of cell cultivation. Such an analysis scheme would allow one to screen contaminated food within working hours without delaying distribution. In this study, we constructed an EOC sensor system and optimized the testing conditions for the detection of the microorganism. In addition, the analytical approach combined with IMS was then assessed by employing a real sample inoculated with trace amounts of *L. monocytogenes* to further test its usability for the early detection of contamination.

## Methods

**Construction of EOC Sensor System. Labeling of Antibody with Horseradish Peroxidase:** Mouse monoclonal antibody LZF7 (HyTest, Turku, Finland) which recognizes the outer membrane fraction and intact cells of *L. monocytogenes* was chemically conjugated to horseradish peroxidase (HRP; EDM chemicals, Gibbstown, NJ), *via* a cross-linker following a protocol described elsewhere.[22] Briefly, the antibody (2.65 nM) was first reduced using 10 mM dithiotheritol (Pierce, Rockford, IL), at 37 °C for 1 h and the excess reagent was removed on a Sephadex G-15 gel column (10 mL volume). HRP (26.45 nM) was activated with a 25-fold molar excess of 661.29 nM succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (Pierce, Rockford, IL), and the excess reagent was separated on the same gel column. The activated antibody was then combined with 5-fold molar excess of the activated HRP and the conjugation was carried out at 4 °C overnight. The synthesized conjugates were stored as aliquots after snap freezing.

**Preparation of Immuno-strip:** An immuno-strip, which was installed within the EOC, was prepared as described elsewhere.[26] The strip consisted of four different types of functional membrane pads consecutively connected by partial superimposition. They were, from the bottom, the glass fiber membrane (4 mm × 15 mm, Ahlstrom 8980; Whatman, Kent, UK) for sample application, glass membrane (4 mm × 5 mm, PT-R5; Advanced Microdevices, Ambala Cantt, India) for the release of the detection antibody labeled with HRP, nitrocellulose membrane (4 mm × 25 mm, 70CNPH-N-SS40; Advanced Microdevices, Ambala Cantt, India) for signal generation, and cellulose membrane (4 mm × 15 mm, 17 CHR; Whatman, Kent, UK) for absorption. The signal generation pad was prepared by dispensing (1.5 μL/cm) the monoclonal antibody (1 mg/mL, LZH1; HyTest, Turku, Finland) for the analyte line and goat anti-mouse IgG (0.1 mg/mL) for the control on the pre-determined sites, 5.5 and 11.5 mm from the bottom, respectively, using a micro-dispenser (BioJet3000, BioDot, Irvine, CA).

**Fabrication of EOC:** The EOC, which was comprised of top and bottom plastic plates made by injecting polycarbonate molding, was fabricated as previously reported.[26] After placing the immuno-strip and a horizontal flow absorption pad (cellulose membrane; 14 mm × 12 mm) within the vertical channel and the absorption pad compartment of the bottom plate, respectively, the two plates were firmly combined using groove joints to produce the EOC. The assembled EOC was stored in a desiccator maintained at room temperature prior to use.

**Analytical Performance of the EOC Biosensor. Analytical Procedure:** The procedure used to detect and analyze the presence of *L. monocytogenes* was previously described.[22,24] Briefly, standard samples of *L. monocytogenes* cells (100 μL) in 10 mM phosphate buffer containing 140 mM NaCl, pH 7.4, (PBS) was applied to the sensor and the vertical flow was maintained for 15 min to complete the immune reactions. The horizontal flow absorption pad was then connected to the lateral side of the signal generation pad, and 150 μL 3,3',5,5'-tetramethylbenzidine for membranes (TMB-M; Moss, Pasadena, ML) was supplied into the substrate supply pot. After the enzyme reaction was allowed to proceed for 5 min, the detectable color signal produced on the signal generation pad was captured as images using a digital camera, installed within the colorimetric detector. The color signals on the captured image were quantified along the center line of the immuno-strip in the vertical direction using software as described elsewhere.[22] The analysis was repeated three times for the same sample and, to establish the calibration curve, the mean values for each sample were plotted against the analyte concentration.

**Cross-reactivity Test:** The specificity of the EOC biosensor was tested by carrying out cross-reactions with the following bacterial species: *Salmonella typhimurium* (*S. typhimurium*), *Salmonella cholerasuis* (*S. cholerasuis*), *Salmonella bongori* (*S. bongori*), *Salmonella enteriaca* subsp. *arizonae* (*S. enteria* subsp. *arizonae*), *Salmonella enterica* subsp. *houtenae* (*S. enterica* subsp. *houtenae*), *Salmonella enteritidis* (*S. enteritidis*), *Shigella flexneri* (*S. flexneri*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Vibrio litoralis* (*V. litoralis*), *Listeria grayi* (*L. grayi*), *Listeria innocua* (*L. innocua*), *Listeria welshmeri* (*L. welshmeri*), *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus*), *Streptococcus mutans* (*S. mutans*), *Lactobacillus plantarum* (*L. plantarum*), *Psuedomonas fluorescens* (*P. fluorescens*), *Clostridium perfringens* (*C. perfringens*), and *Staphylococcus aureus* (*S. aureus*). *L. monocytogenes* (ATCC 19117) was obtained from the American Type Culture Collection (ATCC; Manassas, VA). Other standard bacterium species were supplied by the Korean Collection for Type Cultures (Biological Resource Center, Daejon, Korea). The EOC analyses were performed as described, where each sample contained $10^8$ cells/mL microorganism in PBS.

**Cell Concentration by IMS. Antibody Coating on Magnetic Beads:** The monoclonal antibody (LZF7; total 120 μg) was conjugated to magnetic beads (total $4 \times 10^8$ particles; Dynabeads M-270 Amine, Invitrogen, Carlsbad, CA) containing amine functional groups on the surfaces, according to the protocol

provided by the manufacturer. Briefly, the beads (200 μL of 30 mg/mL) were sufficiently washed with 100 mM 2-(*N*-morpholino)ethanesulfonic acid (Pierce, Rockford, IL), pH 4.5, and then chemically coupled with the antibody (100 μL of 1.2 mg/mL) after addition of a mixture (120 μL) of 0.13 mM *N*-hydroxysulfosuccinimide (Pierce, Rockford, IL) and 0.05 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Pierce, Rockford, IL) in deionized water. The reaction was carried out at room temperature for 2 h. To block the residual active sites on the beads, 1 M ethanolamine, pH 8.5, (12.2 μL) was added and incubated at room temperature for 15 min. The bead surfaces were finally blocked in PBS containing 0.5% casein (0.5% Casein-PBS). After the solution was magnetically separated, the immuno-beads were re-suspended in 1 mL of PBS (the final concentration 6 mg beads per mL and approximately 20 μg antibody per 1 mg bead) and stored at 4 °C until used.

**IMS Procedure:** The *L. monocytogenes* sample (10 mL) was concentrated by adding the immuno-beads (150 μL), reacting for 1 h on a shaker at room temperature, and magnetically separating the supernatant. To recover the cell concentrate from the magnetic beads, 10 mM glycine, pH 1.5, (60 μL) was added, incubated for 30 min, and magnetically separated again. The collected cell suspension was neutralized with 1 M Tris-HCl, pH 8.5, (30 μL) and sequentially stabilized by adding 5% Casein-PBS (10 μL). The concentrated cells (total 100 μL) were then subject to analysis using EOC.

**Real Sample Test. Sample Preparation:** Flatfish was kindly provided by the National Fisheries Research and Development Institute (NFRDI, Busan, Korea) and used to assess the ability of the EOC biosensor system combined with IMS to detect *L. monocytogenes* in a real sample. To this end, the fish intestines were collected and employed as real sample matrices. According to the standard protocol for sample preparation,[6] the intestines (10 g) were grinded in 90 mL *Listeria* enrichment broth (LEB; Merck, Darmstadt, Germany) containing 0.5% yeast extracts.

**Test Procedure.** The prepared samples were artificially inoculated with a 24 colony-forming unit (CFU) of *L. monocytogenes* and then cultivated under continuous shaking at 37 °C for different time periods in the range of 3 to 12 h. At pre-determined times, each sample was concentrated *via* IMS and analyzed using the EOC biosensor system. The same experiment for each sample was repeated three times.

## Results and Discussion

**Analytical Performances of the EOC Biosensor. Analytical Procedure:** Using the EOC, the microorganism can be analyzed and detected in a sequential two-step manner: antigen-antibody binding and enzyme reaction. The sample absorbs to the bottom of the immuno-strip after it is added to the system and the analyte first reacts with the detection antibody labeled with the enzyme, HRP, pre-loaded on the conjugate release pad. The aqueous mixture is then transferred by capillary action to the capture antibody, LZH1, which also recognizes the *L. monocytogenes cells* as a binder of the sandwich pair with LZF7, immobilized on a pre-determined site of the signal generation pad of the strip where the sandwich-type immune complex forms (vertical flow). To generate signals from the enzyme, the enzyme substrate is supplied into the pot after placing the horizontal absorption pad on the lateral side of the signal pad. The substrate then flows in the horizontal direction such that the excess reagents are washed away from the strip and the enzyme reaction is initiated at the same time (horizontal flow). The color signals are produced on the site of antibody immobilization in proportion to the analyte concentration. On the top position of the capture site, a secondary antibody (goat anti-mouse IgG) is bound to produce the control signal that is constant regardless of the analyte concentration. The color signals were quantified by employing a colorimetric detector equipped with a digital camera and software installed in the personal computer to digitize the color image.

**Dose Responses.** By following the pre-determined procedure, immuno-chromatographic assays were conducted to obtain the sensor responses to concentration changes of *L. monocytogenes*. The color signals (at the position indicated as Analyte)

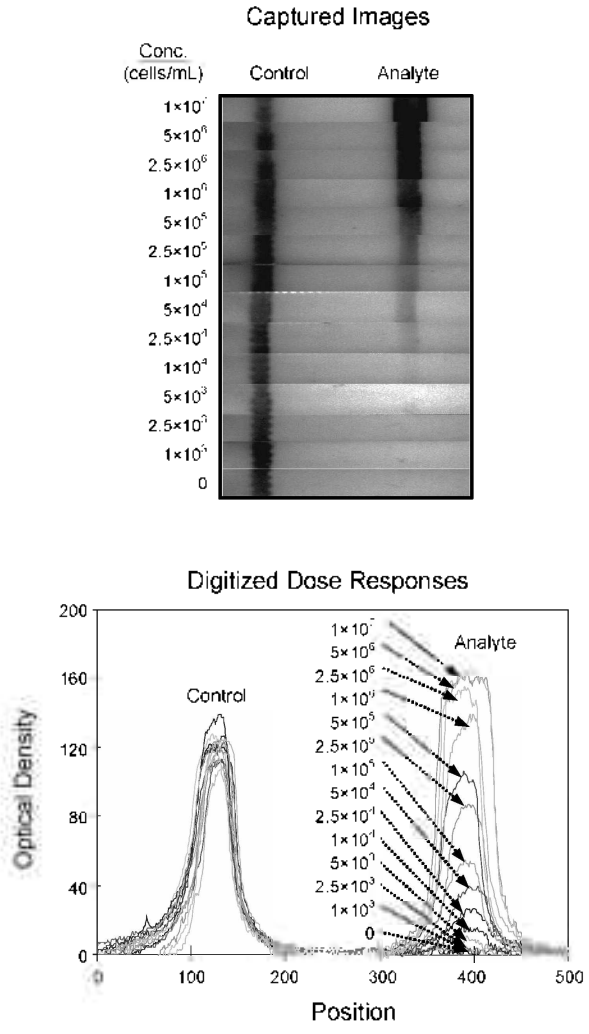


**Figure 1.** Dose responses of the EOC biosensor system to the concentration of *L. monocytogenes*. After analyses, the produced color signals were captured as images, showing a direct proportionality to the analyte dose (upper part). The signals were then digitized to optical densities using software and plotted against the position on the signal generation pad (lower part).

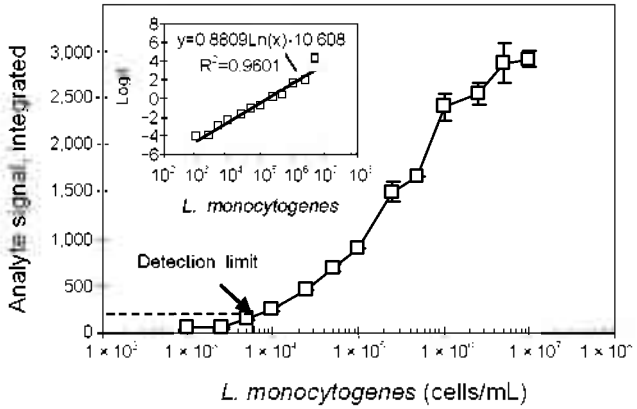


**Figure 2.** Calibration curve of the EOC biosensor for *L. monocytogenes*. The digitized color densities for each analyte dose (refer to Fig. 1, lower part) were integrated and then plotted against the concentration. The sigmoid-type curve was converted to a linearized graph *via* logit- log transformation (the inset). Variation in three repetitive measurements at each determination was indicated. See text for a more detailed explanation.

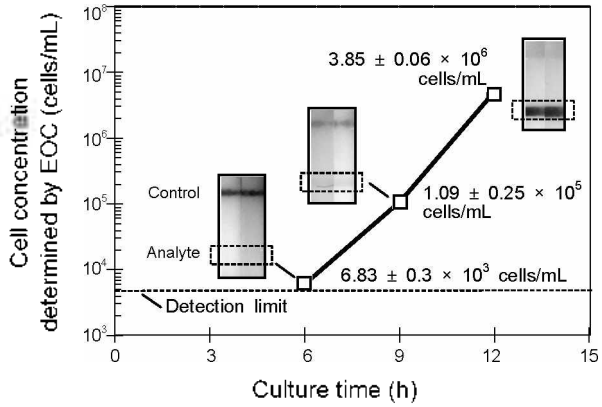


**Figure 4.** Real sample tests by means of the IMS-coupled EOC sensing at different cultivation time after the inoculation of *L. monocytogenes* cells into fish intestines. The inoculum density was 0.24 cells/mL and the sample was cultured in LEB medium. At each pre-determined time, the medium was analyzed and the concentration was determined using the calibration curve obtained for the EOC biosensor shown in Figure 2. Standard variations of three measurements for each determination were indicated.

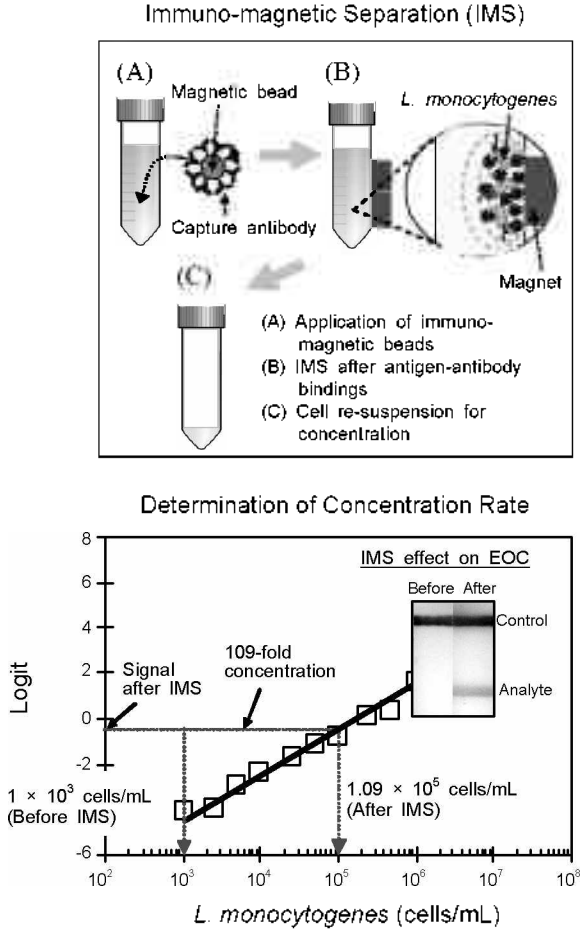


**Figure 3.** Schematic representation of the immuno-magnetic separation (IMS) process (upper part) and determination of the cell concentration rate using the EOC-based analytical method (lower part). The *L. monocytogenes* sample at a dose (e.g., $1 \times 10^3$ cells/mL) below the detection limit was enriched by IMS and then analyzed on the EOC (refer to the results before and after IMS in the inset). The measured signal was used to determine the corresponding concentration of the cells on the linearized calibration curve.

that appeared on each EOC were proportional to the analyte dose (Figure 1, upper part) in the range of approximately $5 \times 10^3$ to $1 \times 10^7$ cells/mL if determined by the naked eyes. The signals were then converted to optical densities *via* digitization of the image using the software developed in this laboratory. The density values were normalized by subtracting them from the mean value between the Control and Analyte bands (i.e., the background color density) and then plotted against the position on the signal generation pad (Figure 1, lower part). The signal curve dimension increased as the dose was elevated in the standard samples (Analyte) while the signal from the control remained relatively constant (Control). This plot showed that the curve measured at a cell concentration of $2.5 \times 10^3$ cells/mL was barely distinguishable from that at the zero dose.

To prepare the calibration curve for *L. monocytogenes*, the normalized optical densities for each peak were integrated to determine the signal values corresponding to the respective analyte concentration. The integrated signal was then plotted against the analyte concentration (Figure 2), which had a sigmoidal pattern identical to those previously reported.[24,26] From this curve, we were able to determine the analytical sensitivity of the EOC biosensor, which was the concentration that matched the signal value obtained by multiplying the standard deviation of the integrated signal at the zero dose by three.[22,23] The resulting detection capability of the EOC biosensor was determined to be $5.9 \times 10^3$ cells/mL and the quantification limit was determined to be $1.06 \times 10^4$ cells/mL by multiplying this value by a factor of five.[26] The calibration curve was linearized (correlation coefficient: $R^2 = 0.9601$) *via* logit-log transformation (the inset of Figure 2)[22] so that it could be used to accurately determine the analyte concentrations in unknown samples.

**Cross-reactivity:** In addition to the detection capability, it was also important to examine the specificity of the biosensor system to the target microorganism. The two monoclonal antibodies used as the capture and detection binders were claimed to show selective reactivities to *L. monocytogenes* by the manu-

**Table 1.** Cross-reactivities of the EOC biosensor system for *L. monocytogenes*.

| | Strain | Analytical results at $10^8$ cells/mL |
|---|---|---|
| 1 | *S. typhimurium* (ATCC 13311) | –[a] |
| 2 | *S. choleraesuis* (ATCC 10708) | – |
| 3 | *S. bongori* (ATCC 43975) | – |
| 4 | *S. bongori* (ATCC 12397) | – |
| 5 | *S. enterica* subsp *arizonae* (ATCC 12323) | – |
| 6 | *S. enterica* subsp *houtenae* (ATCC 43974) | – |
| 7 | *S. enteritidis* (ATCC 13076) | – |
| 8 | *S. flexneri* (ATCC 29903) | – |
| 9 | *V. Parahaemolyticus* (ATCC 27519) | – |
| 10 | *V. Parahaemolyticus* (ATCC 17802) | – |
| 11 | *V. litoralis* (ATCC 12520) | – |
| 12 | *V. parahaemolyticus* (ATCC 27969) | – |
| 13 | *L. monocytogenes* (ATCC 19117) | +[b] |
| 14 | *L. grayi* (ATCC 19120) | + |
| 15 | *L. innocua* (ATCC 33090) | + |
| 16 | *L. welshmeri* (ATCC 35897) | + |
| 17 | *E. coli* (ATCC 43888) | – |
| 18 | *B. cerius* (ATCC 21366) | – |
| 19 | *S. mutans* (ATCC 27607) | – |
| 20 | *L. plantarum* (ATCC 14917) | – |
| 21 | *P. fluorescens* (ATCC 49642) | – |
| 22 | *C. perfringens* (ATCC 3624) | – |
| 23 | *S. aureus* (ATCC 25923) | – |

[a]–: No signal observed. [b]+: Signal obviously observed.

facturer. The antigenic sites of the bacterial cell, however, might be analogous to those on other species such as lipopolysaccharides (LPS) and three types of surface antigens (K-, O-, and H-antigens).[27] The microorganisms used to test the cross-reactivity of the sensor were the *Salmonella* species, *Vibrio* species, *Listeria* species, and other pathogenic species (Table 1). The analyses were carried out for the samples containing a high concentration (e.g., $10^8$ cells/mL) of each species and showed that the EOC biosensor did not cross-react with other bacteria except the *Listeria* species. Nevertheless, since the sensor was not able to discriminate the pathogenic species, *L. monocytogenes*, from the others of the same genus, the specificity was needed to be further improved. This can be achieved, in a future study, by producing and screening appropriate monoclonal antibodies that react solely with the target microorganism.

**Enhancement of Detection Capability.** Since *L. monocytogenes* can rapidly grow when incubated under optimal conditions, the presence of even extremely low concentrations of *L. monocytogenes* (e.g., a single cell per 10 g specimen) in food products is not legally allowed. However, such low titers cannot be directly detected using most analytical means. Thus, the analyte is usually enriched by cell cultivation until the titer reaches detectable levels.[18] To decrease the cultivation period, which is the time-limiting process, we devised an analytical scheme to concentrate the cells immediately prior to detection.

**IMS-coupled Analysis:** IMS can be used to rapidly concentrate *L. monocytogenes* cells and may be carried out in the field where the food specimens are furnished. To examine the ability of this method to concentrate *L. monocytogenes*, we first chemically linked the capture antibody, specific to the microorganism, on the surfaces of magnetic beads and then added the conjugates into an aqueous solution containing the cells (Figure 3, upper part: A). After the antigen-antibody reaction, the complexes were isolated *via* IMS (B), the solution was removed, and the products were re-suspended in a minimal volume of acidic solution (C). The cells that dissociated from the beads under the acidic condition were retrieved using IMS again and then, after neutralization of the pH, the samples were subject to EOC analysis.

A sample containing $1 \times 10^3$ cells/mL of *L. monocytogenes*, which was initially not detectable by the sensor, was concentrated *via* IMS and then analyzed by EOC. The color signal was quantified as previously described and used to determine the concentration after IMS through the linearized calibration curve (Figure 3, lower part). The cell density was increased up to $1.09 \times 10^5$ cells/mL, indicating that concentration rate by IMS was 109-fold. Consequently, the detection capability of the IMS-coupled EOC analysis was as low as 54 cells/mL of *L. monocytogenes* in the original sample. The cell concentration effect even produced on-off color signals after and before IMS, respectively (see the inset).

**Tests with Real Sample.** As mentioned above, *L. monocytogenes* may be present in contaminated food products in trace quantities that cannot be directly detected by means of the IMS-coupled EOC biosensor technology. Thus, cell cultivation for pre-enrichment is generally required before sample analysis,[13] which is a time-consuming step. Several studies have attempted to shorten the cultivation time[18,28] with the goal of developing an early screening method against food contamination that is reliable and rapid.

**Enrichment by Cultivation:** We employed a real sample, e.g., fish intestines, to determine the pre-enrichment time of the bacterium, inoculated into the sample, needed to reach a cell concentration that is detectable by the sensor. The sample may contain a diverse range of different microorganisms that might interfere with the sensors ability to specifically detect the target analyte. The *L. monocytogenes* cells (2.4 CFU/g) were artificially inoculated into the real sample and were cultured (inoculation density: 0.24 CFU/mL) for different time intervals. To determine a minimum cultivation period, the cell culture was analyzed at pre-determined times using the IMS-coupled EOC sensing protocol as described above.

Based on the analytical results (Figure 4), the microorganism inoculated into the sample was detected by the EOC biosensor after concentration by IMS after 6 h from the onset of cultivation. The cell concentration determined from the standard curve was $6.83 \pm 0.3 \times 10^3$ cells/mL after IMS, which was somewhat above the detection limit of the EOC biosensor ($5.9 \times 10^3$ cells/mL). If the condensation factor by IMS (typically, 109-fold) was considered, the cell density in the culture was expanded to 62.7 cells/mL. Thereafter, the growth curve showed a nearly straight line in the semi-log plot during the monitoring time period, indicating that the culture was maintained in the log phase of the growth. When the bacteria cells began to divide into two daughter cells (i.e., binary fission), the cell number was

augmented in the form of logarithmic pattern.[22]

Since the inoculated cells (0.24 cells/mL) expanded to 62.7 cells/mL in the initial 6 h of cultivation, the doubling frequency of the culture was estimated to be 8 times on the basis of 40 min per one binary fission in the LEB medium.[29,30] This estimation also suggested a lag phase period of about 40 min, during which the individual cells matured, but were not yet able to divide.[29] Due to cell growth, the cell concentration should increase up to $1.00 \times 10^3$ cells/mL, if there was an average of 4 doublings over the next 3 h (for total 9 h-culture), and up to $3.21 \times 10^4$ cells/mL with 7 doublings over the next 6 h (for total 12 h). These estimations were very close to the experimental values, $1 \times 10^3$ and $3.53 \times 10^4$ cells/mL after a 9 and 12 h cultivation period, respectively, which was calculated by dividing the measured densities using the EOC by the IMS condensation factor of 109.

It is worth noting that, in regards to the lag phase, the time period was not extended for very long even though an extremely low bacterial concentration was inoculated. According to previous reports,[31,32] a long lag phase was often encountered with cells that were subjected to stress by physical damage, heat treatment, or starvation. Since the cells grown in the middle of the exponential phase were used for the inoculation in this study, they may spend only a short time in the lag phase to allow them to adopt to the new environment and subsequently grow in a logarithmic pattern.

## Conclusions

The EOC sensor combined with IMS was able to detect 0.24 cells/mL of *L. monocytogenes* inoculum in culture (2.4 CFU/g intestines as sample) after 6 h from the onset of pre-enrichment. It was inferred based on a 40-min doubling time of *L. monocytogenes* that the same sample containing 1 CFU/g could be detected within approximately 9 h when this novel analytical method was used. Although the lag phase in the culture may increase with a decrease in the inoculum size, IMS would compensate for the loss in total analysis time due to its ability to concentrate the sample by 109-fold. The EOC sensor performance was superior to those of other commercial rapid test kits currently available in regards to shortening the total assay time for detection of the microorganism to within working hours. It would be expected that the biosensor system can be utilized in places where an early food screening against microbial contamination is required. We are currently devising a system that physically combines both IMS and detection using the EOC biosensor, which would allow one to sequentially conduct the both processes.

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