# Interdigitated microelectrode based impedance biosensor for detection of salmonella enteritidis in food samples



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# Interdigitated microelectrode based impedance biosensor for detection of salmonella enteritidis in food samples

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**Abstract**. *Salmonella enteritidis* outbreaks continue to occur, and *S. enteritidis*-related outbreaks from various food sources have increased public awareness of this pathogen. Conventional methods for pathogens detection and identification are labor-intensive and take days to complete. Some immunological rapid assays are developed, but these assays still require prolonged enrichment steps. Recently developed biosensors have shown great potential for the rapid detection of foodborne pathogens. To develop the biosensor, an interdigitated microelectrode (IME) was fabricated by using semiconductor fabrication process. Anti-*Salmonella* antibodies were immobilized based on avidin-biotin binding on the surface of the IME to form an active sensing layer. To increase the sensitivity of the sensor, three types of sensors that have different electrode gap sizes (2 μm, 5 μm, 10 μm) were fabricated and tested. The impedimetric biosensor could detect $10^3$ CFU/mL of *Salmonella* in pork meat extract with an incubation time of 5 minutes. This method may provide a simple, rapid and sensitive method to detect foodborne pathogens.

## 1. The first section in your paper

*Salmonella enteritidis* is one of the major foodborne pathogens of concern. It is a gram-negative rod-shaped bacterium that causes severe illness in the elderly, infants, and those with weak immune systems. A person infected with this pathogen shows symptoms of fever, abdominal pain, nausea and vomiting, diarrhea, dehydration, weakness, and loss of appetite. The symptoms may begin to appear 12 to 72 hours after consuming a contaminated food or beverage. The pathogen is usually associated with raw or undercooked eggs and poultry. Salmonella enteritidis outbreaks continue to occur, and *S. enteritidis*-related outbreaks from various food sources have increased public awareness of this pathogen.

Conventional methods for *Salmonella* detection and identification involve prolonged multiple enrichment steps. Some immunological rapid assays are available with sensitivity comparable to those of the conventional methods. One common rapid detection method is based on impedance characteristics of electrodes and a medium where bacteria are resides. Impedance measurement method analyzes both the resistive and capacitive properties of a medium or electrodes. Many researches have been conducted with conventional impedimetric method, which has been focused on the changes in electrical impedance of a medium resulting from the bacterial growth [1][2].

Impedance changes of the medium are resulted from the release of ionic metabolites from live bacteria. Some researchers measured impedance changes of both the medium and electrodes [3]. Even though the impedimetric method reduces the time for the detection of bacteria in food, it still requires hours of incubation time to detect bacteria.

Recently, biosensors have shown great potential for rapid detection of foodborne pathogens. They are capable of direct monitoring the receptor-analyte reactions in real time. Among the biosensors, impedimetric biosensors have been widely adapted as an analysis tool for the study of various biological binding reactions because of their high sensitivity and reagentless operation. The impedimetric biosensor, which was devised to increase the selectivity and the sensitivity by incorporating a biologically functionalized detection layer on the surface of the electrode, usually measures electrode or interface impedance. The impedimetric biosensor enables qualitative and quantitative monitoring of bacteria by measuring the changes in the electrical impedance.

A variety of impedimetric biosensors have been constructed to monitor various biological reactions at the surface of electrodes by immobilizing biomolecules such as enzymes, antibodies, nucleic acids, cell, and microorganisms [4]. In fact, some impedimetric biosensors have been used to detect various microorganisms including E. coli O157:H7 [5], still more researches are needed to improve the performance of *Salmonella* detection in food.

In this study, impedimetric biosensors were developed to detect *S. enteritidis* in food samples. To increase sensitivity of the biosensor, different specifications of interdigitated electrodes were fabricated and tested. Developed impedimetric biosensor was evaluated by performing *S. enteritidis* detection in PBS and food samples.

## 2. Materials and Methods

### 2.1. Interdigitated microelectrode (IME) sensor fabrication

The IME sensor was fabricated from a glass wafer with a 50 nm Cr layer as an adhesive layer. A 100 nm thick of interdigitated gold electrode was deposited over the Cr layer using photolithographic processing methods to form an active sensing area. Three different sensor types which have different electrode gap sizes (2 μm, 5 μm, 10 μm) were fabricated to evaluate the effect of electrode specification on the sensitivity of the sensor. Each sensor had a 3 $mm^2$ of active sensing area. Each electrode finger had a length of 990 μm and a width of 10 μm. Each sensor had a different number of electrodes according to electrode spacing. Figure 1. Sensor surface other than electrode finger region was coated with insulation epoxy to maintain fixed sensing-area throughout experiments.

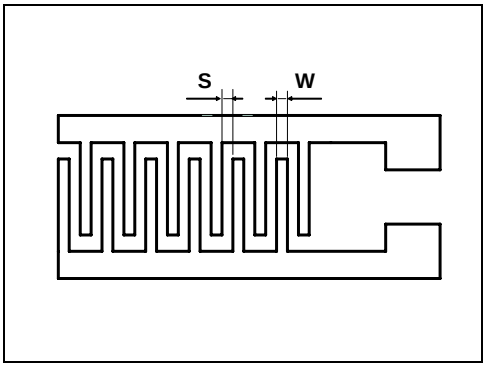



Figure 1. IME sensor design that has a large number of interdigitated electrode pairs. In the figure, S and W denotes electrode gap size and width, respectively.

### 2.2. Impedimetric biosensor preparation

Impedimetric biosensor was fabricated by immobilizing biological binding ligands on the IME sensor. Before immobilization process, surface of the IME sensor was cleaned with acetone, a solution of 0.1

M NaOH and 1% Triton X-100, 100% ethyl alcohol, and deionized water. Impedimetric biosensor was prepared to have active binding sites for *S. enteritidis* as follows. Firstly, 1 mg/ml biotinylated bovine serum albumin (bBSA) was applied and incubated overnight at room temperature to form a linking protein film on the sensor surface. Secondly, 0.1 mg/ml neutravidin was applied and captured onto the physisorbed film of bBSA by incubating 20 min at room temperature. Thirdly, The IME was incubated with 100 μl of 50 μg/ml biotinylated anti-*Salmonella* antibody in phosphate buffered saline (PBS, pH 7.2) at room temperature for 10 min. Finally, the IME were incubated with 100 μl of 1 mg/ml BSA at room temperature for 5 min to block non-specific binding sites. Between each step, the IME were rinsed with PBS to remove unbound reagents.

Most reagents including BSA, PBS, Triton-X 100, acetone, NaOH, and ethyl alcohol were purchased from Sigma (St. Louis, MO, USA). Neutravidin was purchased from Pierce (Rockford, IL, USA). Biotinylated rabbit anti-*Salmonella* polyclonal antibody was purchased from ViroStat Inc. (Portland, ME, USA).

### 2.3. Instrument setup

After immobilization of capture antibody onto the IME, the impedimetric biosensor was connected to impedance measurement set up. Impedance measurements were performed using an HP 4194A impedance analyzer (Hewlett-Packard, now Agilent Technologies, Palo Alto, CA, USA). The impedance magnitude of the biosensor was measured at frequency ranges between 100 Hz to 1 MHz with a 50 mV (amplitude) voltage excitation. To measure only the impedance of the biosensor, impedance calibration for the wiring and probes was done before the measurements. Measured impedance signal was sent to the data processor via GPIB connection. To measure the signal that is most sensitive to the attachment of *S. enteritidis* to the antibodies on the biosensor surface, impedance signal at 100 Hz was used for data analysis.

### 2.4. Bacteria and sample preparation

*Salmonella* enterica serotype *enteritidis* (*S. enteritidis*) ATCC 11076 was used for the experiments. The cells were diluted to appropriate numbers ($10^9$ – $10^1$ cfu/ml) with PBS, and used for the experiment.

Food samples were prepared by inoculating 100 μl of the cell suspension into the food extracts. The cells were also diluted to appropriate numbers ($1.1\times10^8$ – $1.1\times10^3$ cfu/ml) with food extract. For negative control, PBS or plain food extract, which does not contain cells, was used. Enumeration of the enriched *S. enteritidis* was performed using the standard plate count (SPC) method. For the food sample preparation, packages of pork were purchased from a local grocery store.

### 2.5. Detection of *S. enteritidis*

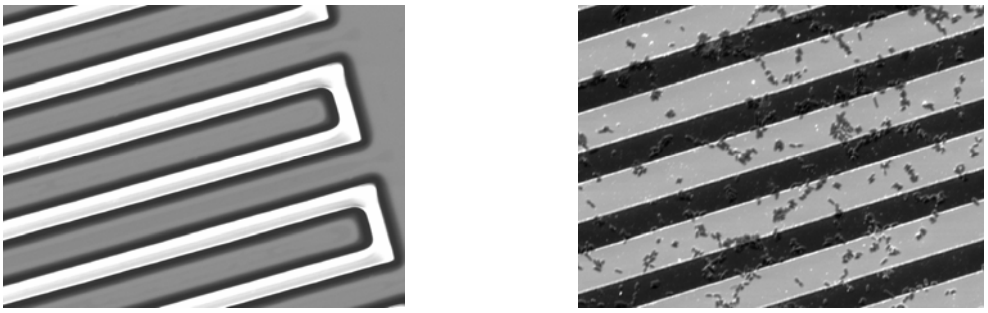
When the impedimetric biosensor and the sample sets were ready, a background signal was measured first using PBS as a sample. With the same biosensor, consecutive measurements were performed using serially diluted bacteria samples in PBS or food extract. To allow the antibody-antigen reaction, the biosensor was incubated with the sample for 5 min before the signal measurement. Between the measurements, the biosensor was rinsed three times with PBS-Triton (0.02 M phosphate buffered saline (PBS) containing 0.05 % Triton X-100) followed by PBS.

## 3. Results and Discussion

*S. enteritidis* was detected by reading impedance changes caused by the attachment of the cells to the anti-*Salmonella* antibodies immobilized on interdigitated gold electrodes. The antibodies were immobilized on the electrode surface by using neutravidin-biotin binding. The impedance across the interdigitated electrodes was measured after the series of sample introduction. Captured bacteria cells by the antibody changed the impedance between the electrodes.

Specification of the biosensor and binding of *S. enteritidis* to antibodies immobilized of the biosensor surface was examined with scanning electron microscopy (SEM). Figure 2 shows the SEM image

(1000×) of the biosensor before (A) and after (B) used in detection assay. Larger magnification (4000×) of Figure 2B clearly shows that bacteria were attached to the used biosensor surface after rinsing with PBS-Triton and PBS.



(A) (B)

Figure 2. SEM images of impedimetric biosensors: (A) clean surface of a biosensor (1000×); (B) bacteria bound to antibodies immobilized to biosensor surface (1100×).

The impedance signal changes over the background of all types of the biosensors were proportional to the number of bacteria in the sample. Since, a sample with high cell concentrations tends to more cells being attached to antibodies immobilized on the sensor surface. Higher concentration of cells in the sample generally increased responses. Because the cells attached onto the surface of the biosensor act as resistors, the larger the number of attached cells increased the impedance.

Both the 2 μm gap and 5 μm gap sensors could detect $10^3$ cfu/ml concentrations of *S. enteritidis* in pork samples. However, the 10 μm gap sensor could detect $10^7$ cfu/ml concentrations of *S. enteritidis* in pork samples. Better detection limits of the 2 μm gap and 5 μm gap sensors can be explained by the characteristics of the micro-sized interdigitated electrodes. Larger area of the sensor surface could be covered by bacteria for small gap IME electrodes and sensitivity of the biosensors could be improved by the big changes of the surface characteristics.

The majority of the published papers on the detection of bacteria using IME impedance sensor focused on the electrical characteristic changes of culture medium. The detection time of the measuring impedance changes in the culture medium method tends to long depends on the number of initial bacterial cells present in the sample. The impedimetric biosensor developed in this research could detect $10^3$ cfu/ml concentrations of *S. enteritidis* in pork samples in less than 10 min. Even though detection time for the impedimetric biosensor is faster than medium impedance measurement method, further research is needed to improve the sensitivity of the impedimetric biosensor.

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