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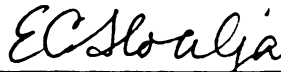
Fabrication and Evaluation of an Electrical Immunoassay
Biosensor for the Rapid Detection of Foodborne Pathogens in
Food and Water

presented by

Zarini Muhammad-Tahir

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**FABRICATION AND EVALUATION OF AN ELECTRICAL IMMUNOASSAY
BIOSENSOR FOR THE RAPID DETECTION OF FOODBORNE PATHOGENS
IN FOOD AND WATER**

By

Zarini Muhammad-Tahir

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ABSTRACT

FABRICATION AND EVALUATION OF AN ELECTRICAL IMMUNOASSAY BIOSENSOR FOR THE RAPID DETECTION OF FOODBORNE PATHOGENS IN FOOD AND WATER

By

Zarini Muhammad-Tahir

The thesis describes the development of an electrical immunoassay (EI) biosensor for detecting foodborne pathogens. The biosensor consists of two components: an immunosensor that is based on an electrical sandwich immunoassay and a reader for signal measurement. The architecture of the immunosensor utilizes the lateral flow format, which allows the movement of fluid from one region to another by capillary action. The EI biosensor performs better than the previous biosensor developed in this laboratory. The biosensor provides a specific, sensitive, low volume, and real-time detection strategy. Initial results show that the biosensor can detect approximately 8×10^1 colony-forming units (CFU) per ml in 6 minutes. Results are presented to highlight the performance of the biosensor for detecting enterohemorrhagic *Escherichia coli* and *Salmonella* species in food and water samples. The ability to change the specificity of the antibodies will enable the biosensor to be used as a semi-quantitative detection device for other types of foodborne pathogens. Such a device can be used to enhance food safety and biosecurity of the food supply and can also be adapted for use in medical diagnostics and bio-defense.

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This thesis is dedicated to my mother and father, Zahirin and Zalina Muhammad-Tahir, for their unconditional love, support and trust.

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INTRODUCTION

Bacterial contamination in food and water supplies is a major food safety concern. Foodborne illnesses cause billions of dollars in medical costs, lost productivity, and product recalls (Hui et al. 1994). In the United States, the incidence of foodborne illness is estimated to be 76 million cases with 5,000 deaths (Mead et al. 1999), and an annual economic burden of 22 billion US dollars (NFSI 1997). Fresh produce and drinking water are some of the food and water sources, which are highly vulnerable to microbial contamination.

In recent years, per capita consumption of fresh produce has increased significantly. During the year 2000, the total cost for fresh produce consumption in the US were 78.5 billion (USDA 1996). Additionally, in the last decade, the amount of imported fresh produce has doubled from countries all over the world (USDA 1996). The increase in fresh fruit and vegetable consumption, unfortunately, has been paralleled with an increase in the number of foodborne illnesses. The Centers for Disease Control and Prevention (CDC) has reported a significant increase in the number of produce-associated outbreaks in the US- 103 outbreaks affecting 6,082 people (CDC 2002). The rate of outbreaks went from 4.6 from 1973 to 1987, to 9.8 from 1988 to 1992 (Turner 1998; Jay 2000). A 1997 *Escherichia coli* O157:H7 outbreak involving unpasteurized apple juice in several Western States and an outbreak involving cider in the Northeast are two examples of the hazardous effect of foodborne pathogens in these food products

(CDC 2002). Other produce-related outbreaks have involved cantaloupe, watermelons, tomatoes, fresh basil, alfalfa sprouts, melon and iceberg lettuce (Beuchat and Jee-Hoon 1996; Thayer and Raykowski 1999).

E. coli O157:H7 and *Salmonella* species have emerged as the most important bacterial foodborne pathogens, especially in fresh fruits and vegetables. These pathogens not only cause serious illnesses but also are also lethal to infants, elderly, and immune-compromised individuals. *E. coli* O157:H7 is a pathogen of particular concern due to its combination of severe consequences of infection, its low infectious dose, and its association with commonly consumed foods (Jay 2000). *Salmonella* species also have been associated with produce and water-related outbreaks and have been identified as the most prevalent foodborne pathogens (Davies 1997). Previous studies have shown that these pathogens are the main cause of outbreaks in most developed countries (Buzby and Roberts 1996) including the United States, Canada and the United Kingdom.

There is no definitive way to tell simply by looking at a food whether or not it is contaminated. To date, the presence of bacteria in food is determined through microbiological testing. These approaches, however, are time consuming and laborious. Therefore, there is a growing need in the food industry for microbial detection systems that are rapid, specific, sensitive, and reliable. In this study, a new microbial detection device, known as a biosensor has been developed to enhance the detection of foodborne pathogens in food and water supplies.

The said biosensor is an electrical immunoassay (EI) biosensor, which comprised of two types of proteins: a capture protein and a reporter protein. The capture protein is immobilized on a platform between two electrodes, while the reporter protein is attached to a conductive polyaniline. After adding the sample, the target protein (analyte) binds to the reporter protein and forms a sandwich complex with the capture protein. The conductive polymer that is attached to the reporter protein acts like a messenger to report the amount of target protein captured. The message is translated into an electrical signal in the form of resistance. By replacing the two types of proteins with antibodies specific to the target bacteria of interest, it is possible to utilize this technology to detect the presence of pathogenic bacteria in a sample.

The overall goal of the research is to design and fabricate an electrical immunoassay (EI) biosensor for foodborne pathogen detection in food and water supplies. The objectives of the study are as follows:

1. To design and fabricate an EI biosensor to detect pathogens in real-time with an electronic data collection system (Chapter 3).
2. To determine the sensitivity and specificity of the biosensor in pure cultures of *E. coli*, *E. coli* O157:H7, and *Salmonella* (Chapter 4).
3. To test the ability of the biosensor to detect *E. coli*, *E. coli* O157:H7, and *Salmonella* in artificially contaminated fresh produce samples (Chapter 5).

The long-term goal of this research is to develop an automated, rapid, inexpensive, and sensitive diagnostic tool to detect any type of foodborne pathogen or spoilage organisms in food and water supplies.

CHAPTER 1

LITERATURE REVIEW

ESCHERICHIA COLI

Escherichia coli is a normal microflora of the intestinal tract of humans and warm-blooded animals. *E. coli* strains are classified based on serology that identifies the O (somatic), H (flagella) and K (capsular) antigens (Doyle 1997). All together there are more than 160 serotypes of *E. coli* (Salyers and Whitt 1994; Neill 2001). Initially, *E. coli* was not considered pathogenic. Over the past decades however, it has become evident that some *E. coli* strains can cause a variety of diseases, such diarrhea, dysentery, septicemia, and hemolytic uremic syndrome (Riemann and Cliver 1998). The pathogenic strains are categorized based on the virulence genes which encode for factors such as the ability to invade and attach to host cells, and the ability to produce toxins (Riemann and Cliver 1998). These genes are often located on plasmids and can be transferred from one *E. coli* strain to another. Categorizing *E. coli* strains based on virulence genes has led to the classification of *E. coli* based on virotypes (Salyers and Whitt 1994; Neill 2001). At present, six virotypes have been identified: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enterohemorrhagic (EHEC) (Riemann and Cliver 1998), and diarrheagenic adherent *E. coli* (DAEC) (Neill 2001).

ESCHERICHIA COLI O157:H7

Escherichia coli O157:H7 has emerged in the past 20 years as a pathogen of major public health importance. This pathogenic serotype produces toxins that cause bloody diarrhea in humans. Shiga toxin-producing strains of *E. coli* O157:H7 (STEC) first gained broad notoriety in the US in 1982 following two outbreaks of bloody diarrhea involving patrons of a fast food restaurant chain (Acheson 1996). This organism has been identified as one of the most dangerous foodborne pathogens based on its virulence and pathogenicity (Buchanan 1997).

E. coli O157:H7 is not in the ETEC group since heat labile and heat stable toxins are not produced (Neill 2001). This organism is non-invasive and thus cannot be classified as EIEC (Riley 1983). *E. coli* O157:H7 strains produce verotoxin, which is shown to have a close structural and antigenic similarity to the Shiga toxin of *Shigella dysenteriae*. Hence, this toxin is also known as Shiga-like toxin (SLT), or Shiga toxin (Stx) (O' Brien et al. 1983; Neill 2001).

EPIDEMIOLOGY

E. coli O157:H7 strains have been reported to cause severe illnesses in healthy individuals as well as those with chronic disease. The current understanding of human infection with *E. coli* O157:H7 illnesses ranges from mild and non-bloody diarrhea, to a severe and life-threatening illness with such complication as hemolytic uremic syndrome (HUS) in children and thrombotic thrombocytopenic purpura (TTP) in adults (Doyle 1997).

Illness typically begins with a short, mild, bloody diarrhea, which progresses within 24 hours to voluminous bloody diarrhea that is often associated with severe abdominal pain and cramp (Ostroff et al. 1989). Hospitalization rates for illness caused by *E. coli* O157:H7 are high, accounting for 50% of all reported cases. Although bloody diarrhea is a common symptom of *E. coli* O157:H7 and other EHEC infections, some strains can also cause non-bloody diarrhea (Slutsker et al. 1997).

A rising incidence of HUS has been noted (Martin et al. 1990) with 6%-90% of all *E. coli* O157:H7 patients developing HUS (Rowe et al. 1991). The increased incidence of HUS following *E. coli* O157:H7 infections may be related to age, antibiotic use, anti-motility agents, and toxin type of the infecting strain (Neill 2001). Symptoms of HUS include intravascular destruction of red blood cells (microangiopathic hemolytic anemia), thrombocytopenia, lack of urine formation (oligo-anuria), edema, and acute renal failure (Buchanan 1997). HUS has a fatality rate of 3-5% and a considerable risk of long-term morbidity, such as hypertension, chronic renal failure and disability (Neill 2001).

Cases of TTP also have been associated with *E. coli* O157:H7 infections (Ramsey and Neill 1986). TTP resembles HUS histologically. Patients who developed TTP after *E. coli* O157:H7 infections are reported to have bloody diarrhea with the overall features of illness resembling those of HUS cases (hemolytic anemia, thrombocytopenia, and renal failure) accompanied with fever and altered mental status (Neill 2001). Few cases of TTP are found in children as this disease is generally restricted to adults (Buchanan and Doyle 1997).

Thrombotic thrombocytopenic purpura, however, is a rare syndrome for *E. coli* O157:H7 infection (Doyle 1997).

A seasonal pattern has been observed for *E. coli* O157:H7 outbreaks with most cases occurring in summer (Ostroff et al. 1989; Griffin and Tauxe 1991). Interestingly, a similar pattern also has been observed for HUS cases even prior to the discovery of *E. coli* O157:H7. The increased incidence of HUS during summer suggests an increase in exposure to *E. coli* O157:H7 probably through such risk factors as barbecuing and swimming (Neill 2001). For example, this pathogen was the causative agent of two outbreaks of severe bloody diarrhea in Oregon and Michigan in which transmission occurred via contaminated hamburgers (Riley et al. 1983). The Centers for Disease Control and Prevention estimates that there are 20,000 illnesses a year due to *E. coli* O157:H7 infections (Jay 2000). Human infections with *E. coli* O157:H7 are usually linked to consumption of contaminated and improperly cooked beef, unpasteurized milk, fresh fruits and vegetables (Griffin and Tauxe 1991; Beesley 1993).

Some *E. coli* O157:H7 cases, however, have involved drinking and swimming water. In 1999, hundreds of people became sick after drinking contaminated water in Washington County, New York and swimming in contaminated water in Clark County, Washington (USEPA 2002). Human and animal wastes are the main vectors for *E. coli* transmission (USEPA 2002). During rainfall, snowmelts, or other types of precipitation, produce-surface runoff may wash *E. coli* into sources of drinking water, such as creeks, rivers, streams, lakes, or groundwater. Additionally, during flood seasons, these pathogenic

bacteria also have been found in wells. When these water sources are not treated or are inadequately treated, *E. coli* may end up in drinking water. The USEPA has outlined that to be considered safe for swimming and drinking, average *E. coli* counts in water must be below 130 cells per 100 ml (USEPA 1986).

SALMONELLAE

Salmonellae are members of a large and varied group of Gram negative, non-spore forming rods, which is commonly known as the Family *Enterobacteriaceae* (Weil and Saphra 1953). *Salmonella Enterica* is recognized as the main cause of foodborne illnesses in most developed countries (Buzby and Roberts and Roberts 1996). In the US, an estimated 8-18 thousand hospitalizations, 2,400 cases of septicemia, and 500 deaths are associated with *Salmonella* infections each year (USDA-FSIS 1998). The United State Department of Agriculture (USDA) also has identified *Salmonella enterica* as the most prevalent and costly of the known foodborne pathogens (Davies 1997).

Salmonella was first found by Gaffky in 1884 (Weil and Saphra 1953). As early as 1886, Gaffky showed that this organism was involved in cases of foodborne illnesses (Weil and Saphra 1953). There are now more than 2,000 serotypes or serovars of the genus, of which 50 –150 serotypes have been linked to disease outbreaks (Ziprin 1994). All *Salmonella* serotypes are classified based on their closeness of features as determined by DNA hybridization (Le Monor and Popoff 1987). Most of the *Salmonella* are classified in two species, namely:

Salmonella enterica and *Salmonella bogori* (Jay 2000). For example, the serotype Typhimurium is classified among *S. enterica* species, and is referred to as *Salmonella enterica* subsp. *enterica* serovar Typhimurium, or *S. Typhimurium* (Buzby and Roberts 1996; Jay 2000). Some *Salmonella* serotypes might produce enterotoxins that are biochemically and immunologically related to Cholera toxin, heat-labile toxin and Shiga toxin (Inoue et al. 1996), with some isolates also producing low levels of enterotoxin (Ziprin 1994).

SALMONELLA TYPHIMURIUM STRAIN DEFINITIVE PHAGE TYPE 104 (DT 104)

Multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium DT 104 has emerged during the last decade as a global health problem because of its association with animal and human diseases (CDC 1996). The DT 104 epidemic has now spread worldwide, with several outbreaks since 1996 in the US and Canada (Davis 1999; Besser 2000). Multidrug-resistant *S. Typhimurium* DT 104 strains are commonly resistant to ampicillin, chloramphenicol/florfenicol, spectinomycin/streptomycin, sulfonamides, and tetracyclines (WHO 1997). This organism initially emerged in cattle in 1988 in England and Wales (WHO 1997).

Studies show that increased use of antibiotics in animals is one of the factors involved in the dramatic increase in drug-resistant microorganisms. Ciprofloxacin resistance in DT104 has increased from 1% in 1994 to 6% in 1995, coincident with the licensing of this drug for veterinary use in the UK in 1994

(Killalea 1996). Surveys of *S. Typhimurium* isolates from cattle and humans in Australia, France, Hong Kong, and Spain all reveal an increased incidence in resistance to multiple antibiotics in this organism (WHO 1997). In addition to acquiring infections from contaminated food, human cases also have occurred where individuals came in contact with infected cattle. Data shows that 34 of 83 DT104 cases studied in a 1993 epidemiological study required hospitalization and 10 died (Glynn et al. 1998).

EPIDEMIOLOGY

Typically, *S. enterica* serotypes cause acute gastroenteritis (Buzby and Roberts 1996). Susceptibility to *Salmonella* depends on several factors; the virulence of the serotype, the individual's immune system, and the quantity of *Salmonella* ingested (Buzby and Roberts 1996; Jay 2000). Individuals at greatest risk of contracting salmonellosis include children, the elderly, and individuals with immune deficiencies (Buzby and Roberts 1996). Many salmonellosis cases however, have been reported among healthy individuals (Ziprin 1994). While a cell concentration of $10^7 - 10^9$ CFU/g is generally required to induce salmonellosis (Jay 2000), some *Salmonella* serotypes can cause illness at levels as low as one cell (Buzby and Roberts 1996).

Initial symptoms of salmonellosis include diarrhea, along with abdominal pain, nausea, vomiting, headaches, chills, myalgia, and low-grade fever (Nisbet and Ziprin 2001). Diarrhea is due to invasion of the intestinal mucosa and a concomitant inflammatory response that leads to secretory diarrhea mediated by

activation of cyclic adenosine monophosphate (Moss 1980). These symptoms develop in 12-36 hours after ingesting the organism (Buzby and Roberts 1996; Jay 2000).

Salmonella infections can also cause a secondary-disease syndrome, or sometimes a chronic disease (Buzby and Roberts 1996). Many *Salmonella* serotypes penetrate the intestinal lining in humans, with this organism also frequently invading the bloodstreams to cause septicemia (Buzby and Roberts 1996). In a study of 74 consecutive cases of salmonellosis, *S. Typhimurium* was the most common strain to cause septicemia. Complications of septicemia include meningitis, pneumonia and endocarditis (Jay 2000).

FACTORS IN THE EMERGENCE OF FOODBORNE DISEASE

Foodborne diseases caused by bacterial pathogens have a major public health impact. Several factors have been associated with the emergence of foodborne disease, including human demographics and behavior, technology and industry, international travel, and microbial adaptation (Altekruse 1998).

1. Changes in human demographics and behavior

Demographic changes occurring in industrialized nations have resulted in increased numbers of people becoming susceptible to foodborne infections. In the US, there is a growing population experiencing immune deficiency as a consequence of aging, HIV and chronic infections (Altekruse 1994). In 1995, an estimated one million people in the US were infected with HIV (Altekruse 1994). *Salmonella* and other foodborne pathogens are more likely to produce

more severe, recurrent, or persistent illnesses in this population. Reported cases of salmonellosis are higher in HIV-infected individuals than in the general population (Altekruse 1994).

Aging of population also contributes significantly in the increase of foodborne illnesses. During the 20th century, the median age of the US population has increased steadily, (USBC 1990) as the “baby boom” generation ages (USBC 1993). Increased susceptibility of the elderly to foodborne infections is reflected by the increased rate in hospitalizations and deaths due to *Salmonella enteritidis* outbreaks in nursing homes (Mishu 1994).

Changes in food-related behavior of consumers also have been recognized as a factor in the increasing rate of foodborne illness. Many individuals have increased their intake of fresh fruits and vegetables in response to health promotion efforts. For example, the National Cancer Institute recommends consuming five servings of fresh fruits and vegetables per day as part of a healthy lifestyle (Bowersox 1992). Because feces or soil may contaminate the surface of produce during growth, harvest and distribution, consumption of fresh produce can cause foodborne infections. Additionally, most fresh produce is likely to be consumed raw without undergoing processing that can inactivate pathogenic bacteria. Since 1990, foodborne outbreaks have been associated with a wide variety of produce including melons, lettuce, alfalfa sprouts, and tomatoes as well as fresh apple

cider and orange juice (Buchanan and Doyle 1997; Thayer and Raykowski 1999).

The shift toward food consumption outside the home is an important factor in the emergence of foodborne disease. In the US, the percentage of income spent on food eaten away from home has increased steadily (Manchester and Clauson 1995). Fast-food restaurants and salad bars were rare 40 years ago but are now primary locations for food consumption in today's fast-paced society (Manchester and Clauson 1995). Outbreaks occurring outside the household account for 80% of the total reported foodborne outbreaks (Bean et al. 1996). *E. coli* O157:H7 infection, for example, was first recognized in 1982 when two outbreaks in the US were associated with undercooked hamburger in a fast-food restaurant chain (Acheson and Gerald 1996).

2. Changes in industry and technology

The consolidation of food industry has a major impact on the emergence of foodborne disease. Large volumes of food products are normally distributed to customers from centralized suppliers. When mass-distributed products are contaminated, the cases of illnesses are scattered rather than localized (MacDonald 1985; Killalea 1996). In 1985, one particular outbreak of salmonellosis from a large Midwest milk processor was estimated to have resulted in approximately 250,000 illnesses (MacDonald 1985)

3. Changes in travel and commerce

International travel has increased dramatically during the 21st century. While 5 million international tourists travelled internationally in 1950, 937

million international travelers are projected in 2010 (WTO 2000). Travelers may become infected with foodborne pathogens that are uncommon in their nation of residence. Acquisition of such infections abroad may complicate diagnosis and treatment of the disease when they return home.

4. Microbial adaptation

In the US, *S. Typhimurium* DT 104 is a growing concern. Ninety percent of *S. Typhimurium* DT 104 isolates are resistant to ampicillin, amphenicol, streptomycin, sulphonamides, and tetracycline. Additional strains resistant to trimethoprim and ciprofloxacin have emerged in the United Kingdom during the 1990s (Altekruse 1998). Surveillance for human *S. Typhimurium* DT 104 infections in the UK indicates higher hospitalization and fatality rates with this organism compared to other *Salmonella* serotypes (Lee 1994; Altekrus 1998).

CONTAMINATION CONTROL IN FOOD PROCESSING

In an effort to reduce the incidence of foodborne illnesses, new regulations require all food processing plants to conduct regular microbial testing. A program known as Hazard Analysis and Critical Control Point, or HACCP, has been adopted by both the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) to identify and prevent biological, chemical and physical hazards in foods (Bernard 1997). HACCP programs are now required for processed food, seafood, and juice industries, as well as meat and poultry processing plants. FDA now is considering developing regulations that would establish HACCP as the food safety standard throughout other areas of

the food industry, including both domestic and imported food products. The system is not only practiced by numerous US food companies but also in most other developed countries.

HACCP involves seven principles (Bernard 1997):

1. **Analyze hazards.** Potential hazards associated with food are identified. The hazard could be biological, such as a microbe; chemical, such as a toxin or allergen; or physical, such as ground glass or metal fragments.
2. **Identify critical control points.** These are points in a food's production at which the potential hazard can be controlled, reduced or eliminated. Examples are cooking, cooling, packaging, and metal detection.
3. **Establish preventive measures with critical limits for each control point.** For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbe.
4. **Establish procedures to monitor the critical control points.** Such procedures would include determining how and by whom cooking time and temperature should be monitored.
5. **Establish corrective actions to be taken when monitoring shows that a critical limit has not been met.** For example, reprocessing or disposing of food if the minimum cooking temperature is not met.

6. Establish procedures to verify that the system is working properly.

For example, testing time-and-temperature recording devices to verify that a cooking unit is working properly.

7. Establish effective record keeping to document the HACCP system.

This would include records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potential problems.

New challenges to the US food supply have prompted FDA to consider adopting a HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. For example, between 1973 and 1988, bacteria not previously recognized as important causes of foodborne illness, such *E. coli* O157:H7, have become more widespread (Jay 2000).

CURRENT CONTROL METHODS AND DETECTION

This section examines current methods for microbial detection especially in the food industry. Microbial detection includes conventional plating methods, modern molecular and immunologic detections, and the application of biosensors. The parameters most often used to evaluate a method are sensitivity, specificity (Barbour 1997), detection time, and user-friendliness of the method.

Sensitivity characterizes the number or concentration of target organisms required for a positive result (Barbour 1997). Increasing the assay sensitivity will

increase the accuracy of the test. Specificity is generally measured by testing a group of bacterial strains within the target genus or species against a group of related non-target strains (Barbour 1997). Following enrichment of a food sample, $10^8 - 10^9$ bacteria per ml may be present in the enrichment broth with only a portion of this population comprising the target pathogen. Therefore, the challenge is to achieve a very low sensitivity without sacrificing the specificity of the detection method. Detection time is critical, especially in the food processing facilities. If the detection time is long, the contaminated food products may have already reached the consumers, which could in turn result in a very expensive product recall. Finally, it is advantageous for microbial detection methods to be relatively simple and easy to use. This feature will definitely expand the application of the detection method not only in research laboratories, but also in a small food industry that cannot afford to have an on-site laboratory.

CONVENTIONAL METHOD

Conventional plating is the most widely used method for determining the number of viable bacteria in a food product (Jay 2000). This method requires cultivation, isolation, morphological examination, and biochemical identification (Deshaonde 1996). This conventional method usually requires hands-on preparation and 24–48 hours of incubation time before suspected pathogens can be identified (Jay 2000). The advantages of this method in identifying viable cells are the accuracy and reproducibility of the results. These method however is laborious and time-consuming.

MOLECULAR AND GENETIC-BASED METHODS

Most genetic and molecular methods for pathogen detection are based on the formation of a nucleic acid hybrid. The nucleic acid hybridization is typically between DNA or RNA molecules present in the target organism and a diagnostic probe with DNA having a sequence complementary to the target pathogen (Barbour 1997). Typically, the molecular and genetic detection methods begin with cell lysis followed by DNA or RNA preparation for hybridization (Jay 2000). Some of the current molecular methods are nucleic acid probes and polymerase chain reaction (PCR) (Jay 2000). The advantages of using these methods are their high sensitivity. The drawbacks are the time and expertise required to perform the test (Barbour 1997; Jay 2000).

IMMUNOLOGIC DETECTION METHODS

Immunologic detection methods are analytical tests that use antibodies. These methods take advantage of the unique binding characteristics of the immunoglobulin molecule to specific antigens (Barbour 1997). A variety of techniques have been used to detect antigen-antibody reactions. The principal techniques presently in use are enzyme immunoassays, immunofluorescence, and radioimmunoassays (D'Souza 2001). In a typical enzyme immunoassay procedure, the antigen-antibody reaction is detected by the formation of a colored product from a colorless substrate. In an immunofluorescence technique, a reaction is indicated by emission of small quantities of light, which must generally be observed microscopically (Cahn 1993; D'Souza 2001).

Radioimmunoassays use radioactive labeled substances that detect antigen-antibody binding based on measured amounts of radioactivity (Cahn 1993). These immunologic methods are reliable but are slow and tedious microscopically (Cahn 1993; D'Souza 2001).

Recently, several types of electrical immunoassays have been developed. Such methods use electrical signals to measure the immune reaction. One strategy involves the use of antibody-labeled colloidal gold particles. Gold particles are used in immunodiagnostic tests where the results are determined optically by observing small amounts of light reflected as a result of the antigen-antibody reaction (Kim et al. 2000). Silver also has been used with or without gold in developing electrical immunoassay techniques (Brunelle 2001),

BIOSENSORS

Biosensors have emerged recently to provide rapid detection methods in addition to the current classical methods. A biosensor is an analytical device that integrate a biological sensing element with an electronic transducer (Turner 1998; Ivnitski et al. 1999). The general function of a biosensor is to convert biological events into quantifying electrical responses (Cahn 1993). Biosensors make use of a variety of transducers, such as electrical, electrochemical, optical, piezoelectric crystal, and acoustic wave. The sensing elements may be enzymes, antibodies, DNA, receptors, organelles, and microorganisms, as well as animal and plant cells.

Some of the major attributes of a biosensor technology are its specificity, sensitivity, reliability, portability, real time analysis, and simplicity of operation (D'Souza 2001).

Two types of biosensors will be described in detail as a comparison to the device investigated in the study. First to be described is the electronic nose (E-nose) developed by Alocilja (2000) at Michigan State University. The E-nose basically consists of several commercialized gas sensors coupled to an artificial neural network. The E-nose technology is cheap, requires less than 24 hours to complete detection, and is shown capable to differentiate *E. coli* O157:H7 from non-O157: H7 *E. coli* by generating a distinctive gas signature (Younts 1999). This technology is easy to use as no sample preparation or pre-enrichment is required. There are several limitations for this technology, however. One of the issues is related to the biosensor specificity. In mixed cultures, it is hard to distinguish the gas signatures between *E. coli* O157:H7 and other non-target bacteria (Younts 1999). The gas signature of other foodborne pathogens such as *S. Typhimurium*, also has not been established.

The second biosensor is the surface plasmon resonance (SPR) biosensor, which has gained popularity in the last couple of years. Meeusen (2000) has converted an off-the-shelf Spreeta™ sensor to a SPR biosensor for *S. Typhimurium* and *E. coli* O157:H7 detection in the pork production chain. The specificity of the SPR biosensor is higher than the E-nose as it utilizes a sandwich immunoassay technique. The detection limit of the biosensor for both

pathogens in pure culture is 10^7 CFU/ml. The SPR biosensor requires 30 minutes for detection after 5 1/2 hours of enrichment (Meeusen 2000).

ELECTRICAL IMMUNOASSAY BIOSENSOR

The focus of this study is to evaluate an electrical immunoassay-based biosensor (EI) as a microbial detection device. The EI biosensor uses antibodies as biological elements that are attached to an electrical transducer. Materials used in EI biosensors are: materials for electrodes and support, materials for immobilizing the biological sensing element, materials for outer membranes, the biological sensing element, and electrically active materials (Zhang 2000).

ELECTRODES AND SUPPORTING SUBSTRATE

Metal is commonly used to fabricate the electrodes and the supporting system. Metals such as platinum, silver, gold, and stainless steel have long been used for making the electrodes because of their excellent electrical and mechanical properties. These chemically inert materials possess low electrical resistivity and have very pure crystal structures that provide low residual currents and high signal-to-noise ratios (Ivnitski et al. 1999).

MATERIALS FOR OUTER MEMBRANES

Electrical immunoassay-based biosensors are usually covered with a thin membrane to control diffusion, reduce interference and to protect the sensing elements from the outer environment. Materials used for outer membranes

include glass (Kim et al. 2000), copper wafers (Sergeyeva et al. 1994), plastics, and semi-conductive platforms (Hatch et al. 1999).

BIOLOGICAL SENSING ELEMENTS

Improvements in biosensor design have been frequently directed at the incorporation of active biological molecules, including enzymes and mediators such as ferrocene and its derivatives, cofactors based on nicotinamide adenine dinucleotide (NADH^+ and NADP^+), catalysts, antibodies, and DNA (Ivnitski et al. 1999).

MATERIALS FOR IMMOBILIZING BIOLOGICAL ELEMENTS

Most materials used for immobilizing biological elements are multifunctional agents, such as glutaraldehyde and hexamethyl diisocyanate, which form crosslinks between the bio-catalytic species or proteins. The process called coreticulation creates complex matrices, which make multi-enzyme immobilization possible (Zhang 2000).

ELECTRICALLY ACTIVE MATERIALS

Materials used as electrical transducers in EI biosensors include silver (Brunelle 2001), gold, and organic conductive polymers (Kim et al. 2000). Organic conductive polymers, such as polyaniline, polypyrrole, polyacetylene, and polythiophene (Hoa 1992), offer several advantages in the development of EI biosensors. Organic conductive polymers provide an appropriate interaction

between the biological element and the electrodes. Therefore, the electrical connection between the sensing region and the electrodes is more efficient.

CONDUCTIVE POLYMERS

Plastic, under certain circumstances, can be made to behave very much like a metal. This is a discovery for which Alan J. Heeger, Alan G. Macdiarmid, and Hideki Shirakawa received the 2000 Nobel Prize in Chemistry with polyacetylene identified as the first conducting polymer (Skotheim 1987).

Plastics are long chain molecules, repeating themselves. In becoming electrically conductive, a polymer must imitate a metal, that is, its electrons need to be free to move rather than bind to atoms. Although, the polymer must contain alternating single and double bonds, called conjugated double bonds (Figure 1.1), to become electrically conductive, electrons must be either removed (oxidation), or inserted (reduction) into the polymer structure. This process is known as doping. In the case of polyaniline, the electrical conductivity of the polymer can be changed either by an acid/base treatment or electrochemical technique (Salaneck 1987).

There is a considerable number of biosensors based on conducting polymers utilizing different biological entities, such as antibodies, DNA, whole cells, enzymes and polyanions. The combination of the selectivity and sensitivity of the bio-molecule with the novel electrochemical properties of conducting polymers provides such a powerful bio-sensing device (Sadik 1995). A summary of conducting polymer-based biosensors is shown in Table 1.2. These

biosensors were shown to exhibit improved sensitivities and faster response times compared to the conventional methods (Sadik 1995). However, due to the complex processes involved in polymer formation, difficulties in obtaining reproducible polymer films have been reported (Barsch et al 1994; West et al 1993).

POLYANILINE

Polyaniline is a family of interesting materials due to its unusual transport, magnetic and optical properties (Sergeyeva et al. 1996). It is the first conducting polymer to be commercialized and now has applications ranging from batteries (Osama et al. 1992) to biosensors (Kim et al. 2000; Killard et al. 2000). In the biosensor development, polyaniline has been used as a label for an enzyme-based method and immunoassay system. The latter method is more preferred as the enzyme-based method requires labeling procedure and several washing steps to separate bound and unbound molecules (Killard et al. 2000). The immunoassay system, with its characteristic of high affinity and excellent selectivity, is ideal for a rapid detection device (Paek et al. 1999). Killard et al. (2000) have focused on an electrochemical immunosensor based on polyaniline in analyzing the pesticide atrazine for environmental monitoring. The detection limit claimed is as low as 1 part per billion. Sergeyeva et al. (1996) have examined the potential of using polyaniline in immunosensor analysis by using interdigitated electrodes as the platform. Kim et al. (2000), on the other hand, has integrated polyaniline with

Table 1.1 Examples of conducting polymer-based biosensors

Biosensor	Analyte	Immobilized biomolecule	Reference
PPy/AHSA	HSA	AHSA	(John et al. 1991)
PPy/ATHAU	THAU	ATHAU	(Sadik et al. 1994)
PPy/P-Cres	Phenol	Phenol	(Barnett et al. 1994)
PANI/GOx	Glucose	Glucose	(Tamiya et al. 1989)
PANI/cell	Whole cell	IgG	(Kim et al. 2000)
PANI/	Attrazine	IgG	(Killard et al. 2000)
PPy/Urease	Urea	Urease	(Adeloju et al. 1993)
PPy/Dopa	Dopamine	Whole cell	(Deshpande and Hall 1990)

IgG=Immunoglobulin, HSA=Human serum albumin, AHSA= anti- human serum albumin, THAU=Thaumatococcus, ATHAU=anti-Thaumatococcus, P-Cres=P-Cresol Bovine Serum albumin conjugate, GOx =Glucose oxidase, Dopa= Dopamine, Ppy=Polypyrrole, PANI= polyaniline

gold colloids to provide a more sensitive colorimetric immunosensor for microbial detection.

Different forms of polyaniline exist based on the oxidation levels. Polyaniline is believed to be composed of the basic chemical units shown in base form (Figure 1.1) (Macdiarmid et al. 1987; Yen Wei 1989).

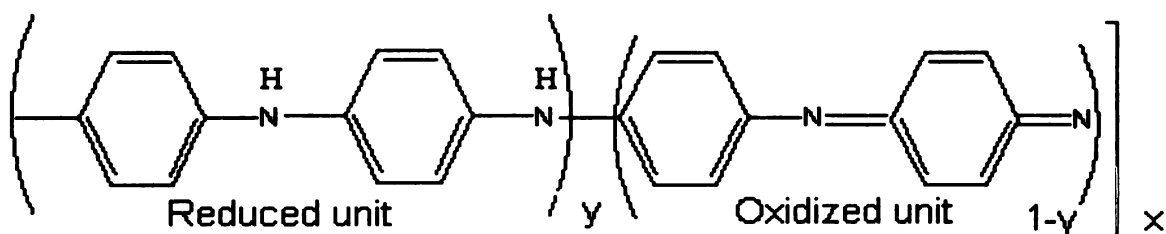


Figure 1.1 Polyaniline structure

When $0 < y < 1$, they are called poly (paraphenyleneamineimines) in which the oxidation state of the polymer increases continuously with decreasing value of y . The fully reduced form, also known as leucoemeraldine, has a y value equal to 1. The most oxidized form (y equal to 0) is called pernigraniline. Finally, the intermediate form, with y equal to 0.5, is called emeraldine. The terms leucoemeraldine, emeraldine, and pernigraniline refer to the different oxidation states of polyaniline (Macdiarmid et al. 1987). Between the three types of polyaniline, emeraldine is the most stable polyaniline in air (Sergeyeva et al. 1994).

The emeraldine base has an equal number of reduced $[-(C_6H_4)-(N(H)-(C_6H_4)-(N(H)-]$ and oxidized $[-(C_6H_4)-N=(C_6H_4)=N-]$ repeat units (Figure 1.2). The electrical properties of the emeraldine base can be interchanged from insulating

(conductance (σ) $< 10^{-10} \text{ ohm}^{-1}\text{cm}^{-1}$) to conducting ($\sigma \cong 5\text{-}10 \text{ ohm}^{-1}\text{cm}^{-1}$) through protonation (Epstein 1987; Macdiarmid et al. 1987). Upon treatment with acid of varying pH levels, protons (H^+) are added to a fraction of the unprotonated nitrogen sites (Macdiarmid et al. 1987). In this case, the number of electrons on the polymer backbone is held constant while varying the number of protons (Epstein 1987). For example, treatment of emeraldine base with aqueous 1M HCl resulted in nearly complete protonation of a conducting emeraldine salt (Macdiarmid et al. 1987) as shown in Figure 1.3. Protonation of the emeraldine base with aqueous acid brings about an increased in conductivity $10 \text{ ohm}^{-1}\text{cm}^{-1}$ (Macdiarmid et al. 1987).

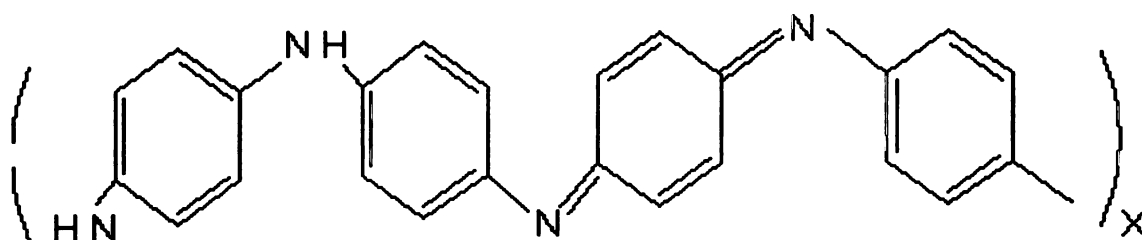


Figure 1.2 Emeraldine base

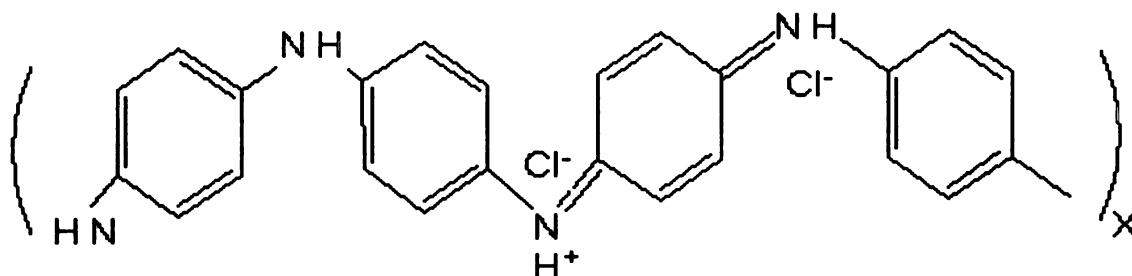


Figure 1.3 Emeraldine base treated with HCl

Synthesized polyaniline is green in its hydrochloride salt form and has an electrical conductivity of $10 \text{ ohm}^{-1}\text{cm}^{-1}$ (Yen Wei 1989). The polymer in the base form is blue, and is insoluble in water and other common organic solvents such as benzene. Polyaniline, however, at room temperature, has a solubility rate of 20-50% in N, N-dimethylformamide (DMF), depending on the reaction time or aniline polymerization (Yen Wei 1989).

Polyaniline was chosen as the electrical transducer in the proposed EI biosensor due to its excellent stability in liquid form, promising electronic properties (Syed and Dinesan 1991), and strong bio-molecular interactions (Imisides 1996).

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS FOR THE FABRICATION OF THE EI BIOSENSOR (CHAPTER 3)

2.1 Construction materials for the biosensor

Experiments were done to identify the most appropriate membranes for each of the following sections of the biosensor: sample application, conjugate, detection and absorption membranes. The membranes were chosen based on different criteria. The sample application membrane must provide easy and quick flow of the sample with no or minimal interference. The conjugate membrane must be able to adsorb the polyaniline-conjugated antibody and yet allow easy flow when dissolved. The capture membrane must have pores of sufficient size to allow non-target antigens to flow through and provide good adsorption properties for the immobilized antibody. The absorption membrane must be able to hold the liquid sample.

Two types of electrodes were tested: copper and silver. Selection was based on the electrical properties and ease of handling during fabrication.

Two kinds of platform materials were evaluated: glass and etched copper wafers. These materials were chosen based on previous studies (Sergeyeva et al. 1996; Kim et al. 2000).

2.2 Bacteria isolates and culturing

A characterized strain of *E. coli* O157:H7 (ATCC #43895) was obtained from the Biosystems Engineering collection, Michigan State University. A 10- μ l loop of the frozen culture was transferred in a 10-ml of nutrient broth (Difco Laboratories, Detroit, MI) and incubated for 24 hours at 37°. Two additional transfers were made with the last sample used as the stock culture. All experiments were performed in a certified Biological Safety Level II laboratory. Cultures were serially diluted in 0.1% of peptone water to generate a varying concentration of the bacteria.

2.3 Antibody preparation

A lyophilized affinity purified polyclonal antibody for *E. coli* O157:H7 was purchased from Kirkegaard & Perry Laboratories Inc. (Gaithersburg, MD) and stored at 4°C until rehydrated. Antibodies were rehydrated with phosphate buffer (Sigma-Aldrich, MO) according to the manufacturer's instruction.

2.4 Conjugate membrane preparation

A water-soluble polyaniline was synthesized by following a standard procedure of oxidative polymerization of aniline monomer (Sigma-Aldrich, MO) in the presence of ammonium persulfate (Sigma-Aldrich, MO) (Sergeyeva et al. 1994). Details of the polymerization process can be found in Appendix A. A mixture of the rehydrated antibody and the synthesized polyaniline was allowed to react for 30 minutes. The antibody-polyaniline conjugate was centrifuged at 13,000 rotation per minute (rpm) for three minutes using 0.1M tris buffer (Sigma-Aldrich, MO) as the blocking reagent. The conjugated antibody was diluted in

0.1M LiCl (Sigma-Aldrich, MO) and stored at 4°C. When ready, 10µl of the conjugated antibody was applied to the conjugate pad and allowed to dry.

2.5 Absorption and application pads preparation

To prepare the absorption and application pads, cellulose membranes were washed three times with distilled water to remove dirt and surface residues. The membranes were allowed to dry and stored inside a petri dish to maintain a clean surface.

2.6 Capture pad preparation

Affinity purified antibodies were immobilized on the nitrocellulose (NC) membrane as follows. First, the NC membrane was saturated with 10% (v/v) methanol in water for 45 min and allowed to dry. The membrane was then saturated with 0.5% (v/v) glutaraldehyde for one hour. After drying, 0.5mg/ml of antibodies was pipetted onto the membrane and incubated at 37°C for one hour. Inactivation of residual functional groups and blocking were done simultaneously by incubating the membrane with 0.1M tris buffer, pH 7.6, containing 0.1% Tween-20 for 45 min. Finally, the membrane was allowed to air-dry.

2.7 Varying antibody concentrations

The optimum concentrations of antibody used on the capture and conjugate pads were determined in this study. The following concentrations of *E. coli* O157: H7 antibodies were prepared in sterile phosphate buffer (pH 7.0): 50µg/ml, 150µg/ml, and 500µg/ml. Permutation of the various concentrations was applied to the conjugate and capture pads. An *E. coli* O157:H7 concentration of about 10¹ CFU/ml was prepared by serial dilution process and confirmed by

plating on CHROM Agar (Microbiology, France). Biosensors prepared with various antibody concentrations (Table 2.1) were tested with 0.1 ml of the *E. coli* O157:H7 solution. After the solution reached the absorption pad, the capture pad was removed from the biosensor and washed with 0.1 ml of distilled water. To identify the optimum antibody concentration, the washed solution was surface plated on the CHROM agar. A total of nine sets of biosensors were prepared and tested as mentioned above. Results were assessed for differences in the number of organisms captured using varying concentrations of antibodies on the conjugate and capture pads.

Table 2.1. Combinations of antibody concentrations ($\mu\text{g/ml}$) used for preparing the conjugate and capture pads.

Conjugate pad ($\mu\text{g/ml}$)	Capture pad ($\mu\text{g/ml}$)
500	500
500	150
500	50
150	150
150	500
150	50
50	50
50	500
50	150

2.8 Varying polyaniline concentrations

A total of five sets of biosensors were prepared containing five different concentrations of polyaniline were used on the conjugate area: 0.001g/ml, 0.01g/ml, 0.1g/ml, 1g/ml, and 10g/ml. The polyaniline was conjugated with the optimum antibody concentration found in the previous experiments. The distance

between electrodes for all biosensors was fixed at 0.5 mm. These biosensors were tested with approximately 10^4 CFU/ml of *E. coli* O157:H7. Data were compared to find the correlation between polyaniline concentration and the signal generated.

2.9 Varying the distance between the electrodes

The next experiment determined the optimum distance between the electrodes. The optimum antibody and polyaniline concentrations found in the previous experiments were used. Various electrode gap-sizes (0.5 mm, 1mm, 2mm, 3mm, 4mm, and 5mm) were fabricated on the capture pads. Results were compared to see which distance would yield the strongest signal using approximately 10^4 CFU/ml of *E. coli* O157:H7.

2.10 Signal measurement

The prepared immunosensor was connected to a multimeter and computer for signal measurement. To begin detection, 0.1 ml of the bacteria serial dilution was pipetted onto the application pad. Measurements were taken at 2-minute interval over a period of 6 minutes. The generated signal was captured using a multimeter (BK multimeter, MA) in the form of resistance. The biosensor was calibrated using a sterile diluted nutrient broth (Difco, Mi).

2.11 Confirmation

CHROM agar (Microbiology, France) was used to plate 0.1 ml of the last dilution of the bacterial culture. After 24 hours of incubation at 37°C, suspected colonies were identified and counted according to the manufacturer's instruction.

The estimated population of the original culture was determined by back-counting the 8-folds dilution.

2.12 Signal processing

A drop in resistance was determined from the difference between the resistance output of the blank and the inoculated samples. The difference in measured resistance (resistance drop) was the reduction in resistance due to the electron transfer facilitated by the polyaniline-labeled antibody between the electrodes.

2.13 Statistical analysis

Statistical analysis was conducted using a single factor analysis of variance (ANOVA). All biosensors were assumed to have the same physical properties. For the purpose of this study, the biosensor responses were considered statistically different when the P-value was less than 0.05 (95% confidence interval).

MATERIALS AND METHODS FOR THE SENSITIVITY AND SPECIFICITY ANALYSIS (CHAPTER 4)

The biosensor consisted of two parts: the immunosensor and the electronic data collection system. The immunosensor contained four different regions: application, conjugate, detection, and absorption. The system was constructed as shown in Figure 2.1: The cellulose membrane was used for sample application and absorption pads, the fiber glass membrane was for the conjugate pad and the nitrocellulose (NC) membrane for the capture pad. Silver

electrodes were fabricated on the NC membrane to electrically connect the immunosensor with the electronic data acquisition system consisting of a copper wafer and an ohmmeter linked to a computer. The four regions were prepared as previously described (Sections 2.3, 2.4, 2.5, and 2.6)

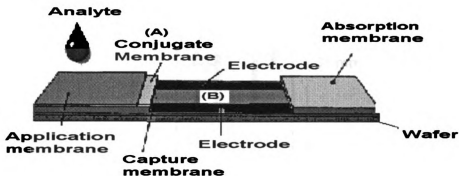


Figure 2.1. Schematic diagram of the immunosensor. Conjugate pad for polyaniline-labeled antibody adsorption (A). Capture pad with two silver electrodes (B). Gap between electrodes is the site for antibody immobilization.

2.14 Construction of the immunosensor

All prepared membranes were arranged in the order mentioned in Figure 2.1 and attached onto an etched copper plate (Figure 2.2) using double-sided tape. *Salmonella*, *E. coli* O157:H7 and *E. coli* biosensors (to be referred to as Sal, EHEC, EC biosensors, respectively) were prepared with antibodies specific to each organism and stored at 4°C.

2.15 Construction of the electronic data collection system

The etched copper wafer served as the platform for connecting the immunosensor to the ohmmeter. The copper wafer was designed as in Figure 2.2 and etched using ferric chloride according to the manufacturer's instruction

(Radio Shack, MI). The ohmmeter, which was linked to a computer, was used for measuring and recording the resistance generated by the biosensor.

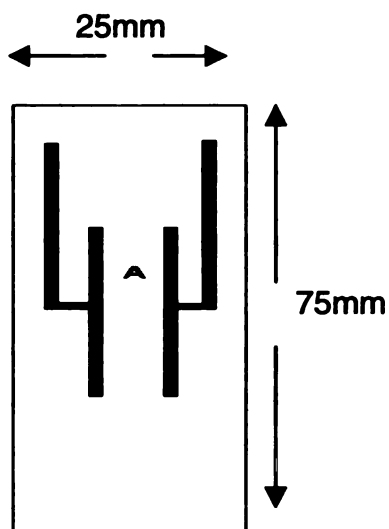


Figure 2.2 An etched copper wafer. The white and black parts indicate the non-conductive and conductive regions, respectively. Region A has a distance of 5 mm.

2.16 Bacteria isolates and culturing

Characterized strains of *E. coli* O157:H7 (ATCC #35150 and ATCC #43895), non-pathogenic *E. coli* (ATCC #25922), *Salmonella* Typhimurium (ATCC #14028) and *Salmomella* Thompson (ATCC # 8391) were obtained from the Biosystems Engineering collection, Michigan State University. All strains were cultured as previously described in section 2.2.

2.17 Mixed culture preparation

A mixed culture was used to determine the specificity and sensitivity of the biosensor in the presence of non-target bacteria. A mixture of five organisms, *E. coli* O157:H7 isolates (ATCC #35150 and ATCC #43895), non-pathogenic *E.*

coli, *S. Typhimurium* and *S. Thompson*, was prepared by culturing each organism in 10 ml of nutrient broth at 37°C for 24 hours. Cells from 1 ml of each microbial culture were harvested by centrifugation at 2000 rpm for 15 minutes. The resulting pellets were suspended in 2 ml of peptone water. All five suspended mixtures were combined to make a 10 ml solution. The bacterial suspension was serially diluted in 0.1% peptone water. A duplicate bacterial suspension was plated on Chrom, McConkey, and Brilliant Green agars and incubated at 37°C to determine the number of organism present.

2.18 Sensitivity testing

Sensitivity testing was conducted to determine the detection limit of the biosensors. A series of 10-fold serial dilutions was prepared from the stock culture using 0.1% peptone water. The *E. coli* O157:H7 dilution was tested with the EHEC biosensor, the non-pathogenic *E. coli* dilution with the EC biosensor, and the *Salmonella* dilution with the Sal biosensor. The mixed culture dilutions were used to test all biosensors. Serial dilutions were also plated on Chrom, McConkey, and Brilliant Green agars and incubated at 37°C to determine the number of organism present.

2.19 Specificity testing

Specificity testing was performed to determine the ability of the biosensors to select for the target organisms. The Sal, EHEC, and EC biosensors were tested with sample diluents accordingly:

1. Non-inoculated samples (negative control)
2. Serial dilution of the target organism that the antibody was not specific for:

- *E. coli* O157:H7 dilutions using Sal and EC biosensors
- *S. Typhimurium* dilutions using EC and EHEC biosensors
- *S. Thompson* dilutions using EC and EHEC biosensors
- Non- pathogenic *E. coli* dilutions using Sal and EHEC biosensors

3. Serial dilution of the target organism for which the biosensor was prepared for:

- *E. coli* O157:H7 dilutions using EHEC biosensors
- *S. Typhimurium* dilutions using Sal biosensors
- *S. Thompson* dilutions using Sal biosensors
- Non-pathogenic *E. coli* dilutions using EC biosensors

2.20 Plate count

Numbers of the target organisms were determine by surface plating 0.1 ml of *E. coli* O157:H7, *Salmonella* species, and the non-pathogenic *E. coli* dilutions on CHROM (Microbiology, France), Brilliant Green, and McConkey agar (Difco Laboratories, Detroit, MI) respectively. After incubating at 37°C for 24 hours, suspected organisms were confirmed following a standard protocol in the Bacteriological Analytical Manual of the Food and Drug Administration (BAM) and manufacturer's instructions.

Table 2.2: Interpretation of results on differential media

Differential Medium	Target organism	Colony color
McConkey Agar	<i>E. coli</i>	Dark purple
CHROM Agar	<i>E. coli</i> O157	Mauve
Brilliant Green Agar	<i>Salmonella</i> species except for <i>S. Typhi</i>	Pinkish white or red

MATERIALS AND METHODS FOR THE PERFORMANCE OF THE BIOSENSOR IN EVALUATING FRESH PRODUCE SAMPLES (CHAPTER 5)

In this chapter, the biosensor was prepared and analyzed according to the previous section (Materials and Methods for Chapter 4).

2.21 Mixed culture preparation

Bacterial cultures containing a mixture of three organisms were prepared and analyzed to determine the specificity of the biosensors in the presence of non-target bacteria. *E. coli* O157:H7, non-pathogenic *E. coli*, and *S. Typhimurium* were cultured and mixed as previously described in section 2.17.

2.22 Preparation of produce sample

Alfalfa sprouts, iceberg lettuce and strawberries were purchased from a local supermarket. Produce visibly free from mechanical damage and decay were used. Twenty-five grams of each sample were weighed and placed in a petri dish under a biosafety hood.

For inoculation, 1ml of fully-grown bacteria or the mixed culture was applied to the surface of each produce sample. The samples were left for 45 minutes under the biosafety hood to allow for cell attachment. The samples were then washed to discard any unattached cells. The samples were processed to recover the cells. For alfalfa sprout and strawberries, the cell recovery process was performed by hand-rubbing the sprouts in 100 ml of 0.1% peptone water for 2 minutes. For iceberg lettuce, a stomacher was used.

After the cell recovery process, the solution was filtered using a bio-filter membrane (BIOPath, FL) to concentrate the cells. Then, the membrane filter was aseptically transferred to a test tube containing 10 ml of 0.1% peptone water. The test tube was vortexed thoroughly for 4 minutes^{*} to release the trapped cells from the membrane. The concentrated cells were serially diluted with 0.1% peptone water, hereinafter referred to as bacterial dilution. For example, an *E. coli* O157:H7 dilution would mean the serial dilution of a produce sample inoculated with the *E. coli* O17:H7 bacteria.

2.23 Sensitivity testing

Sensitivity testing using the inoculated produce samples was done following similar procedure as previously described for pure culture (section 2.18).

2.24 Specificity testing

Specificity testing using the inoculated produce samples was also done as previously described for pure culture (section 2.19).

^{*} Experiment leading to this finding is available in Appendix F

CHAPTER 3

FABRICATION OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR

This chapter reports the results of the fabrication of the electrical immunoassay (EI) biosensor. The objective of this chapter was to identify the initial architecture of the biosensor by evaluating the construction materials, the best concentrations of polyaniline and antibodies, and the distance between electrodes. These factors were then used to assess the performance of the biosensor in detecting a model pathogen.

RESULTS

3.1 Biosensor construction

The biosensor consisted of two parts: The immunosensor (Figure 3.1) and the electronic data acquisition system (Figure 3.2). The immunosensor was comprised of four different pads (Figure 3.3): sample application, conjugate, capture, and absorption. A cellulose membrane was chosen for both the sample application and absorption pads. A fiber glass membrane grade G6 was for the conjugate pad, and a nitrocellulose membrane (NC), with a flow rate of 160 sec per 4 cm, was determined to be the best pad for the capture section.

The electrodes were fabricated on the NC membrane to electrically connect the immunosensor to the electronic data acquisition system. Silver paste was the easiest to handle and thus was used in subsequent development of the biosensor.

When attaching the immunosensor on the platform, an etched copper wafer generated more stable signal and was used as the platform for the later experiments.

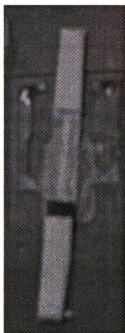


Figure 3.1 Immunosensor

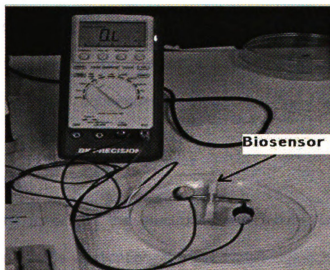


Figure 3.2. Electrical immunoassay biosensor

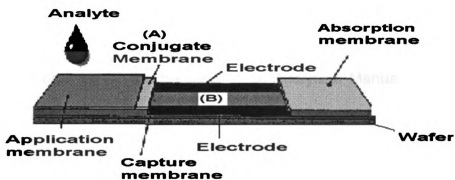


Figure 3.3. Schematic diagram of the immunosensor. Conjugate pad for polyaniline-labeled antibody adsorption (A). Capture pad with two silver electrodes (B). Gap between electrodes is the site for antibody immobilization.

Detection concept

To begin the detection process, 0.1 ml of solution containing the antigen was added onto the sample application pad. By capillary action, the solution flowed to the conjugate pad and dissolved the polyaniline-labeled antibody. The antibody-antigen reaction occurred and formed a complex. This complex moved to the capture pad containing the immobilized antibody. A second antibody-antigen reaction occurred and formed a sandwich immune complex. The unbound materials were subsequently transported by capillary flow to the absorption pad.

Signal Generation

The resistance across the electrodes was infinite prior to the sample application. After sample application, the generated signal fluctuated while the sample flowed by capillary action to the absorption pad. Dispersion time from the sample pad to the absorption pad was less than one minute. In the presence of

sufficient antigens, the signal stabilized and was recorded. The presence of microorganisms was confirmed by the standard plate count method according to the protocol outlined by the Bacteriological Analytical Manual of the Food and Drug Administration.

Optimum antibody concentration

Table 3.1 shows the result of the antibody concentration experiment. Cell concentrations ranging from 8.8×10^1 to 9.3×10^1 CFU/ml of *E. coli* O157:H7 were used to test nine different sets of biosensors. After plating the capture pad along with the washed solution, the maximum number of bacterial cells ranging from 1.0×10^1 to 7.3×10^1 CFU/ml were found. The antibody concentration of 500µg/ml on the capture pad and 150µg/ml on the conjugate pad were resulted in the highest ratio between the number of captured cells and the actual cell concentration tested (Table 3.1).

Optimum polyaniline concentration

An average of 8.7×10^4 CFU/ml of cells were added onto the application pad of five sets of biosensors as detailed in Chapter 2. Figure 3.4 shows that the biosensor generated the most signal when 1g/ml of the soluble polyaniline was used. An excess amount of polyaniline (i.e. 10g/ml in Figure 3.4) however, had an adverse effect, generating a lower signal than the 1g/ml concentration.

Table 3.1. Bacterial counts on the capture pad with varying antibody concentration ($\mu\text{g/ml}$) on the conjugate and capture pads.

Antibody concentrations used on the conjugate pad ($\mu\text{g/ml}$)	Antibody concentrations used on the capture pad ($\mu\text{g/ml}$)	Plate counts of sample (CFU/ml)	Bacterial count on the capture pad (CFU/ml)			Ratio (Captured/Actual)			Average Ratio
			Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
500	500	88	30	30	40	0.34	0.34	0.45	0.38
500	150	90	0	10	20	0.00	0.11	0.22	0.11
500	50	89	0	0	0	0.00	0.00	0.00	0.00
150	150	88	40	30	20	0.45	0.34	0.23	0.34
150	50	93	0	10	10	0.00	0.11	0.11	0.07
50	50	89	0	0	0	0.00	0.00	0.00	0.00
50	500	91	20	20	30	0.22	0.22	0.33	0.26
50	150	91	10	10	0	0.11	0.11	0.00	0.07

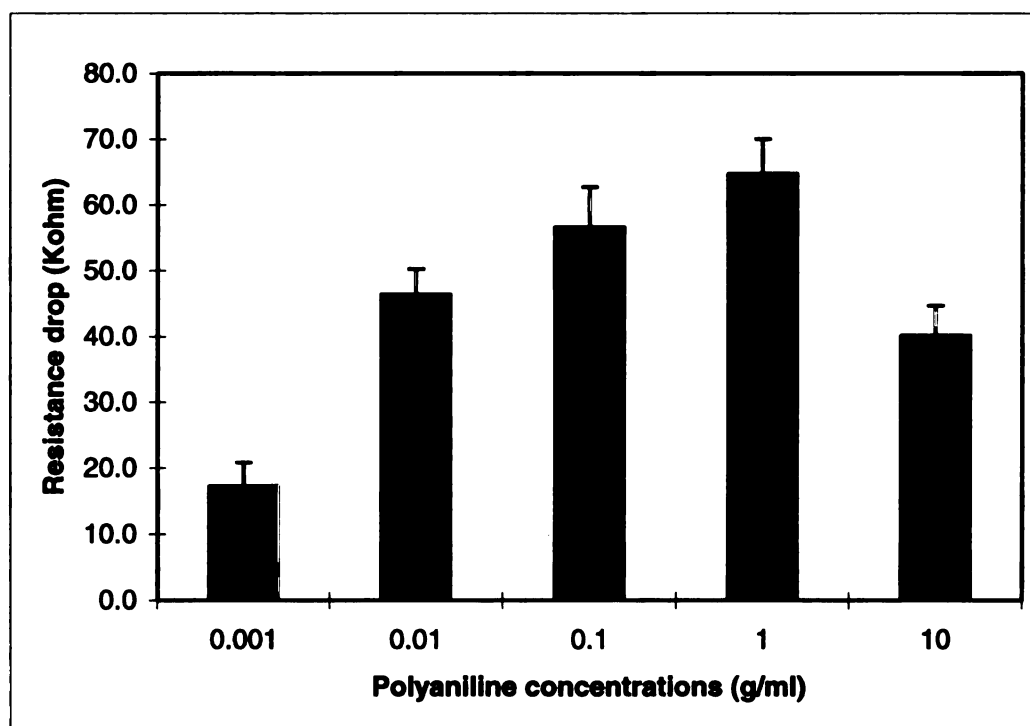


Figure 3.4. Resistance drop of the various polyaniline concentrations.

Optimum distance between electrodes

A silver pen (Chemtronics, GA) was used to fabricate the varying distances between electrodes on the capture pad. An average cell concentration of 7.6×10^4 CFU/ml was used. Overall, the 0.5-mm distance yielded the most signal (drop in resistance) (Figure 3.5).

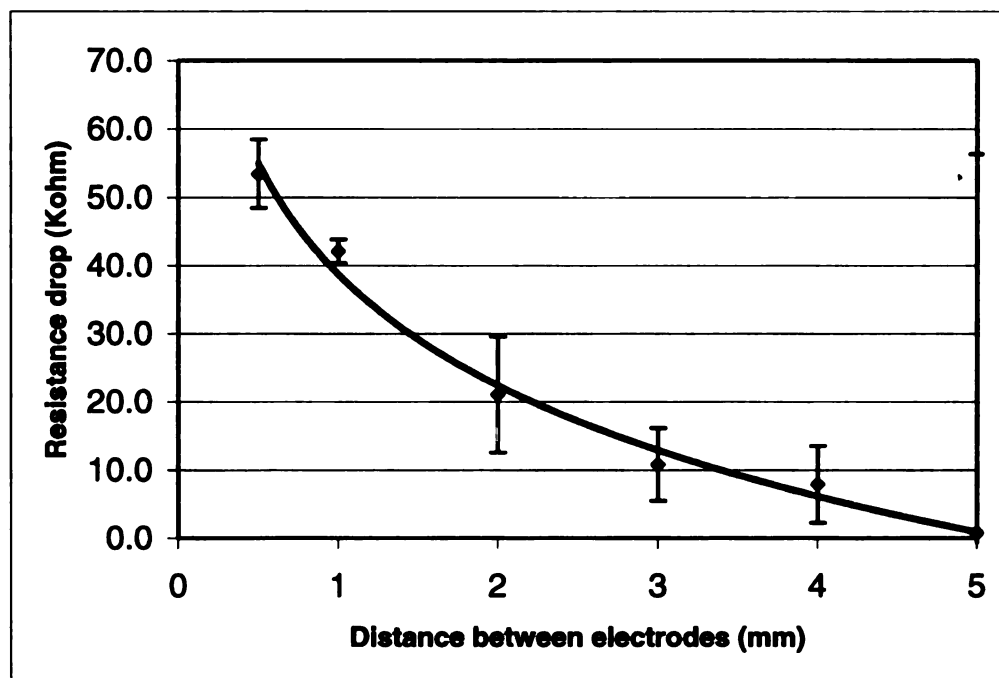


Figure 3.5. Resistance drop of varying distances between electrodes

DISCUSSION

The sensitivity of an immunoassay system is usually defined as the minimum concentration of analytes that can produce a signal distinguishable from the background (Chard 1987). Therefore, the difference between the noise and signal is used as a representation of the biosensor sensitivity. To attain a sensitive biosensor, the best architecture of the biosensor and the optimal assay conditions were evaluated through literature review and experimentations.

The biosensor allowed the liquid sample to laterally flow from one pad to another, through capillary action. The biosensor performance depended on the type of membrane chosen. For application and absorption pads, cellulose membranes were chosen for their excellent thickness and strength properties when exposed to the sample volume. The nitrocellulose membrane was chosen for the capture pad based on several factors: NC membranes bind with protein by electrostatic force, which is stronger than other types of membranes (Millipore 1999). Furthermore, the NC membrane is completely neutral and its binding property is independent from the pH of the immobilization solution. This allows high antibody binding capacity on the membrane irrespective of the pH of the sample applied. The fiber glass membrane was used for the conjugate pad because of its low non-specific binding and consistent flow characteristics (Millipore 1999). Silver was chosen as the material for fabricating the electrode because of its high chemical inertness and low resistivity. This material also has a pure crystal structure that provides low residual currents and a high signal to noise ratio (Ivnitski et al. 1999).

An experiment was performed to determine the optimum antibody concentrations to be used on the conjugate and capture pads. Antibody concentrations ranging from 50-500 $\mu\text{g/ml}$ were typically used in other immunoassay techniques (Watts 1994; Elkind 1998; Kim et al. 2000). It was hypothesized that the highest antibody concentration used in the experiment (i.e. 500 $\mu\text{g/ml}$) for both pads would result in the highest capture ratio (the ratio between the number of captured cells and the actual cell concentration tested).

Figure 3.7 graphically shows the capture ratios for varying antibody concentrations on the conjugate and capture pads. Capture pads with an antibody concentration of 500 μ g/ml resulted in the highest capture ratio regardless of the antibody concentration used on the conjugate pads. Of all antibody concentrations used on the conjugate pads, a concentration of 150 μ g/ml resulted in the highest capture ratio. Therefore, the optimum concentration of antibodies was found to be 500 μ g/ml and 150 μ g/ml for the capture and conjugate pads, respectively.

A water-soluble polyaniline was prepared by oxidative polymerization of aniline monomer (Sergeyeva et al. 1994). The synthesized polyaniline was green in the acidic medium and turned dark blue after treatment with ammonia.

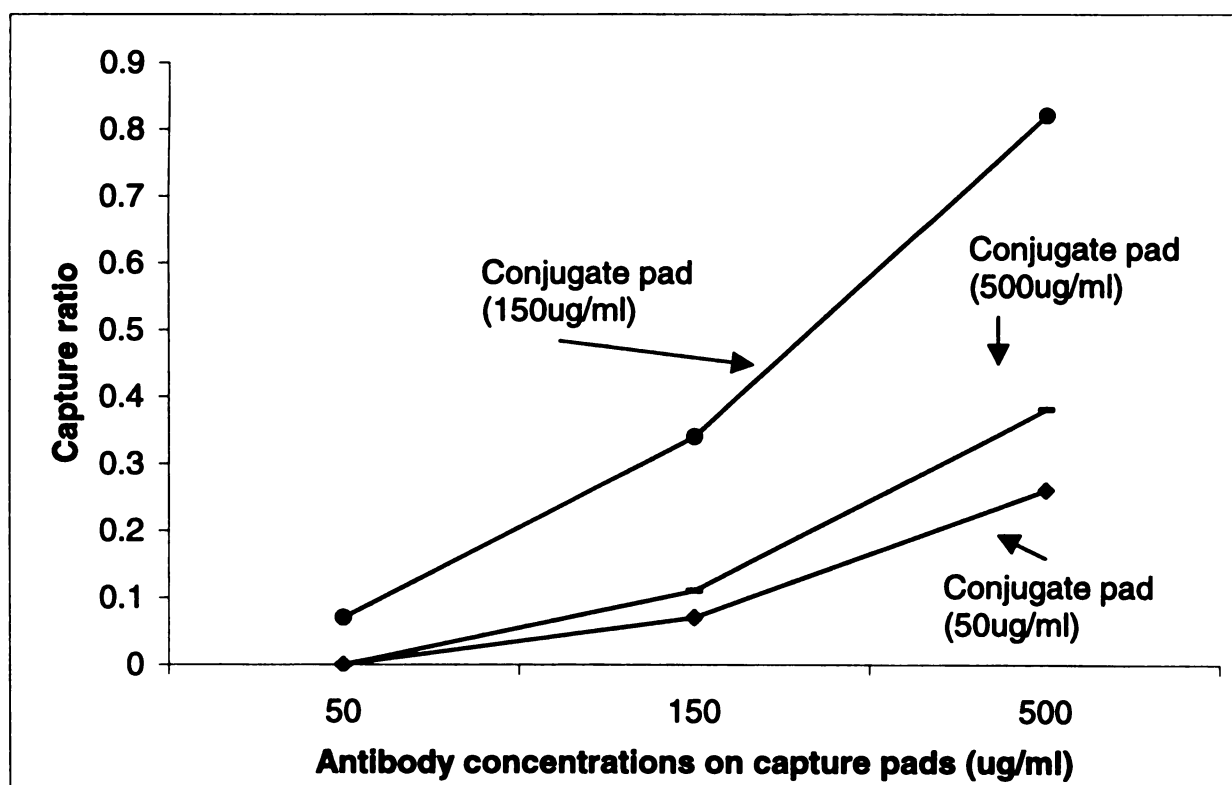


Figure 3.7 Result of the antibody experiments.

These changes correspond to the transformation of emeraldine salt into the base form (Sergeyeva et al. 1994). An increase in signal (resistance drop) with increasing concentration of polyaniline (Figure 3.4) appears to be reasonable, as more polyaniline can form more contact with neighboring polyaniline chains. A decreased signal at the concentration of 10g/ml (Figure 3.4) may be explained by the over-packing of polyaniline molecules which cause some steric hindrance in the interaction between the polyaniline and the antibody molecules (Sergeyeva et al. 1994). A polyaniline concentration of 1g/ml yielded the most reduction in resistance. A scanning electron microscope (SEM) image of polyaniline is shown in Figure 3.8.

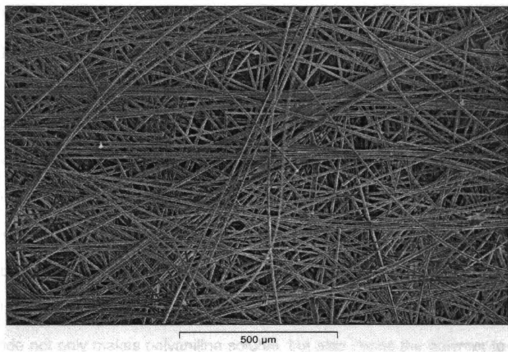


Figure 3.8. Scanning electron microscope image of polyaniline

After the antigen reached the capture pad and formed a sandwich complex, the polyaniline acted as a bridge between the electrodes. The polymer

structure extended out to bridge adjacent cells for signal generation (Kim et al. 2000). This electrical event initially caused a fluctuation in the generated signal which ranged from a few seconds to one minute depending on the level of antigen attached to the antibody. The resistance signal produced was stable for approximately 10 minutes, but started to increase significantly thereafter. Chiang (1986) suggested that drying of the membrane decreased the ineffectiveness of polyaniline, resulting in the increased resistance.

In order to improve the sensitivity of the biosensor, the optimum distance between the electrodes was investigated. The shortest distance of 0.5 mm resulted in the greatest reduction in resistance. Shortening the average distance between the electrodes enhanced the electron transfer by hopping (Heller 1990; Ryder 1997). Half a millimeter was the shortest distance that could be fabricated using the silver pen.

The chemical composition and temperature of the assay were optimized to reduce background noise and increase the performance of the biosensor. The antibody-antigen reaction is optimal at pH 7.0 (Paek et al. 1999). Hence, tris buffer was used to maintain a neutral pH. Numerous immunoassays have used tris buffer not only to sustain the neutral environment for antibody-antigen binding and but also to reduce the background noise (Sergeyeva et al. 1996; Paek et al. 1999; Kim et al. 2000). A previous study shows that an inorganic salt, lithium chloride not only makes polyaniline soluble, but also dopes the polymer to make it more conductive (Angelopoulos 1997). The conductivity however, is highly

affected by temperature (Quadrat 1998). In this study, the assays were maintained at room temperature (25°C).

CHAPTER 4

SENSITIVITY AND SPECIFICITY ANALYSIS OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR

RESULTS

Sensitivity

Figures 4.1-4.4 show the signal responses (resistance drop) of the EI biosensor to varying analyte concentrations. The biosensor generated a parabolic curve with two parts. The resistance drop was proportional to the analyte concentration between 10^1 and 10^4 or 10^5 or 10^6 CFU/ml, and inversely proportional to the higher analyte concentrations. Table 4.1 shows the responses of Sal, EHEC, and EC biosensors to various bacterial samples. ANOVA revealed that the detection limits for the Sal, EHEC, and EC biosensors were $8.3 \pm 0.6 \times 10^1$, $7.9 \pm 0.3 \times 10^1$, and $7.5 \pm 0.3 \times 10^1$ CFU/ml, respectively. The data also showed that signals generated from one concentration level were not significantly different than the next level of cell concentration. The signal (resistance drop) from samples inoculated with 10^5 CFU/ml of *S. Thompson* was not statistically different than that of 10^6 CFU/ml (Table 4.1).

Specificity

Table 4.1 also shows the biosensor specificity to target organisms. The Sal biosensors responded only to *Salmonella* cultures, and not to the *E. coli* cultures. The EHEC biosensors responded only to the *E. coli* O157:H7 culture and not to others. The EC biosensors were found responding to both *E. coli*

O157:H7 and non-pathogenic *E. coli*, but not to *Salmonella* cultures. All biosensors also were shown responding to the mixed sample inoculated with all target organisms. The signal generated by the biosensor to the non-target bacteria was not significantly different from the blank (Table 4.1).

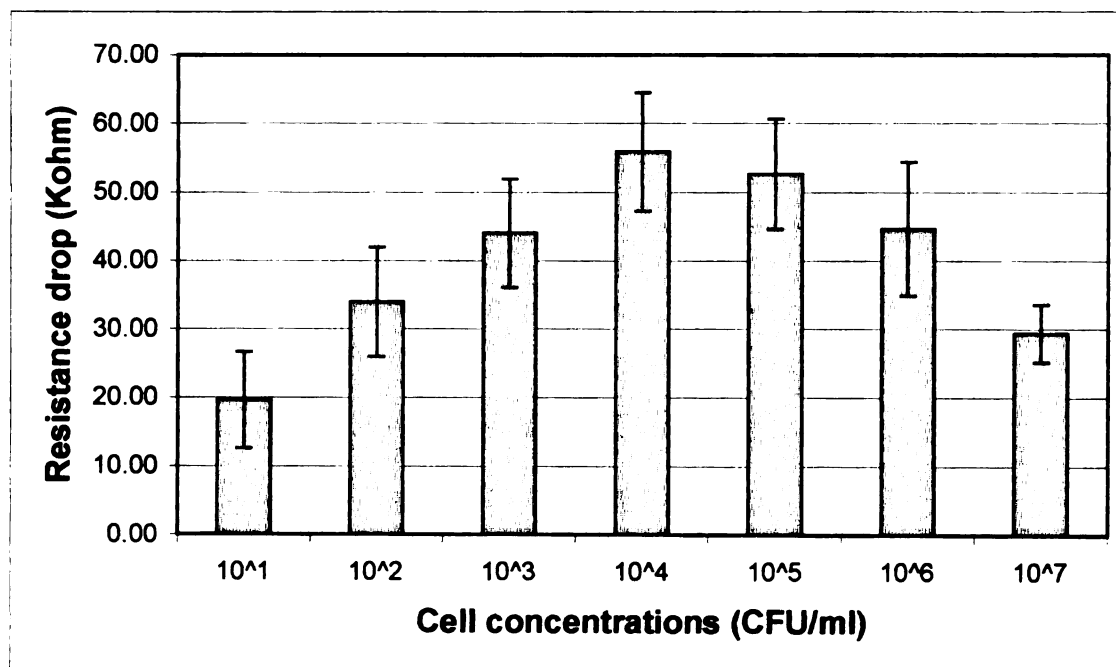


Figure 4.1. Resistance drop of the Sal biosensor tested with *S. Typhimurium*.

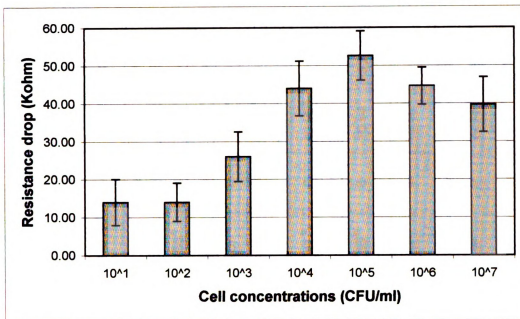


Figure 4.2. Resistance drop of the EHEC biosensor tested with *E. coli* O157:H7 (ATCC 35150).

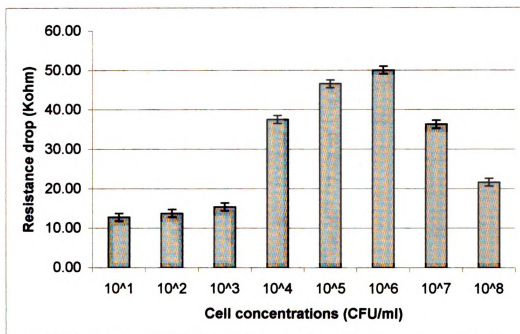


Figure 4.3 Resistance drop of the EC biosensor tested with non-pathogenic *E. coli*

Table 4.1. Resistance drop of the biosensors tested with various bacterial samples.

BIOSENSOR TYPES	INOCULA											
	S. Typhimurium		S. Thompson		E. coli O157:H7 (ATCC #35150)		E. coli O157:H7 (ATCC #43895)		Non O157:H7 E. coli		Mixed sample	
	Signal	Plate count	Signal	Plate count	Signal	Plate count	Signal	Plate count	Signal	Plate count	Signal	Plate count
Sal Biosensor	19.63 ^a	*8 X 10 ¹	15.89 ^a	7.9 X 10 ¹	1.12 ^b	0	1.56 ^b	0	-6.50 ^b	0	15.11 ^a	*9 X 10 ¹
	33.93 ^c	*8 X 10 ²	29.53 ^c	*7.9 X 10 ²	-1.53 ^b	0	-1.18 ^b	0	-6.97 ^b	0	28.63 ^c	*9 X 10 ²
	44.03 ^d	*8 X 10 ³	42.76 ^d	*7.9 X 10 ³	-8.32 ^b	0	-1.37 ^b	0	-0.07 ^b	0	41.77 ^d	*9 X 10 ³
	55.89 ^e	*8 X 10 ⁴	55.64 ^e	*7.9 X 10 ⁴	-4.68 ^b	0	-2.34 ^b	0	-5.16 ^b	0	53.54 ^e	*9 X 10 ⁴
	52.66 ^f	*8 X 10 ⁵	51.80 ^f	*7.9 X 10 ⁵	-1.37 ^b	0	-2.52 ^b	0	2.03 ^b	0	51.67 ^f	*9 X 10 ⁵
	44.63 ^g	*8 X 10 ⁶	43.77 ^g	*7.9 X 10 ⁶	5.20 ^b	0	-0.57 ^b	0	-1.41 ^b	0	42.96 ^g	*9 X 10 ⁶
	29.39 ^h	*8 X 10 ⁷	36.09 ^g	*7.9 X 10 ⁷	-4.83 ^b	0	-3.16 ^b	0	-1.99 ^b	0	36.53 ^h	*9 X 10 ⁷
	13.82 ⁱ	*8 X 10 ⁸	17.77 ⁱ	*7.9 X 10 ⁸	-5.27 ^b	0	-3.10 ^b	0	-2.38 ^b	0	16.94 ⁱ	*9 X 10 ⁸
EHEC Biosensor	0.12 ^b	0	-5.31	0	14.01 ^a	7.7 X 10 ¹	17.90 ^a	7.8 X 10 ¹	-4.48 ^b	0	14.80 ^a	8.3 X 10 ¹
	1.30 ^b	0	-9.15	0	14.06 ^{c,a}	*7.7 X 10 ²	17.94 ^{c,a}	*7.8 X 10 ²	-5.62 ^b	0	13.89 ^{c,a}	*8.3 X 10 ²
	5.56 ^b	0	1.95	0	26.01 ^d	*7.7 X 10 ³	29.90 ^d	*7.8 X 10 ³	-1.84 ^b	0	24.75 ^d	*8.3 X 10 ³
	2.24 ^b	0	-6.00	0	43.98 ^e	*7.7 X 10 ⁴	44.64 ^e	*7.8 X 10 ⁴	-3.16 ^b	0	40.46 ^e	*8.3 X 10 ⁴
	-3.99 ^b	0	1.79	0	52.58 ^f	*7.7 X 10 ⁵	56.47 ^f	*7.8 X 10 ⁵	4.50 ^b	0	53.09 ^f	*8.3 X 10 ⁵
	4.31 ^b	0	0.36	0	44.60 ^{g,i}	*7.7 X 10 ⁶	48.49 ^{g,i}	*7.8 X 10 ⁶	-1.91 ^b	0	44.22 ^{g,i}	*8.3 X 10 ⁶

	-0.36 ^b	0	-1.44	0	39.64 ^{h,g}	^{7.7} X 10 ⁷	43.53 ^{h,g}	^{7.8} X 10 ⁷	-1.46 ^b	0	39.47 ^{h,g}	^{8.3} X 10 ⁷
	0.29 ^b	0	-1.35	0	29.72 ⁱ	^{7.7} X 10 ⁶	33.61 ⁱ	^{7.8} X 10 ⁶	2.21 ^b	0	30.20 ⁱ	^{8.3} X 10 ⁸
EC Biosensor	0.12 ^b	0	-5.93	0	14.01 ^a	^{7.8} X 10 ¹	17.06 ^a	^{7.6} X 10 ¹	12.78 ^a	^{7.7} X 10 ¹	12.78 ^a	^{7.2} X 10 ¹
	2.41 ^b	0	-8.89	0	14.05 ^c	^{7.8} X 10 ²	20.79 ^{c,a}	^{7.6} X 10 ²	13.77 ^{c,a}	^{7.7} X 10 ²	13.77 ^{c,a}	^{7.2} X 10 ²
	5.56 ^b	0	-5.05	0	26.01 ^{d,c}	^{7.8} X 10 ³	26.55 ^d	^{7.6} X 10 ³	15.40 ^{d,e}	^{7.7} X 10 ³	15.40 ^{d,c}	^{7.2} X 10 ³
	2.99 ^b	0	-11.38	0	43.98 ^{e,d}	^{7.8} X 10 ⁴	43.66 ^e	^{7.6} X 10 ⁴	37.53 ^e	^{7.7} X 10 ⁴	37.53 ^e	^{7.2} X 10 ⁴
	0.12 ^b	0	0.81	0	52.58 ^{f,e}	^{7.8} X 10 ⁵	50.78 ^{f,e}	^{7.6} X 10 ⁵	46.57 ^f	^{7.7} X 10 ⁵	46.57 ^{f,e}	^{7.2} X 10 ⁵
	4.78 ^b	0	-5.5	0	44.60 ^{g,f}	^{7.8} X 10 ⁶	43.24 ^{g,f}	^{7.6} X 10 ⁶	50.01 ^{g,f}	^{7.7} X 10 ⁶	50.01 ^{g,f}	^{7.2} X 10 ⁶
	-0.86 ^b	0	-4.39	0	39.64 ^h	^{7.8} X 10 ⁷	37.16 ^{h,g}	^{7.6} X 10 ⁷	36.31 ^h	^{7.7} X 10 ⁷	36.31 ^h	^{7.2} X 10 ⁷
	2.12 ^b	0	-6.49	0	29.72 ^{i,h}	^{7.8} X 10 ⁶	27.74 ^{a,i}	^{7.6} X 10 ⁶	21.62 ⁱ	^{7.7} X 10 ⁶	21.62 ⁱ	^{7.2} X 10 ⁶

-Average signal (resistance drop) with different superscripts are significantly different at 95% confidence level (p<0.05).

-The signal for the calibrated blank was equal to 0^b

* Estimated bacterial populations by back-counting every step from the last dilution.

Performance in the mixed culture

The data in Table 4.1 also show the results of the biosensors in samples inoculated with a mixture of five organisms. The responses of Sal, EHEC, and EC biosensors tested with the mixed culture were statistically different than the blank. In this experiment, the response pattern was found similar to Figures 4.1-4.3 (Figure 4.4).

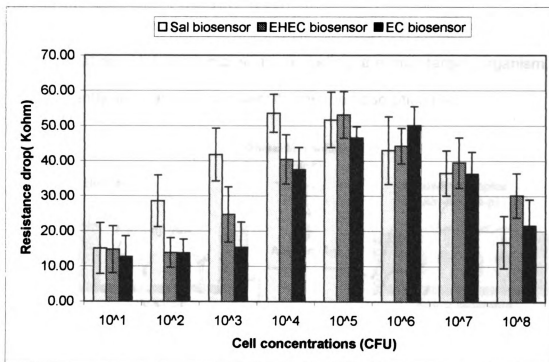


Figure 4.4. Resistance drop of the three biosensors tested with a mixture of *E. coli* O157:H7, *Salmonella* species, and non-pathogenic *E. coli*.

DISCUSSION

Immediately after sample application, the solution carrying the antigen move to the conjugate pad and dissolved the polyaniline-labeled antibody (Ab-P) to form an antibody-antigen complex (Figure 4.5B). This complex moved to the capture pad containing the immobilized antibody. A second antibody-antigen reaction occurred and formed a sandwich (Figure 4.5C). The polyaniline in the sandwich formed a molecular wire and bridges the two silver electrodes by polymerization. Unbound materials, including the non-target organism, were subsequently separated by capillary flow to the absorption pad.

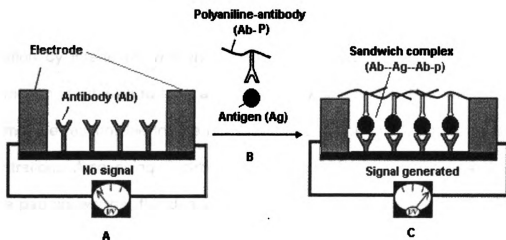


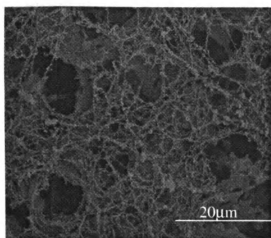
Figure 4.5. Cross-section of a capture pad before (A) and after (C) analyte application.

Antibodies used in the Sal biosensor were specific to a wide range of *Salmonella* species as listed in the manufacturer's manual. Similarly, the antibodies used in the EC biosensor were specific to all *E. coli* serotypes. The antibodies used in the EHEC biosensor, however, were only specific to *E. coli*

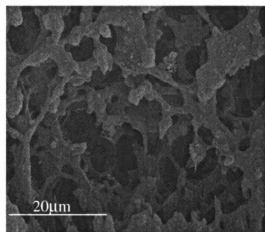
O157:H7. Data showed the EI biosensor was highly specific to select target antigen even in samples with multiple organisms (Table 4.1).

The biosensor response however, shows a parabolic curve instead of a linear relationship (Figures 4.1-4.4). At concentrations above 10^4 , 10^5 or 10^6 CFU/ml, signal was shown to decrease. This phenomenon may be explained by the nature of sandwich immunoassays which rely on the interaction between the labeled antibodies attached to the antigen and the limited number of antibodies fixed on the capture site (Hatch et al. 1999). At high concentrations (i.e. above 10^4 , 10^5 or 10^6 CFU/ml), the binding site may be over occupied with the antigen, thus obstructing the charge transfer within the conductive polymer structure. Cavalier (1995) also indicated that protein molecules served as a barrier against conduction by interfering with the electron hopping between electrodes. This phenomenon is referred to here as an over-crowding effect. The over-crowding effect may be responsible for the low signal (resistance drop) generated at high concentrations. A scanning electron microscope (SEM) image (Figure 4.6) of the capture pad shows that the density of bound cells varied between 10^1 (Figure 4.6B), 10^5 (Figure 4.6C), and 10^7 CFU/ml (Figure 4.6D).

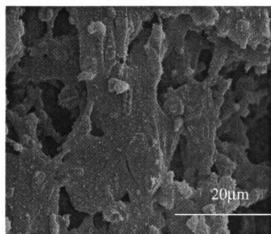
The detection limit of the Sal, EHEC, and EC biosensors was $8.3 \pm 0.6 \times 10^1$, $7.9 \pm 0.3 \times 10^1$, and $7.5 \pm 0.3 \times 10^1$ CFU/ml, respectively. Statistical results, however show that the biosensor is not yet suitable for quantitative analysis as the signal generated from one level of bacterial concentration to another was not statistically different.



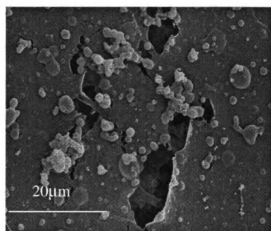
(A)



(B)



(C)



(D)

Figure 4.6. SEM images of the capture pads. (A) Before sample application. (B) After applying with 10^1 CFU/ml (C) 10^5 CFU/ml, and (D) 10^7 CFU/ml of bacterial cultures.

CHAPTER 5

PERFORMANCE OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR IN FRESH PRODUCE SAMPLES

The purpose of this study was to assess the feasibility of the EI biosensor to detect *E. coli* O157:H7, non-pathogenic *E. coli*, and *Salmonella* Typhimurium in the selected produce samples.

RESULTS

Biosensor sensitivity assays

Tables 5.1-5.3 show the results of the biosensor sensitivity experiments. The resistance drop for each test was calculated. An average from the three replicates and a standard deviation were also determined. For most of the samples tested, the drop in resistance was proportional to the analyte concentration from 10^1 to 10^4 or 10^5 CFU/ml (Figures 5.1-5.3). Tables 5.1-5.3 show the responses of the Sal, EHEC, and EC biosensors to varying inocula. Table 5.1 shows the detection limit of the Sal, EHEC, and EC biosensors in lettuce samples inoculated with the target bacteria were $8.4 \pm 0.2 \times 10^1$, $7.3 \pm 0.06 \times 10^1$, and $8.9 \pm 0.1 \times 10^1$ CFU/ml, respectively. Signals generated from the EC and EHEC biosensors to bacteria cell concentration ranging from 5 to 6.3×10^1 CFU/ml were not statistically different than the blank (highlighted in Table 5.1). The detection limits of the Sal, EHEC, and EC biosensors for the inoculated sprout samples were $7.8 \pm 0.2 \times 10^1$, $7.8 \pm 0.1 \times 10^1$, and $8.1 \pm 0.4 \times 10^1$ CFU/ml,

Table 5.1. Average signal (resistance drop) of biosensors used in lettuce samples inoculated with varying inocula.

Biosensor	Inoculum									
	S. Typhimurium			E. coli O157:H7			Non-pathogenic E. coli			Mixed culture
	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)
Sal Biosensors	21.6 ^a	8.2 X 10 ¹	-3.3 ^b		0	1.00 ^b		0	20.5 ^a	8.5 X 10 ¹
	32.6 ^{c,a}	*8.2 X 10 ²	-1.3 ^b		0	-1.53 ^b		0	30.7 ^c	*8.5 X 10 ²
	38.2 ^{d,c}	*8.2 X 10 ³	1.1 ^b		0	5.30 ^b		0	28.4 ^{d,c}	*8.5 X 10 ³
	42.9 ^{a,d}	*8.2 X 10 ⁴	-10.3 ^b		0	3.90 ^b		0	23.8 ^{a,d}	*8.5 X 10 ⁴
	52.1 ^f	*8.2 X 10 ⁵	1.4 ^b		0	10.43 ^b		0	22.5 ^{f,e}	*8.5 X 10 ⁵
	34.5 ^g	*8.2 X 10 ⁶	-1.7 ^b		0	4.30 ^b		0	15.6 ^g	*8.5 X 10 ⁶
	-2.08 ^b	0				2.67 ^b		0	18.5 ^a	7.3 X 10 ¹
EHEC Biosensors	-4.64 ^b	0	37.3 ^a		*5 X 10 ²	-1.10 ^b		0	28.6 ^c	*7.3 X 10 ²
	-6.87 ^b	0	56.1 ^c		*5 X 10 ³	2.10 ^b		0	30.1 ^{d,c}	*7.3 X 10 ³
	-3.98 ^b	0	48.4 ^d		*5 X 10 ⁴	-7.50 ^b		0	29.0 ^{a,d}	*7.3 X 10 ⁴
	-0.39 ^b	0	47.2 ^{a,d}		*5 X 10 ⁵	0.97 ^b		0	26.5 ^{f,e}	*7.3 X 10 ⁵
	-5.47 ^b	0	42.1 ^{f,e}		*5 X 10 ⁶	2.37 ^b		0	22.0 ^g	*7.3 X 10 ⁶
	-2.71 ^b	0							18.5 ^a	8.9 X 10 ¹
	-7.64 ^b	0	37.3 ^a		*6 X 10 ²	47.70 ^a		*6.3 X 10 ²	26.0 ^c	*8.9 X 10 ²
EC Biosensors	1.72 ^b	0	56.1 ^c		*6 X 10 ³	59.10 ^c		*6.3 X 10 ³	30.1 ^{d,c}	*8.9 X 10 ³
	3.43 ^b	0	48.4 ^d		*6 X 10 ⁴	51.00 ^d		*6.3 X 10 ⁴	30.2 ^{a,d}	*8.9 X 10 ⁴
	5.61 ^b	0	33.1 ^{a,d}		*6 X 10 ⁵	49.37 ^{a,d}		*6.3 X 10 ⁵	27.0 ^{f,e}	*8.9 X 10 ⁵
	-2.03 ^b	0	28.8 ^{f,e}		*6 X 10 ⁶	46.23 ^{f,e}		*6.3 X 10 ⁶	19.5 ^{g,f}	*8.9 X 10 ⁶

-Average signals (resistance drop) with different superscripts are significantly different at 95% confidence level (p<0.05).

-The signal for the calibrated blank was equal to 0^b.

*Estimated bacterial populations by back-counting every step from the last dilution.

Table 5.2. Average signal (resistance drop) of biosensors used in alfalfa sprout samples inoculated with varying inocula.

Biosensor	Inoculum								
	S. Typhimurium			E. coli O157:H7			Non-pathogenic E. coli		
	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)	Mixed culture
Sal Biosensors	18.16 ^a	8 X 10 ¹	-2.71 ^b	0	-5.47 ^b	0	21.3 ^a	7.6 X 10 ¹	
	24.52 ^{c,a}	*8 X 10 ²	-4.83 ^b	0	-4.82 ^b	0	28.0 ^{c,a}	*7.6 X 10 ²	
	26.88 ^{d,c}	*8 X 10 ³	2.33 ^b	0	-0.20 ^b	0	25.6 ^{d,c}	*7.6 X 10 ³	
	33.68 ^{e,d}	*8 X 10 ⁴	4.41 ^b	0	-9.29 ^b	0	25.1 ^{e,c}	*7.6 X 10 ⁴	
	33.72 ^{f,e}	*8 X 10 ⁵	5.52 ^b	0	0.66 ^b	0	21.6 ^{f,e}	*7.6 X 10 ⁵	
	27.43 ^{g,f}	*8 X 10 ⁶	-1.42 ^b	0	-3.71 ^b	0	13.9 ^g	*7.6 X 10 ⁶	
EHEC Biosensors	-4.28 ^b	0	12.78 ^a	7.7 X 10 ¹	-2.71 ^b	0	36.1 ^a	7.8 X 10 ¹	
	-4.74 ^b	0	13.77 ^{c,a}	*7.7 X 10 ²	-7.64 ^b	0	43.0 ^c	*7.8 X 10 ²	
	1.04 ^b	0	15.40 ^{d,c}	*7.7 X 10 ³	1.72 ^b	0	46.6 ^{d,c}	*7.8 X 10 ³	
	-2.93 ^b	0	37.53 ^e	*7.7 X 10 ⁴	3.43 ^b	0	39.7 ^e	*7.8 X 10 ⁴	
	4.26 ^b	0	46.57 ^{f,e}	*7.7 X 10 ⁵	5.61 ^b	0	36.0 ^f	*7.8 X 10 ⁵	
	-0.30 ^b	0	50.01 ^{g,f}	*7.7 X 10 ⁶	-2.03 ^b	0	31.2 ^{g,f}	*7.8 X 10 ⁶	
EC Biosensors	-5.39 ^b	0	15.89 ^a	7.8 X 10 ¹	14.0 ^a	8.2 X 10 ¹	29.4 ^a	8.9 X 10 ¹	
	-5.86 ^b	0	29.53 ^c	*7.8 X 10 ²	14.1 ^{c,a}	*8.2 X 10 ²	36.8 ^{c,a}	*8.9 X 10 ²	
	1.04 ^b	0	42.76 ^d	*7.8 X 10 ³	26.1 ^d	*8.2 X 10 ³	39.1 ^{d,c}	*8.9 X 10 ³	
	-4.04 ^b	0	55.64 ^e	*7.8 X 10 ⁴	44.0 ^e	*8.2 X 10 ⁴	35.1 ^{e,d}	*8.9 X 10 ⁴	
	3.14 ^b	0	51.80 ^{f,e}	*7.8 X 10 ⁵	53.0 ^f	*8.2 X 10 ⁵	28.3 ^f	*8.9 X 10 ⁵	
	-1.41 ^b	0	43.77 ^g	*7.8 X 10 ⁶	44.6 ^{g,f}	*8.2 X 10 ⁶	22.4 ^g	*8.9 X 10 ⁶	

-Average signals (resistance drop) with different superscripts are significantly different at 95% confidence level (p<0.05).

-The signal for the calibrated blank was equal to 0^b

*Estimated bacterial populations by back-counting every step from the last dilution.

Table 5.3. Average signal (resistance drop) of biosensors used in strawberry samples inoculated with varying inocula.

Biosensor	Inoculum							
	S. Typhimurium		E. coli O157:H7		Non-pathogenic E. coli		Mixed culture	
	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)	Signal (Kohm)	Plate count (CFU/ml)
Sal Biosensors	24.21 ^a	8.3 X 10 ¹	-2.71 ^b	0	1.84 ^b	0	22.3 ^a	7 X 10 ¹
	26.82 ^{c,a}	*8.3 X 10 ²	-7.22 ^b	0	-3.51 ^b	0	28.7 ^{c,a}	*7 X 10 ²
	26.09 ^{d,c}	*8.3 X 10 ³	4.38 ^b	0	2.37 ^b	0	27.4 ^{d,c}	*7 X 10 ³
	26.82 ^{e,d}	*8.3 X 10 ⁴	4.10 ^b	0	1.72 ^b	0	27.0 ^{e,d}	*7 X 10 ⁴
	25.34 ^{f,e}	*8.3 X 10 ⁵	5.04 ^b	0	2.37 ^b	0	23.6 ^{f,e}	*7 X 10 ⁵
	21.68 ^g	*8.3 X 10 ⁶	0.62 ^b	0	0.17 ^b	0	16.2 ^g	*7 X 10 ⁶
EHEC Biosensors	-2.11 ^b	0	12.32 ^a	8.1 X 10 ¹	0.27 ^b	0	28.5 ^a	8.3 X 10 ¹
	-6.31 ^b	0	13.10 ^{c,a}	*8.1 X 10 ²	1.23 ^b	0	35.7 ^{c,a}	*8.3 X 10 ²
	-6.21 ^b	0	16.91 ^{d,c}	*8.1 X 10 ³	3.62 ^b	0	37.2 ^{d,c}	*8.3 X 10 ³
	-0.49 ^b	0	38.93 ^a	*8.1 X 10 ⁴	3.86 ^b	0	32.0 ^{e,c}	*8.3 X 10 ⁴
	-4.69 ^b	0	47.41 ^f	*8.1 X 10 ⁵	0.39 ^b	0	24.7 ^f	*8.3 X 10 ⁵
	-3.33 ^b	0	49.89 ^{g,f}	*8.1 X 10 ⁶	0.66 ^b	0	19.4 ^{g,f}	*8.3 X 10 ⁶
EC Biosensors	-2.42 ^b	0	19.83 ^a	8.2 X 10 ¹	13.72 ^a	7.9 X 10 ¹	23.5 ^a	8.9 X 10 ¹
	-6.93 ^b	0	32.50 ^c	*8.2 X 10 ²	15.12 ^{c,a}	*7.9 X 10 ²	30.9 ^c	*8.9 X 10 ²
	4.67 ^b	0	46.58 ^d	*8.2 X 10 ³	24.90 ^d	*7.9 X 10 ³	33.2 ^{d,c}	*8.9 X 10 ³
	4.39 ^b	0	58.21 ^e	*8.2 X 10 ⁴	43.31 ^e	*7.9 X 10 ⁴	28.5 ^e	*8.9 X 10 ⁴
	5.33 ^b	0	56.40 ^{f,e}	*8.2 X 10 ⁵	52.24 ^f	*7.9 X 10 ⁵	23.6 ^f	*8.9 X 10 ⁵
	0.91 ^b	0	47.87 ^g	*8.2 X 10 ⁶	44.38 ^{g,f}	*7.9 X 10 ⁶	20.9 ^{g,f}	*8.9 X 10 ⁶

-Average signals (resistance drop) with different superscripts are significantly different at 95% confidence level (p<0.05).

-The signal for the calibrated blank was equal to 0^b

*Estimated bacterial populations by back-counting every step from the last dilution.

respectively (Table 5.2). As for the strawberry samples, the detection limits for the Sal, EHEC, and EC biosensors were $7.8 \pm 0.4 \times 10^1$, $8.2 \pm 0.07 \times 10^1$, and $8.3 \pm 0.4 \times 10^1$ CFU/ml, respectively (Table 5.3).

Biosensor specificity assays

Tables 5.1, 5.2, and 5.3 also show the biosensor specificity to the target organisms. The Sal biosensors responded only to *S. Typhimurium* and not to *E. coli*. The EHEC biosensors responded only to *E. coli* O157:H7 and not to other organisms. The EC biosensor responded to both *E. coli* and *E. coli* O157:H7 but not to *S. Typhimurium*. All biosensors also were shown to respond to the mixed sample inoculated with all three-target organisms. The resistance drop from the non-target bacteria was not significantly different from the blank.

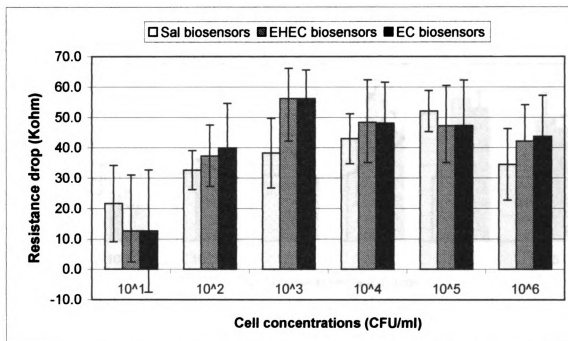


Figure 5.1. Resistance drop of the biosensors used in lettuce samples inoculated independently with *E. coli* O157: H7, non-pathogenic *E. coli* and *S. Typhimurium*

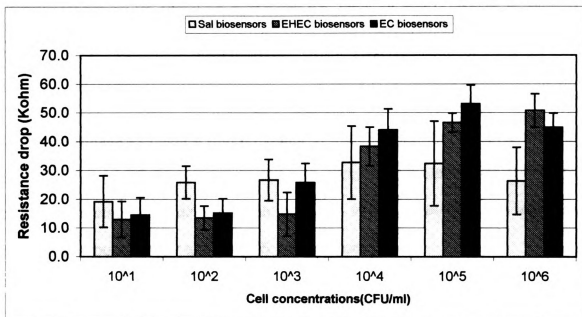


Figure 5.2. Resistance drop of the biosensors used in sprout samples inoculated independently with *E. coli* O157: H7, non-pathogenic *E. coli* and *S. Typhimurium*

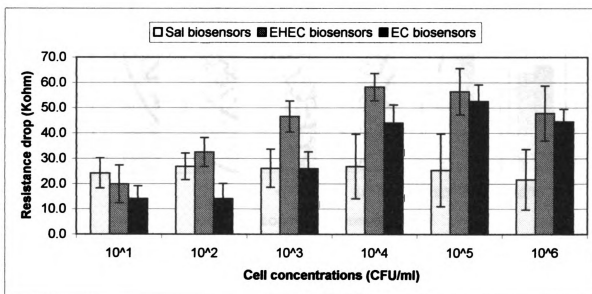


Figure 5.3. Resistance drop of the biosensors used in strawberry samples inoculated independently with *E. coli* O157: H7, non-pathogenic *E. coli* and *S. Typhimurium*.

PERFORMANCE IN PRODUCE SAMPLES INOCULATED WITH A MIXED CULTURE

The data in Tables 5.1-5.3 also show the results of the biosensors in produce samples inoculated with a mixture of three organisms. The responses of Sal, EHEC, and EC biosensors tested with samples inoculated with the mixed culture were statistically different than the blank. In this experiment, the response pattern was found similar to Figures 5.1-5.3 (Figure 5.4).

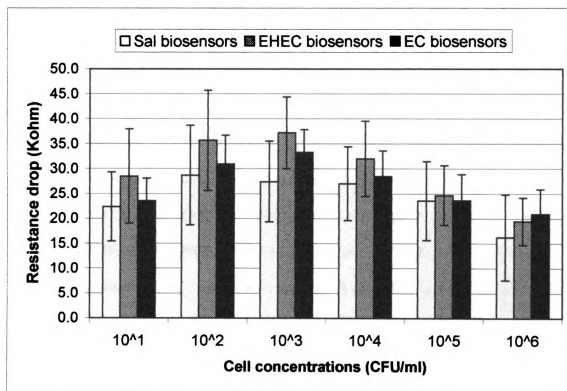


Figure 5.4. Resistance drop of the biosensors used in strawberry samples inoculated with a mixture of *E. coli* O157: H7, non-pathogenic *E. coli* and *S. Typhimurium*. Figures for the rest of the samples are available in Appendix E.

DISCUSSION

The biosensor used the method of lateral flow technique to enable the liquid sample to move from one membrane to another through capillary action. The biosensor also utilized the immunoassay technique, in which its specificity depends on the unique binding characteristics of the immunoglobulin molecule (Barbour 1997).

Each sensitivity test displays a similar pattern of signal response to varying bacterial concentration when plotted on a semi-log scale. Instead of being linear, a parabolic curve was observed with two distinct regions. A linear increasing response was observed at low cell concentrations (i.e. below 10^4 or 10^5 CFU/ml). Above this point, the opposite signal response was observed. Similar patterns had been noted in previous studies. In a study using gold to label the antibody in a sandwich-immunoassay, a similar parabolic curve was observed (Kim et al. 2000). The cause for this is not yet fully understood, but steric hindrances that can occur as binding sites are saturated at high concentrations may be partly responsible (Sergeyeva et al. 1994; Kim et al. 2000).

The EI biosensor is still in the developmental stage and several obstacles remain to be overcome. Figures 5.1- 5.4 reveal the inconsistency in the signal pattern between biosensors, such as in the case of the biosensor response to inoculated strawberry samples. The signal from the EHEC biosensor was proportional to cell concentrations of 10^1 to 10^4 CFU/ml and inversely proportional thereafter. The signal from the Sal biosensor, however, was proportional to the

target organism at concentrations up to 10^5 CFU/ml before decreasing gradually. This inconsistency is not understood yet. Factors such as biosensor-to-biosensor variability and variation in the sample matrix may have contributed to the variation of the results. Particles that detached from the produce samples during homogenizing may also contribute to the inconsistency of the signal pattern. These detached particles may interfere with the conduction of polyaniline or even clog the biosensor.

The inoculum levels used to inoculate the produce samples were high ($\sim 10^8$ CFU/ml), however, only about 10^6 CFU/ml of cells were attached to the samples. This significant loss of bacteria in the sample mainly due to the sample preparation method used this study. Therefore, a new efficient way of preparing the sample needs to be investigated in order to further increase the reliability of the biosensor in testing a food matrix.

The issue of sample size was also addressed in the study. To run a single biosensor, only 0.1 ml of sample was needed. If a very small number of organisms were present in the original sample, the probability of capturing those organisms in a 0.1-ml sample size was low. Therefore, a method utilizing bio-filtration was chosen to concentrate a 100-ml sample to 10-ml. This technique is commonly used in water filtration studies as a way to increase the sample size without having to sacrifice the biosensor sensitivity. In this experiment, no pre-enrichment was needed. Therefore, detection was completed in less than 20 minutes, including the time required to homogenize and filter the sample.

Finally, this study shows that the performance of the biosensor is equal to or even better than other analytical systems which normally have sensitivities and specificities ranging from 10^3 to 10^5 CFU/ml (Ivnitski et al. 1999; D'Souza 2001). The detection limits for the Sal, EHEC, and EC biosensors for inoculated produce samples were approximately $8 \pm 0.3 \times 10^1$, $7.8 \pm 0.4 \times 10^1$, and $8.3 \pm 0.4 \times 10^1$ CFU/ml, respectively. The high sensitivity of the biosensor is an excellent characteristic that should be researched further in the development of a rapid detection tool for food and water samples.

CONCLUSION

The electrical immunoassay (EI) biosensor described in this research represents a novel technique for microbial detection. The combination of lateral flow system and an electrical immunoassay method produced a versatile, rapid, highly sensitive and specific biosensor to detect foodborne pathogens. The major findings and obstacles found in the study are summarized below.

This study contained four distinct objectives. The first objective was to determine the best architecture for the biosensor and the optimum parameters associated with the type of assay to be used. Experimental results showed that the cellulose membrane was superior for both the sample application and absorption pads. The fiberglass membrane was evaluated for the conjugate pad, and the nitrocellulose membrane was chosen for the capture area. Between the glass and copper wafer, the copper wafer provided the best platform to connect the immunosensor to a multimeter. Silver paste was used to construct the electrodes on the etched copper wafer. An antibody concentration of 500 μ g/ml on the capture pad, 150 μ g/ml on the conjugate pad, a polyaniline concentration of 1g/ml, and a 0.5-millimeter distance between electrodes were optimal for the biosensor.

The second objective was to further evaluate the performance of the biosensor in multiple types of microbial cultures. Biosensor specificity and sensitivity were assessed. The response curve had a parabolic shape. ANOVA revealed that the detection limits for the Sal, EHEC, and EC biosensors to target organisms were $8.3 \pm 0.6 \times 10^1$, $7.9 \pm 0.3 \times 10^1$, and $7.5 \pm 0.3 \times 10^1$ CFU/ml,

respectively. The biosensor was specific only to the target organism even in samples with multiple inocula. Using ANOVA, the response signals (resistance drop) generated in samples inoculated with target bacteria were significantly different than those of the blank. These response signals however, were not statistically different between various inoculum levels of concentration.

The third objective of the study was to test the biosensor using produce samples inoculated with different target organisms. Inoculated produce samples were first filtered through a bio-membrane filter to concentrate the cells. This method was used to increase the probability of capturing the target organism in a small (0.1ml) volume of sample. The specificity and sensitivity testing in this experiment were consistent with the result found in the second objective. The detection limits for the Sal, EHEC, and EC biosensors for inoculated produce samples were $8.0 \pm 0.3 \times 10^1$, $7.8 \pm 0.4 \times 10^1$, and $8.3 \pm 0.4 \times 10^1$ CFU/ml, respectively. Sample preparation and detection were completed in approximately 20 minutes.

The EI biosensor faces several issues that need to be addressed before it can be adopted as a rapid microbial detection device. The first issue concerns the over-crowding effect. As previously discussed, the effect causes a decrease in the generated signal at high contamination levels (i.e. above 10^5 CFU/ml). This is a common finding especially when dealing with immunoassay techniques. The explanation for the parabolic curve, however, is not yet fully understood. In this study, it was hypothesized that cell crowding might have hindered the charge transfer within the polyaniline structure, leading to the decreased signal.

The second issue concerns the effect of variability between biosensors. Biosensor-to-biosensor variability does occur at the manufacturing stage, especially when preparing the biosensor without the use of machines. As shown from the data, variability may have contributed to inconsistencies in the signal response. Evidently, there is a need for precision manufacturing of the EI biosensor. This will not only eliminate biosensor-to-biosensor variability effect, but will also increase the reliability of the biosensor as a diagnostic tool.

The third issue concerns the sample preparation method used in testing food matrices, such as fresh fruits and vegetables. The method used in the study was not sufficient to recover very low levels of organisms in the samples tested. Although the biosensor exhibits high specificity and sensitivity, these characteristics may be less efficient if the method used to recover microbial contaminants from the sample is ineffective.

Lastly, the biosensor gives no information about the viability of organisms captured. The antibodies used in this study detected only surface antigens. Therefore, it is impossible to investigate whether the biosensor captured dead or viable cells. The ability to give information on cell viability is critical especially when testing samples in food processing plants, in which the product is subjected to multiple bacteria-reduction processes.

Outcomes from this study are promising for the development of a rapid, sensitive, and specific device for microbial detection in food and water supplies. A preliminary testing of water samples obtained from the Red Cedar river and MSU dairy farm are demonstrated in Appendix E. The biosensor can be

optimized to be an affordable, portable, user friendly and field-based device as an alternative to bulky and more expensive detection methods. With further research and refinement of the biosensor architecture and fabrication, the EI biosensor may be adaptable as a rapid diagnostic tool in food safety.

APPENDICES

APPENDIX A

RESEARCH PROCEDURES AND PROTOCOLS

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Appendix A.1: Media/reagents preparation

Brilliant Green Agar (Difco Laboratories, NJ)

1. Add 58 g of powder in 1 L of distilled water.
2. Autoclave 15 min at 121°C.

McConkey Agar (Difco Laboratories, NJ)

1. Add 50 g of powder in 1 L of distilled water.
2. Autoclave 15 min at 121°C.

CHROM Agar (Microbiology, France)

1. Add 29 g of powder in 1 L of distilled water.
2. Bring to boil (100°C).
3. Cool in water bath to 48°C.

Nutrient broth (Difco Laboratories, NJ)

1. Add 8 g of powder in 1 L of distilled water.
2. Distribute 10 ml per test tube
3. Autoclave 15 min at 121°C.

0.1%(w/v) Peptone water (Difco Laboratories, NJ)

1. Add 8 g of powder in 1 L of distilled water.
2. Distribute 10 ml per test tube
3. Autoclave 15 min at 121°C.

10% (v/v) methanol solution

1. Mix 10ml methanol (Sigma-Aldrich, MO) with 100 ml of distilled water.
2. Vortex.

0.5% (v/v) glutaraldehyde solution

1. Mix 0.5ml glutaraldehyde (Sigma-Aldrich, MO) with 100ml-distilled water
2. Vortex.

100mM tris buffer containing 0.1% tween-20 (or 0.1% casein)

1. Add 12.1 g of tris in 1 L distilled water.
2. Adjust the pH to 7.6 using 1 N sodium hydroxide (NaOH) or hydrochloride acid (HCl).
3. Autoclave for 45 min at 121°C.
4. Add 1ml tween-20 (0.1g of casein).

Appendix A.2: Creating pure cell cultures and plate counting

To create stock cultures of *E. coli* 0157:H7, the non-pathogenic *E. coli*, and *Salmonella* species

1. Aseptically transfer the isolate to 10ml of nutrient broth (NB) (Difco, laboratories) in a sterile 14 ml tube culture.
2. Incubate the inoculated tube culture for 24 hours at 37° C.

Plate counting

1. Prepare eight 15-ml test tubes with 9 ml 0.1% peptone water (Sigma-Aldrich, MO). Label the test tubes C1, C2,.....C8.
2. Aseptically transfer 1 ml of the grown bacterial culture into C1.
3. Vortex.
4. Transfer 1 ml of C1 into C2.
5. Vortex.
6. Repeat steps 4 and 5 until reaching C8.
7. Transfer 0.1ml of C8 on a differential medium.
8. Spread the bacterial solution with a sterile hockey stick on the surface of the medium.
9. Incubate the plate at 37°C for 24 hours.
10. Count the plate.
11. Back count 8-fold dilutions for every step, to determine the original culture concentration.

Appendix A.3: Polyaniline polymerization

1. Mix 5 ml of 0.4 M of Aniline (Sigma-Aldrich) and 40 ml of 0.2 mM ammonium persulfate (APS), with 80ml of 1M HCl.
2. Leave to react for 30 min or until polymerization is complete (i.e. the clear solution turns blue)
3. Filter the product using a Whatman filter paper.
4. Collect the resulting product from the filter paper using a scraper.
5. Dissolve the product in a 5-ml of N, N Dimethylformamide (DMF).
6. Filter the above solution using a Whatman filter paper.
7. Discard the product trapped on the filter paper.
8. Add 2 ml of 2M HCl in the resulting filtrate.
9. Filter the above solution again.
10. Dissolved the final product in phosphate buffer containing 10% (v/v) DMF.

Appendix A.4: Antibody preparation

1. Antibodies were hydrated with phosphate buffer pH 7.0 (PB) to make the desired concentrations

For example: Making 500µg/ml of *E. coli* O157:H7 antibody (Kirkegaard & Perry Laboratories, Inc)

1. Mix 1g/ml of the antibody with 2ml of PB in a vial.
2. Vortex thoroughly.
3. Store at 4°C.

Appendix A.5: Conjugating polyaniline to antibodies

1. Add 800 μ l of the antibody in 8 ml of polyaniline.
2. Leave to react for 30 min.
3. Add 1 ml of tris buffer containing 0.1% of casein.
4. Leave to react for another 30 min.
5. Centrifuge at 13000 rpm for 3 min in the 1.5 ml of centrifuge vial.
6. Discard the liquid.
7. Add 1 ml of tris buffer with 0.1% casein into each vial.
8. Centrifuge again.
9. Repeat the above procedure twice.
10. Dilute the resulting pellets in 1 ml of 0.1M lithium chloride (LiCl).
11. Store at 4°C before use

Appendix A.6: Immunosensor preparation

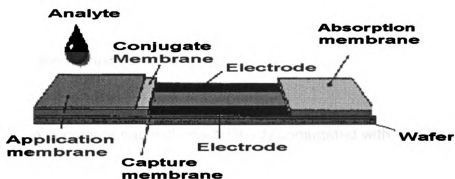


Figure A.5: Schematic diagram of the immunosensor.

Table A.5: Dimension of the immunosensor

Section	Dimension (Length mm X Width mm)
Application membrane	15 X 5
Conjugate membrane	10 X 5
Capture membrane	20 X 5
Absorption membrane	20 X 5

All the membranes were purchased from Millipore (Massachusetts)

Capture pad preparation

1. Cut the nitrocellulose membranes (NC) into smaller pieces (6-7 cm) to fit into a petri dish.
2. Wash the membranes with distilled water three times
3. Immerse the membranes in 10% methanol. The membranes are left to air-dry for 30 min.
4. Proceed with washing the membranes with 0.5% (v/v) glutaraldehyde solution. The membranes are left to dry for 60 min.
5. Apply 0.5mg/ml of antibodies on the membranes. Seal the petri dish with a parafilm, and incubate for 60 min.
6. Wash the membrane with 100mM tris buffer, containing 0.1% (v/v) tween-20. Seal the petri dish with parafilm again, and incubate for 45 min.
7. Store the prepared NC membranes at 4°C.

Application and absorption pad preparation

1. Cut the cellulose membranes into smaller pieces (6-7 cm) to fit into a petri dish.
2. Wash the membranes with distilled water three times.
3. Allow the membrane to air dry.
4. Store at membranes at 4°C.

Conjugate pad preparation

1. Soak the conjugate pad with the antibody conjugated with polyaniline and left to air dry.
2. The pads must be completely dry before the membranes are assembled.

Assembling the immunosensor

1. All the membranes are arranged and cut according to the dimension in Table A.5.
2. Use silver pen to make two electrodes, 0.5mm apart on the capture pad.
3. Attach the immunosensor on the etched copper wafer using a double sided tape.

Appendix A.7: Detection

1. Attach alligator clips to both electrodes.
2. Connect the immunosensor to an ohmmeter.
3. Transfer 0.1 ml of the sample onto the application pad.
4. Record reading at 2 min, 4 min, and 6 min with the multimeter.

Appendix A.8: Mixed culture preparation

For Chapter 4

1. Grow five organisms (*E.coli* 0157:H7 ATCC#43895 and 35150, non-pathogenic *E. coli*, *S. Typhimurium*, and *S. Thompson*) in 10 ml nutrient broth.
2. Transfer cultures by loop inocula at 3 consecutive 24-hr intervals to 10 ml NB.
3. Centrifuge 1 ml of each microorganism at 2000rpm X 15 min using a 1.5-ml centrifuge vial.
4. Discard the liquid.
5. Add 1 ml of 0.1 % peptone water into the vial.
6. Centrifuge.
7. Repeat the above procedure for 2 more times.
8. Suspend each pellet in 2 ml of 0.1 % peptone water.
9. Combine all five suspended mixtures to make a 10-ml solution.
10. Serially dilute the suspended solution.

For Chapter 5

1. Grow three organisms (*E.coli* 0157:H7, non-pathogenic *E. coli*, and *S. Typhimurium*) in 10 ml nutrient broth.
2. Transfer cultures by loop inocula at 3 consecutive 24-hr intervals to 10 ml NB.
3. Centrifuge 1 ml of each microorganism at 2000rpm X 15 min using the 1.5- ml centrifuge vial.
4. Discard the liquid.
5. Add 1 ml of 0.1 % peptone water into the vial.
6. Centrifuge.
7. Repeat the above procedure for 2 more times.
8. Suspend each pellet in 3.33 ml of 0.1 % peptone water.
9. Combine all five suspended mixtures to make a 10-ml solution.
10. Serially dilute the suspended solution.

Appendix A.9: Produce sample preparation (For chapter 5)

Produce preparations

1. Weigh 25 g of lettuce, strawberries, and alfalfa sprouts samples. Prepare two batches of each sample.
2. Mix the first batch of each sample with 225ml of nutrient broth.
3. Incubate for 24 hours at 37° C.

Inoculation

1. Pipette 1 ml of inoculum onto the surface of the sample (second batch).
2. Leave for 45 min
3. Wash three times with 100 ml distilled water.

Sample processing

1. Mix the inoculated sample with 100ml of 0.1% peptone water.
2. Use stomacher for 2 min to homogenize the lettuce samples.
3. Repeat the above method with hand-rubbing technique for alfalfa sprouts and strawberries samples.

Filtering

1. Attach the Biopath SP™ Analytical Filter Funnel to a pump.
2. Transfer 100ml of processed produce samples into the funnel.
3. Filter.
4. Aseptically transfer the membrane to a test tube with a 10-ml of 0.1% peptone water
5. Vortex for 4 min.
6. Aseptically transfer the membrane from the test tube to a plating agar.
7. Serially dilute the suspended solution.

Appendix A.10: Water sample preparation

To inoculate distilled water

1. Prepare eight 15-ml test tubes with 9 ml 0.1% peptone water. Label the test tubes w1, w2,.....w8.
2. Aseptically transfer 1 ml of grown bacterial culture in w1.
3. Vortex.
4. Transfer 1 ml of w1 into w2.
5. Vortex.
6. Repeat the above procedure until reaching w8.

To test field samples

1. Attach the Biopath SP™ Analytical Filter Funnel to a pump.
2. Transfer 100ml of processed produce samples into the funnel.
3. Filter.
4. Aseptically transfer the membrane to a test tube with a 10-ml of 0.1% peptone water.
5. Vortex for 4 min.
6. Aseptically transfer the membrane from the test tube to a plating agar.
7. Serially dilute the suspended solution.

APPENDIX B

RESULT FOR CHAPTER 3

FABRICATION OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR

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Abbreviations

1. Normalization was calculated by subtracting the outputs of the samples from the blank.
2. *-Estimated bacterial populations by back-counting every step from the last dilution

Polyaniline concentrations (g/ml)	Resistance measured in the sample containing 10^4 CFU/ml of <i>E. Coli</i> O157:H7			Average	Standard Deviation	Resistance measured in the blank sample			Average	Standard Deviation
	Trial 1	Trial 2	Trial 3			Trial 1	Trial 2	Trial 3		
0.001	99	101	98	99.33	1.53	120	118	112	116.67	4.16
0.01	79.3	74.2	78.4	77.3	2.72	123	125	123	123.67	1.15
0.1	67.5	71.3	73.4	70.73	2.99	128	131	123	127.33	4.04
1	62.8	64.1	61	62.63	1.56	132	123	127	127.33	4.51
10	76.3	78.3	79.1	77.9	1.44	112	123	119	118	5.57

Figure B.1.a: Biosensor results with varying polyaniline concentration in $\sim 10^4$ CFU/ml of *E. coli* O157:H7 in pure culture.

Polyaniline concentrations (g/ml)	Resistance drop (K ohm)			STD	Plate counts (CFU/ml)
	Trial 1	Trial 2	Trial 3		
0.001	21.0	17.0	14.0	3.5	$*8.7 \times 10^4$
0.01	43.7	50.8	44.6	3.9	$*8.7 \times 10^4$
0.1	60.5	59.7	49.6	6.1	$*8.7 \times 10^4$
1	69.2	58.9	66.0	5.3	$*8.7 \times 10^4$
10	35.7	44.7	39.9	4.5	$*8.7 \times 10^4$

Figure B.1.b: Normalized data for Figure B.1.a

Measured resistance tested with samples										
Distance between electrodes (mm)	Plate counts (CFU/ml)	2min (trial 1)	2min (trial 2)	2 min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3) Average
0.5	*7.6 X 10 ⁴	65.3	61.2	59.1	67.7	73.2	69.1	74.3	75.4	71.2
1	*7.6 X 10 ⁴	78.1	81.2	83.2	89.3	90.3	84.2	91.2	96.5	87.0
2	*7.6 X 10 ⁴	122.5	123.6	113.6	127.7	130.7	128.6	137.6	136.9	140.8
3	*7.6 X 10 ⁴	148.7	153.8	153.7	159.9	170.9	178.8	178.8	177.1	180.0
4	*7.6 X 10 ⁴	209.1	214.2	234.1	220.3	241.3	259.2	272.2	277.5	270.4
5	*7.6 X 10 ⁴	462.1	457.5	523.1	526.1	579.4	872.1	808.5	984.2	980.4

Measured resistance tested with the blank										
Distance between electrodes (mm)	Plate counts (CFU/ml)	2min (trial 1)	2min (trial 2)	2 min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3) Average
0.5	0.0	116.3	112.2	121.1	121.3	120.1	125.4	121.2	131.2	121.9
1	0.0	121.1	121.4	125.4	133.1	132.4	128.9	133.2	135.5	129.1
2	0.0	136	132.2	137.2	142	150.2	155.3	158.3	162.5	150.2
3	0.0	160.3	164.4	168.6	181.2	175.5	185.4	190.4	188.9	177.7
4	0.0	213.2	228.4	244.1	238.5	250.9	260.4	276.2	280.3	252.1
5	0.0	450.1	437.5	539.1	536.1	678.4	769.1	768.5	1003.2	688.9

Figure B.2.a: Biosensor results with varying distance between electrodes.

Distance between electrodes (mm)	Plate counts (CFU/ml)	2min (trial 1)	2min (trial 2)	2 min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	STD
0.5	*7.6 X 10 ⁴	51.0	51.0	62.0	53.6	46.9	56.3	46.9	55.8	57.5	53.4	5.0
1	*7.6 X 10 ⁴	43.0	40.2	42.2	43.8	42.1	44.7	42.0	39.0	41.8	42.1	1.7
2	*7.6 X 10 ⁴	13.5	8.6	23.6	14.3	19.5	26.7	20.7	25.6	37.1	21.1	8.5
3	*7.6 X 10 ⁴	11.6	10.6	14.9	21.3	4.6	6.6	11.6	11.8	4.3	10.8	5.3
4	*7.6 X 10 ⁴	4.1	14.2	10.0	18.2	9.6	1.2	4.0	2.8	6.9	7.9	5.6
5	*7.6 X 10 ⁴	-12.0	-20.0	16.0	10.0	99.0	-103.0	-40.0	19.0	38.0	0.8	55.6

Figure B.2.b: Normalized data for Figure B2.a.

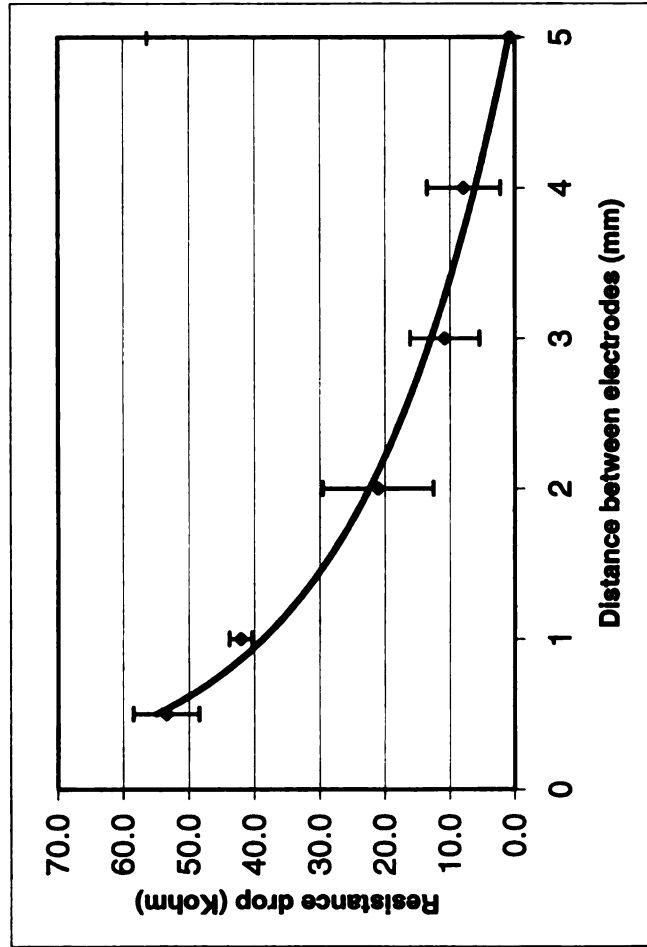


Figure B.2.c. Result of varying distances between electrodes.

Plate counts (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	P-value
Blank	112	99.5	112	124	110.7	124	132	123	131	118.7	1
7.8×10^1	94.5	93.2	92.3	97.6	98.3	99.4	112.5	108.9	110.4	100.8	0.000933645
7.8×10^2	90.3	88	93.6	98.3	96.3	102.4	110.4	112.1	115.3	100.7	0.002018625
7.8×10^3	81.8	85.8	89.4	88	90.1	88.7	90	92.1	93.23	88.8	6.00327E-07
7.8×10^4	71.5	69.5	66	76.3	75.2	72.3	77.5	79.8	78.3	74.0	3.92059E-09
7.8×10^5	55.3	58.2	57.7	55.4	59.4	61.1	69.2	71.3	72.4	62.2	4.63721E-10
7.8×10^6	61.5	59.5	58	71.3	74.2	72.6	77.5	78.1	79.1	70.2	1.08129E-08
7.8×10^7	69.2	72.2	76.2	73.9	75.2	71.2	77.7	80.9	79.9	75.2	4.30483E-09
7.8×10^8	76.4	80.5	81.5	84.5	82.3	82.6	89.1	93.9	94.9	85.1	4.70021E-07

Figure B.3.a: *E. coli* O157:H7 biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #43895).

Plate counts (CFU/ml)	Resistance drop (K ohm)										Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.8×10^1	17.50	6.30	19.70	26.40	12.40	24.60	19.50	14.10	20.60	17.90	5.87
7.8×10^2	21.70	11.50	18.40	25.70	14.40	21.60	21.60	10.90	15.70	17.94	4.83
7.8×10^3	30.20	13.70	22.60	36.00	20.60	35.30	42.00	30.90	37.77	29.90	8.68
7.8×10^4	40.50	30.00	46.00	47.70	35.50	51.70	54.50	43.20	52.70	44.64	7.75
7.8×10^5	56.70	41.30	54.30	68.60	51.30	62.90	62.80	51.70	58.60	56.47	7.58
7.8×10^6	50.50	40.00	54.00	52.70	36.50	51.40	54.50	44.90	51.90	48.49	6.12
7.8×10^7	42.80	27.30	35.80	50.10	35.50	52.80	54.30	42.10	51.10	43.53	8.77
7.8×10^8	35.60	19.00	30.50	39.50	28.40	41.40	42.90	29.10	36.10	33.61	7.17

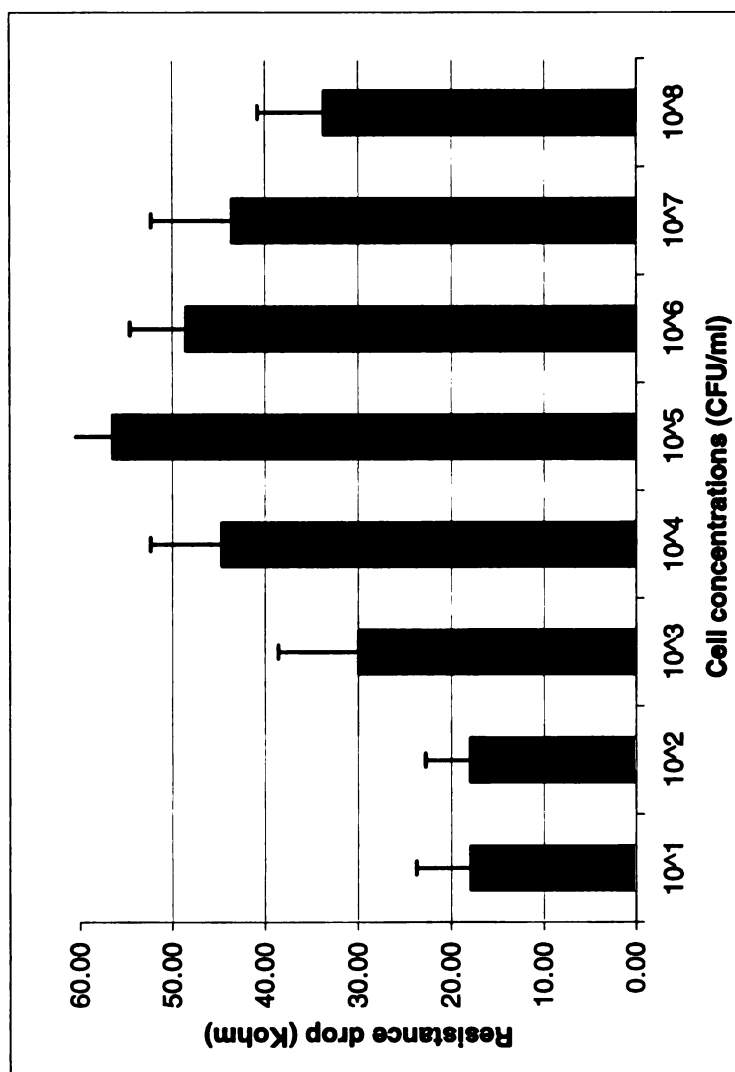


Figure B.3.c: Result of the *E. coli* O157:H7 biosensor with varying concentration of *E. coli* O157:H7 (ATCC #43895).

APPENDIX C

RESULT FOR CHAPTER 4

SENSITIVITY AND SPECIFICITY ANALYSIS OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR

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Abbreviations

1. Normalization was calculated by taking the difference between the outputs of the blank and the sample.
2. Plate counts $^{\alpha}$ = Standard plating on differential media according to the type of biosensors used. Plating on Brilliant Green Agar for Sal biosensor, CHROM Agar for EHEC biosensor, and McConkey Agar for EC biosensor.
3. Plate counts $^{\alpha 1}$ = Standard plating on differential media according to samples tested. Plating on Brilliant Green Agar for *S. Typhimurium* and *S. Thompson*, CHROM Agar for *E. coli* O157:H7, and McConkey Agar for non-pathogenic *E. coli*.
4. STD=Standard deviation
5. *Estimated bacterial populations by back-counting every step from the last dilution.

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	P-value to the blank
Blank	114	102.5	122	127.4	129.1	137.4	135.4	136.4	134.4	1
7.7×10^1	106.5	96.2	102.3	111	111.7	112.8	125.9	122.3	123.8	0.025439
7.7×10^2	102.3	91	103.6	111.7	108.7	115.8	123.8	125.5	128.7	0.015584
7.7×10^3	93.8	88.8	99.4	101.4	103.5	102.1	103.4	105.5	106.63	2.11E-05
7.7×10^4	73.5	75.5	75	79.7	81.6	84.7	89.9	91.2	91.7	82.5
7.7×10^5	67.3	61.2	67.7	68.8	72.8	74.5	82.6	84.7	85.8	73.9
7.7×10^6	73.5	62.5	68	84.7	87.6	86	90.9	91.5	92.5	81.9
7.7×10^7	81.2	75.2	86.2	87.3	88.6	84.6	91.1	94.3	93.3	86.9
7.7×10^8	88.4	83.5	91.5	97.9	95.7	96	102.5	107.3	108.3	96.8

Figure C.1.a: EHEC biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #35150).

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.7×10^1	7.50	6.30	19.70	16.40	17.40	24.60	9.50	14.10	10.60	14.01
7.7×10^2	11.70	11.50	18.40	15.70	19.40	21.60	11.60	10.90	5.70	14.06
7.7×10^3	20.20	13.70	22.60	26.00	25.60	35.30	32.00	30.90	27.77	26.01
7.7×10^4	40.50	27.00	47.00	47.70	47.50	52.70	45.50	45.20	42.70	43.98
7.7×10^5	46.70	41.30	54.30	58.60	56.30	62.90	52.80	51.70	48.60	52.58
7.7×10^6	40.50	40.00	54.00	42.70	41.50	51.40	44.50	44.90	41.90	44.60
7.7×10^7	32.80	27.30	35.80	40.10	40.50	52.80	44.30	42.10	41.10	39.64
7.7×10^8	25.60	19.00	30.50	29.50	33.40	41.40	32.90	29.10	26.10	29.72

Figure C.1.b: Normalized value for data in Figure C.1.a

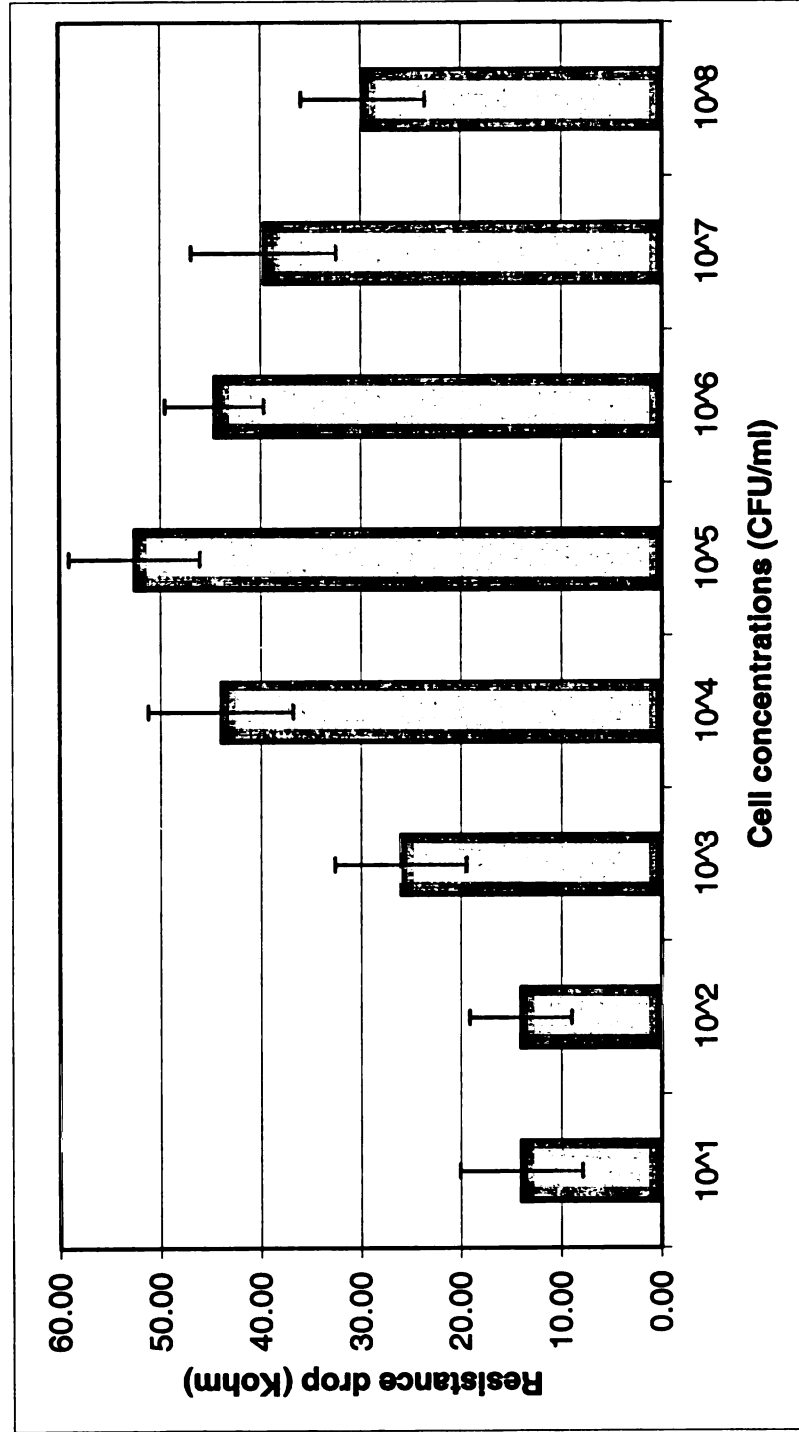


Figure C.1.c: Result of EHEC biosensor with varying concentrations of *E. coli* O157:H7 (ATCC #35150) in pure culture

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average (trial 3)	P-value to the blank
Blank	Blank	113.1	125.2	114.4	126.5	124.5	113.9	117.3	120.0	1
0	7.7 X 10 ¹	110.3	124.2	133.5	111.6	125.5	134.8	111.1	114.5	0.784924
0	7.7 X 10 ²	128.3	120.1	121.2	129.6	121.4	122.5	112.4	121.2	0.545204
0	7.7 X 10 ³	126.5	135.8	113.9	127.8	137.1	133.7	130.4	135.4	0.027405
0	7.7 X 10 ⁴	122.4	123.5	131.9	123.7	124.8	121.4	128.6	113.5	0.09479
0	7.7 X 10 ⁵	115.2	121.1	124.5	125.6	111.8	111.8	124.5	127.3	0.644665
0	7.7 X 10 ⁶	112.4	102.5	122	113.2	109	109	112.4	126.3	0.134542
0	7.7 X 10 ⁷	130.4	111.8	115.2	127.3	127	127	130.4	122.2	0.123232
0	7.7 X 10 ⁸	128.6	109	112.4	126.3	125.2	125.2	128.6	137.9	0.1609341

Figure C.2.a: Sal biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #35150).

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD	
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0	2.80	1.00	-10.30	2.80	1.00	-10.30	2.80	17.50	1.12	8.22
0	-15.20	5.10	2.00	-15.20	5.10	2.00	-3.90	4.80	-1.53	8.23
0	-13.40	-10.60	9.30	-13.40	-10.60	9.30	-16.50	-18.10	7.60	9.93
0	-9.30	1.70	-8.70	-9.30	1.70	-8.70	3.80	-10.40	-4.68	7.12
0	-2.10	4.10	-1.30	-11.20	14.70	12.70	-10.60	-8.60	-1.37	9.97
0	0.70	22.70	1.20	1.20	17.50	15.50	1.50	-9.00	5.20	10.75
0	-17.30	13.40	8.00	-12.90	-0.50	-2.50	-16.50	-4.90	-10.30	10.65
0	-15.50	16.20	10.80	-11.90	1.30	-0.70	-14.70	-12.30	-5.27	12.77

Figure C.2.b: Normalized values for data in Figure C.2.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	117.5	106.2	125.5	130.9	132.6	140.9	138.9	139.9	137.9	130.0	1
*7.8 X 10 ¹	110.0	99.9	105.8	114.5	115.2	116.3	129.4	125.8	127.3	116.0	0.01523907
*7.8 X 10 ²	105.8	94.7	107.1	115.2	113.2	119.3	127.3	129.0	132.2	116.0	0.02497128
*7.8 X 10 ³	97.3	92.5	102.9	104.9	107.0	105.6	106.9	109.0	110.1	104.0	1.9977E-05
*7.8 X 10 ⁴	77.0	79.2	78.5	83.2	85.1	88.2	93.4	94.7	95.2	86.1	5.0647E-08
*7.8 X 10 ⁵	70.8	64.9	71.2	72.3	76.3	78.0	86.1	88.2	89.3	77.5	9.4179E-09
*7.8 X 10 ⁶	77.0	66.2	71.5	88.2	91.1	89.5	94.4	95.0	96.0	85.4	3.4874E-07
*7.8 X 10 ⁷	84.7	76.9	89.7	90.8	92.1	88.1	94.6	97.8	96.8	90.4	1.1603E-07
*7.8 X 10 ⁸	91.9	87.2	95.0	101.4	98.2	99.5	106.0	110.8	111.8	100.3	1.29E-05

Figure C.3.a: EC biosensor responses with varying concentration of *E. coli*/O157:H7 (ATCC #35150)

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*7.8 X 10 ¹	7.49	6.30	19.70	16.40	17.40	24.60	9.50	14.10	10.60	14.01	14.05	6.08
*7.8 X 10 ²	11.69	11.50	18.40	15.70	19.40	21.60	11.60	10.90	5.70	14.05	14.05	5.06
*7.8 X 10 ³	20.19	13.70	22.60	26.00	25.60	35.30	32.00	30.90	27.77	26.01	26.01	6.58
*7.8 X 10 ⁴	40.49	27.00	47.00	47.70	47.50	52.70	45.50	45.20	42.70	43.98	43.98	7.22
*7.8 X 10 ⁵	46.69	41.30	54.30	58.60	56.30	62.90	52.80	51.70	49.60	52.58	52.58	6.50
*7.8 X 10 ⁶	40.49	40.00	54.00	42.70	41.50	51.40	44.50	44.90	41.90	44.60	44.60	4.92
*7.8 X 10 ⁷	32.79	27.30	35.80	40.10	40.50	52.80	44.30	42.10	41.10	39.64	39.64	7.23
*7.8 X 10 ⁸	25.59	19.00	30.50	29.50	33.40	41.40	32.90	29.10	26.10	29.72	29.72	6.19

Figure C.3.b: Normalized values for data in Figure C.3.a

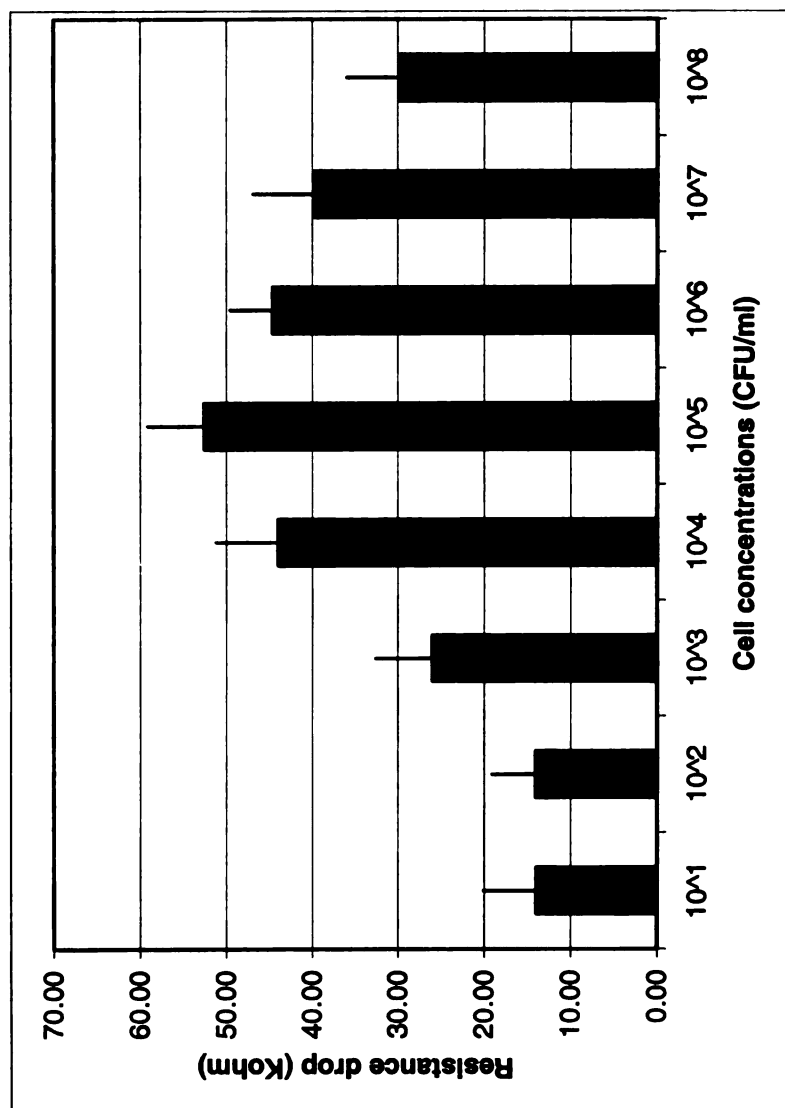


Figure C.3.c: Result of EC biosensor with varying concentrations of *E. coli* O157:H7 (ATCC# 35150) in pure culture

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	112	99.5	112	124	110.7	124	132	123	131	118.7	1
7.8×10^1	94.5	93.2	92.3	97.6	98.3	99.4	112.5	108.9	110.4	100.8	0.000933645
7.8×10^2	90.3	88	93.6	98.3	96.3	102.4	110.4	112.1	115.3	100.7	0.002018625
7.8×10^3	81.8	85.8	89.4	88	90.1	88.7	90	92.1	93.23	88.8	6.00327E-07
7.8×10^4	71.5	69.5	66	76.3	75.2	72.3	77.5	79.8	78.3	74.0	3.92059E-09
7.8×10^5	55.3	58.2	57.7	55.4	59.4	61.1	69.2	71.3	72.4	62.2	4.63721E-10
7.8×10^6	61.5	59.5	58	71.3	74.2	72.6	77.5	78.1	79.1	70.2	1.08129E-08
7.8×10^7	69.2	72.2	76.2	73.9	75.2	71.2	77.7	80.9	79.9	75.2	4.30483E-09
7.8×10^8	76.4	80.5	81.5	84.5	82.3	82.6	89.1	93.9	94.9	85.1	4.70021E-07

Figure C.4.a: EHEC biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #43895).

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.8×10^1	17.50	6.30	19.70	26.40	12.40	24.60	19.50	14.10	20.60	17.90	5.87	5.87
7.8×10^2	21.70	11.50	18.40	25.70	14.40	21.60	21.60	10.90	15.70	17.94	4.83	4.83
7.8×10^3	30.20	13.70	22.60	36.00	20.60	35.30	42.00	30.90	37.77	29.90	8.68	8.68
7.8×10^4	40.50	30.00	46.00	47.70	35.50	51.70	54.50	43.20	52.70	44.64	7.75	7.75
7.8×10^5	56.70	41.30	54.30	68.60	51.30	62.90	62.80	51.70	58.60	56.47	7.58	7.58
7.8×10^6	50.50	40.00	52.70	36.50	51.40	54.50	54.50	44.90	51.90	48.49	6.12	6.12
7.8×10^7	42.80	27.30	35.80	50.10	35.50	52.80	54.30	42.10	51.10	43.53	8.77	8.77
7.8×10^8	35.60	19.00	30.50	39.50	28.40	41.40	42.90	29.10	36.10	33.61	7.17	7.17

Figure C.4.b: Normalized values for data in Figure C.4.a

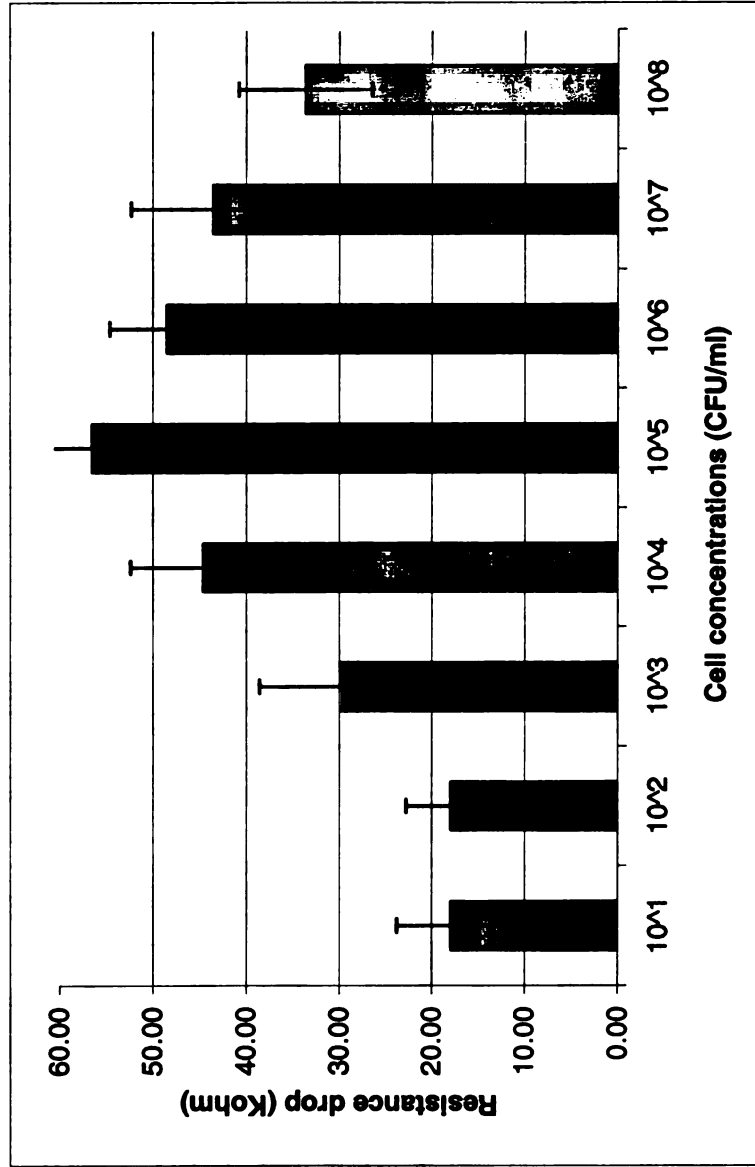


Figure C.4.c: Result of EHEC biosensor with varying concentrations of *E. coli* O157:H7 (ATCC#43895) in pure culture

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	1
0	7.8 X 10 ¹	126.6	125.6	121.5	137.2	124.9	122.5	103.9	113.2	110.4	85.1
0	7.8 X 10 ²	124.6	134.9	122.6	115.3	133.3	125.9	123.4	116.6	113.8	85.1
0	7.8 X 10 ³	115.8	113	131	129.2	125.1	127	114.6	128.7	127.7	85.1
0	7.8 X 10 ⁴	127.9	126.9	122.8	138.5	126.2	113.2	110.4	128.4	126.6	124.5
0	7.8 X 10 ⁵	125.9	136.2	123.9	135.1	122.8	113.2	110.4	128.4	126.6	124.7
0	7.8 X 10 ⁶	115.3	112.5	113.8	131.8	130	125.9	113.8	131.8	130	122.8
0	7.8 X 10 ⁷	118.7	115.9	122.6	136.8	114.9	128.7	127.7	123.6	139.3	125.4
0	7.8 X 10 ⁸	123.5	106	118.7	115.9	133.9	132.1	128	133.8	135.8	125.3

Figure C.5.a: Sal biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #43895) in pure culture.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-12.10	-13.90	8.20	-9.30	-1.10	-5.90	9.90	18.60	19.60	1.56	12.18	12.18
0	-10.10	-23.20	7.10	12.60	-9.50	-9.30	-9.60	15.20	16.20	-1.18	13.33	13.33
0	-1.30	-1.30	-1.30	-1.30	-1.30	-10.40	-0.80	3.10	2.30	-1.37	3.58	3.58
0	-13.40	-15.20	6.90	-10.60	-2.40	3.40	3.40	3.40	3.40	-2.34	7.98	7.98
0	-11.40	-24.50	5.80	-7.20	1.00	3.40	3.40	3.40	3.40	-2.52	9.46	9.46
0	-0.80	-0.80	15.90	-3.90	-6.20	-9.30	0.00	0.00	0.00	-0.57	6.59	6.59
0	-4.20	-4.20	7.10	-8.90	8.90	-12.10	-13.90	8.20	-9.30	-3.16	8.48	8.48
0	-9.00	5.70	11.00	12.00	-10.10	-15.50	-14.20	-2.00	-5.90	-3.10	9.85	9.85

Figure C.5.b: Normalized values for data in Figure C.5.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	121.4	124.1	123.4	128.8	130.5	128.8	126.8	128.3	137.8	127.8	9.87187E-05
7.6 X 10 ¹	101.3	99.4	104.1	102.5	113.5	118.7	117.7	118.6	120.6	110.7	9.64141E-07
7.6 X 10 ²	92.1	94.2	105.4	111.2	111.5	111.7	115.6	108.6	112.5	107.0	9.94403E-10
7.6 X 10 ³	95.6	92	101.2	100.9	105.3	108	105.2	94.3	108.43	101.2	4.26668E-07
7.6 X 10 ⁴	75.3	78.7	76.8	79.2	83.4	90.6	91.7	87.8	93.5	84.1	3.00208E-07
7.6 X 10 ⁵	69.1	64.4	69.5	68.3	74.6	80.4	84.4	94.6	87.6	77.0	9.63511E-06
7.6 X 10 ⁶	75.3	65.7	69.8	84.2	89.4	91.9	92.7	97.4	94.3	84.5	2.42885E-05
7.6 X 10 ⁷	83	78.4	88	86.8	90.4	90.5	92.9	110.4	95.1	90.6	0.002981499
7.6 X 10 ⁸	90.2	86.7	93.3	97.4	97.5	101.9	104.3	118.8	110.1	100.0	0.002981499

Figure C.6.a: EC biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #43895) in pure culture.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD 0.00
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Blank												
7.6 X 10 ¹	20.10	24.70	19.30	26.30	17.00	10.10	9.10	9.70	17.20	17.06	6.36	
7.6 X 10 ²	29.30	29.90	18.00	17.60	19.00	17.10	11.20	19.70	25.30	20.79	6.16	
7.6 X 10 ³	25.80	32.10	22.20	27.90	25.20	20.80	21.60	34.00	29.37	26.55	4.68	
7.6 X 10 ⁴	46.10	45.40	46.60	49.60	47.10	38.20	35.10	40.50	44.30	43.66	4.72	
7.6 X 10 ⁵	52.30	59.70	53.90	60.50	55.90	48.40	42.40	33.70	50.20	50.78	8.52	
7.6 X 10 ⁶	46.10	58.40	53.60	44.60	41.10	36.90	34.10	30.90	43.50	43.24	8.86	
7.6 X 10 ⁷	38.40	45.70	35.40	42.00	40.10	38.30	33.90	17.90	42.70	37.16	8.09	
7.6 X 10 ⁸	31.20	37.40	30.10	31.40	33.00	26.90	22.50	9.50	27.70	27.74	8.01	

Figure C.6.b: Normalized values for data in Figure C.5.a

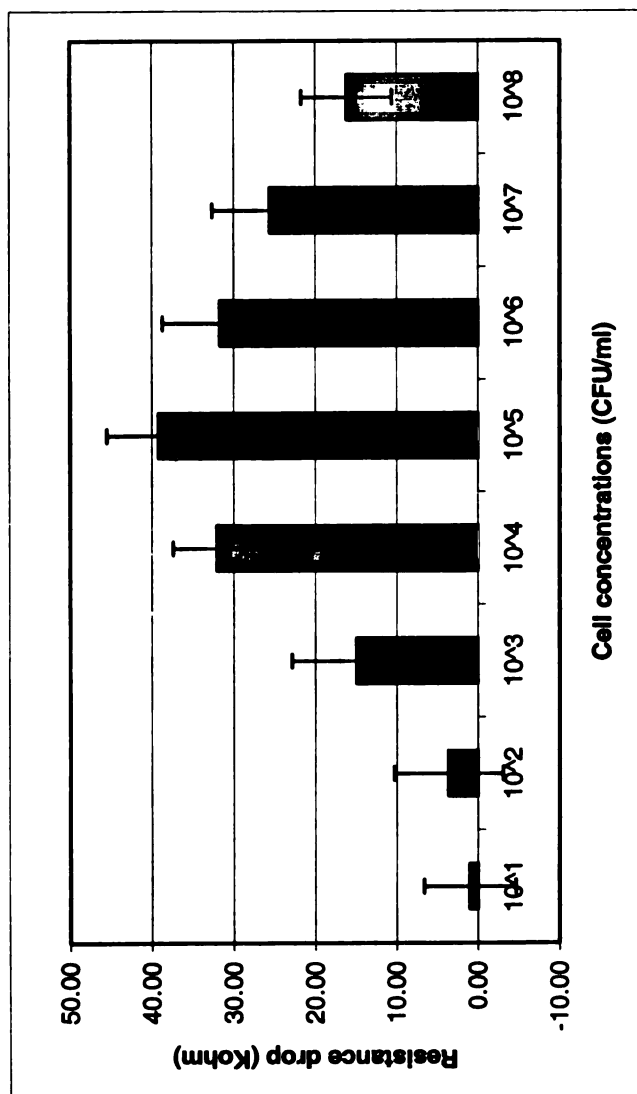


Figure C.6.c: Results of EC biosensor responses with varying concentration of *E. coli* O157:H7 (ATCC #43895) in pure culture.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	98.6	110.3	99.1	110.2	114.1	112.3	123.5	124.3	132.2	113.8	1
8×10^1	87.3	90.3	89.4	94.3	93.2	94.4	99.7	99.8	99.5	94.2	0.000188
8×10^2	72.4	76.5	78.4	76.5	81.2	79.1	82.3	89.4	83.4	79.9	3.55E-07
8×10^3	65.4	68.3	65.4	67.5	71.3	69.4	71.3	74.3	75.4	69.8	5.81E-09
8×10^4	51	57.4	55.4	54.6	57.2	61.4	58.9	65.4	60.3	58.0	2.3E-10
8×10^5	58.2	57.1	54.3	61.2	61.3	59.2	68.4	67.8	63.2	61.2	6.94E-10
8×10^6	67.5	65.4	68.4	69.3	66.3	71.2	71.2	69.3	74.3	69.2	3.64E-09
8×10^7	72.4	78.5	74.2	83.2	81.9	85.3	92.1	98.2	94.3	84.5	1.46E-05
8×10^8	87.5	86.4	89.4	98.3	96.4	101.3	123.4	105.4	112.1	100.0	0.023969

Figure C.7.a: Sal biosensor responses with varying concentration of S. Typhimurium

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8×10^1	11.3	20	9.7	15.9	20.9	17.9	23.8	24.5	32.7	19.63	6.65	7.54
8×10^2	26.2	33.8	20.7	33.7	32.9	33.2	41.2	34.9	48.8	33.93	7.44	8.12
8×10^3	33.2	42	33.7	42.7	42.8	42.9	52.2	50	56.8	44.03	7.44	8.12
8×10^4	47.6	52.9	43.7	55.6	56.9	50.9	64.6	58.9	71.9	55.89	8.12	7.55
8×10^5	40.4	53.2	44.8	49	52.8	53.1	55.1	56.5	69	52.66	7.55	9.17
8×10^6	31.1	44.9	30.7	40.9	47.8	41.1	52.3	55	57.9	44.63	9.17	3.98
8×10^7	26.2	31.8	24.9	27	32.2	27	31.4	26.1	37.9	29.39	3.98	6.72
8×10^8	11.1	23.9	9.7	11.9	17.7	11	0.1	18.9	20.1	13.82	6.72	

Figure C.7.b: Normalized values for data in Figure C.7.a.

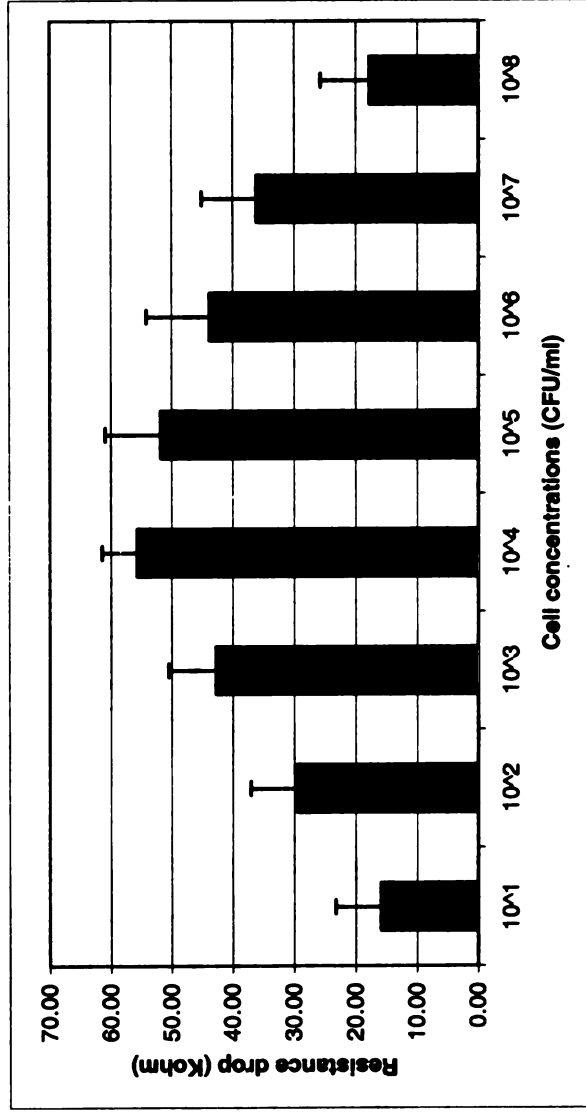


Figure C.7.c: Result of Sal biosensor with varying concentrations of *S. Typhimurium* in pure culture

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	P-value to the blank
0	Blank	122.7	121.7	114.4	132.4	125	122.5	133.4	126	123.5
0	8 X 10 ¹	131.1	130.1	128.3	124.2	126.1	113.7	125.2	127.1	114.7
0	8 X 10 ²	122.9	138.6	126.3	113.3	110.5	128.5	126.7	122.6	110.5
0	8 X 10 ³	124	123	134.2	121.9	112.3	109.5	122.9	113.3	110.5
0	8 X 10 ⁴	113.9	112.9	130.9	129.1	125	112.9	130.1	126	113.9
0	8 X 10 ⁵	122.7	121.7	135.9	114	127.8	126.8	115	128.8	127.8
0	8 X 10 ⁶	113.6	127	117.4	114.6	132.6	115.3	112.5	113.8	131.8
0	8 X 10 ⁷	117	134.2	130.1	118	136	118.7	115.9	122.6	136.8
0	8 X 10 ⁸	116.7	128.8	128.5	131.9	123.7	130	114.9	109.7	123.6

Figure C.8.a: EC biosensor responses with varying concentration of S. Typhimurium

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD	Total STD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-8.40	-8.40	-13.90	8.20	-1.10	8.80	8.20	8.80	0.12	8.85
0	-0.20	-16.90	-11.90	19.10	14.50	-6.00	6.70	13.00	2.41	12.31
0	-1.30	-1.30	-19.80	10.50	12.70	13.00	10.50	12.70	13.00	11.17
0	8.80	8.80	-16.50	3.30	0.00	9.60	3.30	0.00	9.60	8.31
0	0.00	0.00	-21.50	18.40	-2.80	18.40	-2.80	-4.30	0.12	12.21
0	9.10	-5.30	-3.00	17.80	-7.60	7.20	20.90	12.20	-8.30	4.78
0	5.70	-12.50	-15.70	14.40	-11.00	3.80	17.50	3.40	-13.30	12.58
0	0.90	-7.30	-10.50	19.60	-5.80	9.00	12.70	8.60	-8.10	10.74

Figure C.8.b: Normalized values for data in Figure C.8.a.

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)
0	Blank	115.4	114.4	107.1	125.1	117.7	115.2	126.1	118.7	116.2
0	8 X 10 ¹	123.8	122.8	121	116.9	118.8	106.4	117.9	119.8	107.4
0	8 X 10 ²	115.6	131.3	119	106	113.2	121.2	119.4	115.3	103.2
0	8 X 10 ³	116.7	115.7	126.9	114.6	105	102.2	115.6	106	103.2
0	8 X 10 ⁴	126.2	118	107.1	115.7	122.9	107.8	108.4	111.8	117.8
0	8 X 10 ⁵	118.8	119.9	114.3	106.1	118.8	121.6	126.4	129.8	136.1
0	8 X 10 ⁶	116.3	107.5	122.3	103.3	106.7	120.6	109.1	112.5	118.8
0	8 X 10 ⁷	127.2	119	120.5	116.7	123.9	108.8	106.3	109.7	127
0	8 X 10 ⁸	119.8	120.9	116.4	107.1	119.8	122.6	107.6	116.4	122.7
									Average	117.3
										117.2
										0.960009
										0.704482
										0.114524
										0.460858
										0.269085
										0.164456
										0.91427
										0.917596

Figure C.9.a: EHEC biosensor responses with varying concentration of S. Typhimurium

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average
0	-8.40	-8.40	-13.90	8.20	-1.10	8.80	8.20	-1.10	8.80	0.12
0	-0.20	-16.90	-11.90	19.10	4.50	-6.00	6.70	3.40	13.00	1.30
0	-1.30	-1.30	-19.80	10.50	12.70	13.00	10.50	12.70	13.00	5.56
0	-10.80	-3.60	0.00	9.40	-5.20	7.40	17.70	6.90	-1.60	2.24
0	-3.40	-5.50	-7.20	19.00	-1.10	-6.40	-0.30	-11.10	-19.90	-3.99
0	-0.90	6.90	-15.20	21.80	11.00	-5.40	17.00	6.20	-2.60	4.31
0	-11.80	-4.60	-13.40	8.40	-6.20	6.40	19.80	9.00	-10.80	-0.36
0	-4.40	-6.50	-9.30	18.00	-2.10	-7.40	18.50	2.30	-6.50	0.29
										10.73

Figure C.9.b: Normalized values for data in Figure C.9.a.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	98.1	107.3	99.1	126.3	114.6	118.7	133.1	128.5	137.6	118.1	1
7.9 X 10 ¹	89.6	99.3	89.2	94.5	97.3	101.2	118.4	113.4	117.4	102.3	0.019748
7.9 X 10 ²	82.4	78.5	79.4	88.4	83.4	89.2	95.4	97.6	103.2	88.6	7.67E-05
7.9 X 10 ³	69.4	67.9	62.3	72.3	76.5	75.3	84.5	81.2	89.1	75.4	9.88E-07
7.9 X 10 ⁴	51.6	51.4	49.3	61.6	62.4	61.5	73.2	75.1	78.4	62.5	7.04E-08
7.9 X 10 ⁵	57.5	59.9	58.4	61.3	68.9	65.4	76	76.5	73.2	66.3	5.39E-08
7.9 X 10 ⁶	62.5	67.6	72.4	69.4	75.3	79.3	78.4	83.2	81.3	74.4	4.05E-07
7.9 X 10 ⁷	77.4	71.5	75.4	82.4	79.2	83.4	92.5	87.2	89.5	82.1	4.54E-06
7.9 X 10 ⁸	87.5	88.6	89.4	93.3	95.4	98.3	123.4	115.4	112.1	100.4	0.015088

Figure C.10.a: Sal biosensor responses with varying concentration of S. Thompson

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.9 X 10 ¹	8.50	8.00	9.90	31.80	17.30	17.50	14.70	15.10	20.20	15.89	7.34	7.53
7.9 X 10 ²	15.70	28.80	19.70	37.90	31.20	29.50	37.70	30.90	34.40	29.53	7.53	7.75
7.9 X 10 ³	28.70	39.40	36.80	54.00	38.10	43.40	48.60	47.30	48.50	42.76	7.75	9.14
7.9 X 10 ⁴	46.50	55.90	49.80	64.70	52.20	57.20	59.90	53.40	61.20	55.64	5.79	10.40
7.9 X 10 ⁵	35.60	39.70	26.70	56.90	39.30	39.40	54.70	45.30	56.30	43.77	8.97	7.95
7.9 X 10 ⁶	20.70	35.80	23.70	43.90	35.40	35.30	40.60	41.30	48.10	36.09	8.97	7.95
7.9 X 10 ⁷	10.60	18.70	9.70	33.00	19.20	20.40	9.70	13.10	25.50	17.77	7.95	7.95

Figure C.10.b: Normalized values for data in Figure C.10.a.

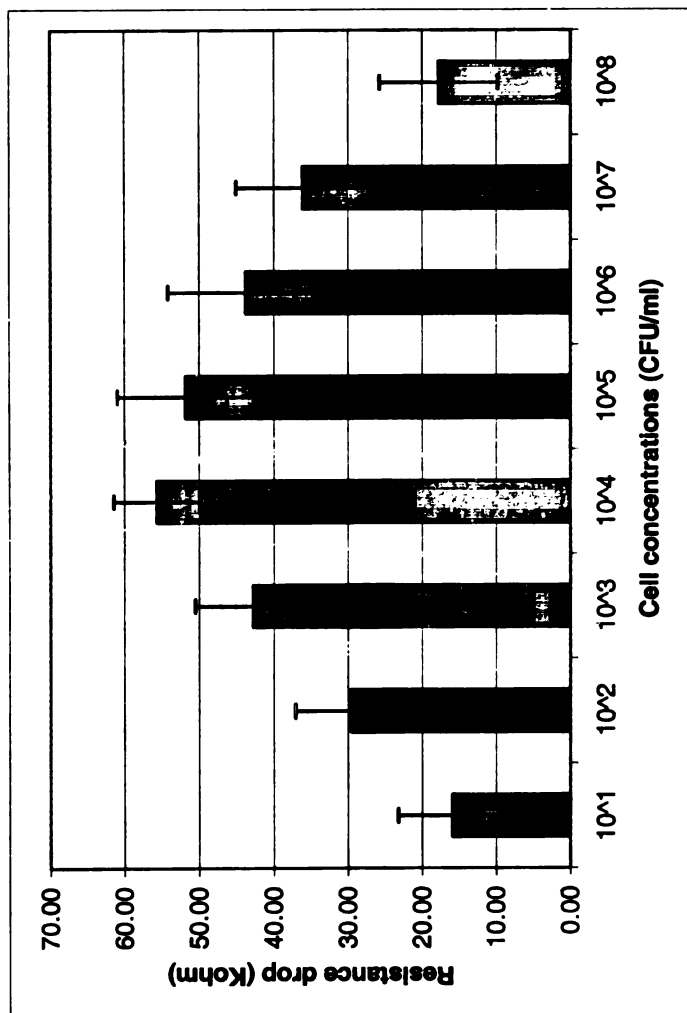


Figure C.10.c: Result of Sal biosensor with varying concentrations of *S. Thompson* in pure culture

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	
0	Blank	123.6	132	123.8	124.9	114.8	123.6	114.5	117.9	122.0	121.9
0	7.9×10^1	122.6	131	139.5	123.9	113.8	122.6	127.9	135.1	127.4	127.1
0	7.9×10^2	115.3	129.2	127.2	135.1	131.8	136.8	118.3	131	127.8	128.1
0	7.9×10^3	133.3	125.1	114.2	122.8	130	114.9	115.5	118.9	120.9	121.7
0	7.9×10^4	125.9	127	111.4	113.2	125.9	128.7	133.5	136.9	126.0	125.4
0	7.9×10^5	123.4	114.6	129.4	110.4	113.8	127.7	116.2	119.6	118.8	119.3
0	7.9×10^6	134.3	126.1	127.6	123.8	131	115.9	113.4	116.8	123.4	123.6
0	7.9×10^7	126.9	128	123.5	114.2	126.9	129.7	114.7	123.5	122.9	123.4
0	7.9×10^8	117.6	129.7	129.4	129.4	132.8	124.6	130.9	115.8	124.5	126.1

Figure C.11.a: EC biosensor responses with varying concentration of *S. Thompson*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)		
0	Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	-15.70	1.00	1.00	1.00	1.00	-13.40	-17.20	-5.39	-5.93	9.25
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	-3.80	-13.10	-5.86	-8.89	-8.89	4.03
0	-9.70	6.90	9.60	2.10	-15.20	8.70	-1.00	-1.00	1.04	-5.05	-5.05	5.30
0	-2.30	5.00	12.40	11.70	-11.10	-5.10	-19.00	-19.00	-19.00	-4.04	-11.38	8.53
0	0.20	17.40	-5.60	14.50	1.00	-4.10	-1.70	-1.70	-1.70	3.14	0.81	3.38
0	-10.70	5.90	-3.80	1.10	-16.20	7.70	1.10	1.10	1.10	-1.41	-5.50	4.82
0	-3.30	4.00	0.30	10.70	-12.10	-6.10	-0.20	-5.60	-0.88	-4.39	-4.39	2.54
0	-0.80	16.40	12.40	13.50	0.00	-5.10	-18.20	-19.80	-1.27	-6.49	-6.49	10.15

Figure C.11.b: Normalized values for data in Figure C.11.a.

Resistance measured (K ohm)												
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	P-value to the blank
Blank	Blank	112.1	120.5	112.3	113.4	103.3	112.1	103	106.4	113.4	110.4	1
0	7.9×10^1	111.1	119.5	128	112.4	102.3	111.1	116.4	123.6	113.6	115.6	0.1629154
0	7.9×10^2	103.8	117.7	115.7	123.6	120.3	125.3	106.8	119.5	128.7	116.6	0.1599022
0	7.9×10^3	121.8	113.6	102.7	111.3	118.5	103.4	104	107.4	107.9	110.3	0.75827357
0	7.9×10^4	114.4	115.5	109.9	101.7	114.4	117.2	122	125.4	126.3	115.1	0.17383822
0	7.9×10^5	111.9	103.1	117.9	98.9	102.3	116.2	104.7	108.1	118.9	107.9	0.34973359
0	7.9×10^6	122.8	114.6	116.1	112.3	119.5	104.4	101.9	105.3	107.4	112.1	0.64969104
0	7.9×10^7	115.4	116.5	112	102.7	115.4	118.2	103.2	112	115.9	111.9	0.753393903
0	7.9×10^8	112.9	104.1	99.9	99.9	103.3	117.2	121.2	126.2	123.4	110.6	0.97348599

Figure C.12.a: EHEC biosensor responses with varying concentration of S. Thompson

Resistance drop (K ohm)												
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD	
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0	1.00	1.00	-15.70	1.00	1.00	1.00	-13.40	-17.20	-0.20	-5.31	8.45	
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	-3.80	-13.10	-15.30	-9.15	6.94	
0	-9.70	6.90	9.60	2.10	-15.20	8.70	-1.00	-1.00	5.50	1.95	8.04	
0	-2.30	5.00	2.40	11.70	-11.10	-5.10	-19.00	-19.00	-12.90	-6.00	11.45	
0	0.20	17.40	-5.60	14.50	1.00	-4.10	-1.70	-1.70	-5.50	1.79	9.04	
0	-10.70	5.90	-3.80	1.10	-16.20	7.70	1.10	1.10	6.00	0.36	7.65	
0	-3.30	4.00	0.30	10.70	-12.10	-6.10	-0.20	-5.60	-2.50	-1.44	6.92	
0	-0.80	16.40	12.40	13.50	0.00	-5.10	-18.20	-19.80	-10.00	-1.35	14.34	

Figure C.12.b: Normalized values for data in Figure C.12.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	109.8	106.8	126.3	112.4	117.8	123.2	121.4	125.6	129.8	119.2	1
*7.7 X 10 ¹	98.2	100.5	106.6	105.6	111	116.4	102.4	106.6	110.8	106.5	0.001282
*7.7 X 10 ²	93.8	95.3	107.9	103.6	109	114.4	104.2	108.4	112.6	105.5	0.001394
*7.7 X 10 ³	89.3	93.1	97.7	98.5	97.9	103.3	118.1	115.2	121.4	103.8	0.004683
*7.7 X 10 ⁴	78.8	79.8	79.3	75.8	81.2	76.6	88.4	85.6	89.8	81.7	2.4E-09
*7.7 X 10 ⁵	63.2	65.5	72	66.3	71.7	77.1	75.2	79.4	83.6	72.7	4.81E-10
*7.7 X 10 ⁶	65.6	66.8	72.3	69.2	64.6	70	67.3	71.5	75.7	69.2	1.06E-11
*7.7 X 10 ⁷	75.2	72.5	74.5	79.9	81.3	82.7	91.2	95.4	93.6	82.9	7.77E-08
*7.7 X 10 ⁸	85.2	87.8	95.8	89.2	94.6	90	101.1	115.3	119.5	97.6	0.000415

Figure C.13.a: EC biosensor responses with varying concentration of non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*7.7 X 10 ¹	11.60	6.30	19.70	6.80	6.80	6.80	19.00	19.00	19.00	12.78	6.27	6.27
*7.7 X 10 ²	16.00	11.50	18.40	8.80	8.80	8.80	17.20	17.20	17.20	13.77	4.20	4.20
*7.7 X 10 ³	20.50	13.70	28.60	13.90	19.90	19.90	3.30	10.40	8.40	15.40	7.63	7.63
*7.7 X 10 ⁴	31.00	27.00	47.00	36.60	36.60	46.60	33.00	40.00	40.00	37.53	6.71	6.71
*7.7 X 10 ⁵	46.60	41.30	54.30	46.10	46.10	46.10	46.20	46.20	46.20	46.20	3.33	3.33
*7.7 X 10 ⁶	44.20	40.00	54.00	43.20	53.20	53.20	54.10	54.10	54.10	50.01	5.77	5.77
*7.7 X 10 ⁷	34.60	34.30	51.80	32.50	36.50	40.50	30.20	30.20	36.20	36.31	6.65	6.65
*7.7 X 10 ⁸	24.60	19.00	30.50	23.20	23.20	33.20	20.30	10.30	10.30	21.62	7.84	7.84

Figure C.13.b: Normalized values for data in Figure C.13.a.

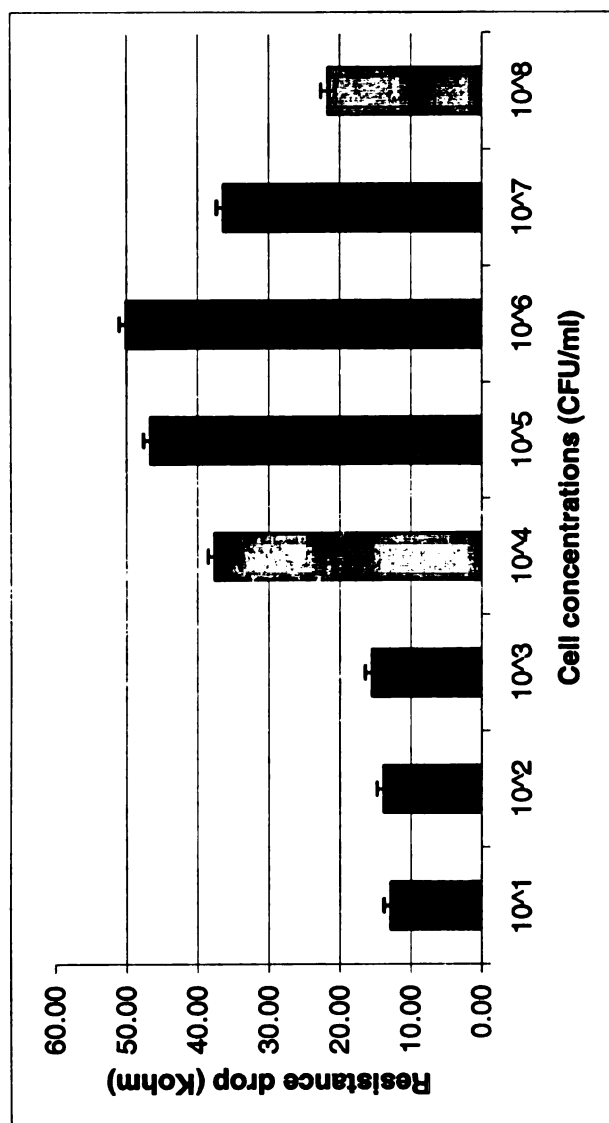


Figure C.13.c: Result of EC biosensor with varying concentrations non-pathogenic *E. coli* in pure culture

Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
		2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	130.5	138.9	130.7	131.8	121.7	130.5	121.4	129.6	132.7	130.0	1
0	7.7×10^1	129.5	137.9	146.4	130.8	120.7	129.5	134.8	146.8	149.9	134.3	0.221445
0	7.7×10^2	122.2	136.1	134.1	142	138.7	143.7	125.2	142.7	145.8	134.7	0.163257
0	7.7×10^3	140.2	132	121.1	129.7	136.9	121.8	122.4	130.6	133.7	127.8	0.518961
0	7.7×10^4	132.8	133.9	118.3	120.1	132.8	135.6	140.4	148.6	151.7	132.9	0.428405
0	7.7×10^5	130.3	121.5	136.3	117.3	120.7	134.6	123.1	131.3	134.4	125.7	0.190648
0	7.7×10^6	141.2	133	134.5	130.7	137.9	122.8	120.3	128.5	131.6	130.3	0.927106
0	7.7×10^7	133.8	134.9	130.4	121.1	133.8	136.6	121.6	135.2	138.3	129.8	0.837333
0	7.7×10^8	131.3	122.5	118.3	118.3	121.7	135.6	139.6	149.4	152.5	130.1	0.969708

Figure C.14.a: Sal biosensor responses with varying concentration of non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	-15.70	1.00	1.00	1.00	-13.40	-17.20	-17.20	-17.20	-6.50	8.96
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	-3.80	-13.10	-13.10	-13.10	-6.97	8.49
0	-9.70	6.90	9.60	2.10	-15.20	8.70	-1.00	-1.00	-1.00	-1.00	-0.07	8.28
0	-2.30	5.00	12.40	11.70	-11.10	-5.10	-19.00	-19.00	-19.00	-19.00	-5.16	12.82
0	0.20	17.40	-5.60	14.50	1.00	-4.10	-1.70	-1.70	-1.70	-1.70	2.03	8.17
0	-10.70	5.90	-3.80	1.10	-16.20	7.70	1.10	1.10	1.10	1.10	-1.41	7.68
0	-3.30	4.00	0.30	10.70	-12.10	-6.10	-0.20	-5.60	-5.60	-5.60	-1.99	6.64
0	-0.80	16.40	12.40	13.50	0.00	-5.10	-18.20	-19.80	-19.80	-19.80	-2.38	14.56

Figure C.14.b: Normalized values for data in Figure C.14.a.

Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
		2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	125.7	132.2	140.6	132.4	133.5	123.4	142.9	123.1	126.5	131.1	1
0	7.7×10^1	124.7	131.2	139.6	148.1	132.5	122.4	141.9	136.5	143.7	135.6	0.249766
0	7.7×10^2	142.7	123.9	137.8	135.8	143.7	140.4	140.1	126.9	139.6	136.8	0.109468
0	7.7×10^3	140.9	141.9	133.7	122.8	131.4	138.6	136	124.1	127.5	133.0	0.590039
0	7.7×10^4	136.8	134.5	135.6	120	121.8	134.5	137.9	142.1	145.5	134.3	0.40494
0	7.7×10^5	124.7	132	120	132.1	130.1	122.4	125.5	124.8	128.2	126.6	0.124661
0	7.7×10^6	141.9	142.9	117.2	131.1	140.4	139.6	137	122	125.4	133.1	0.636829
0	7.7×10^7	137.8	135.5	135.2	127	128.1	135.5	138.9	123.3	132.1	132.6	0.632132
0	7.7×10^8	125.7	133	124.2	120	120	123.4	126.5	141.3	146.3	128.9	0.581454

Figure C.15.a: EHEC biosensor responses with varying concentration of non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)												Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)					
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	1.00	-15.70	1.00	1.00	1.00	-13.40	-17.20	-4.48	8.27	-5.62	9.22	9.48
0	-17.00	8.30	2.80	-3.40	-10.20	-17.00	2.80	-3.80	-13.10	-1.00	-1.84	9.48	-1.84	9.48
0	-15.20	-9.70	6.90	9.60	2.10	-15.20	6.90	-1.00	-1.00	-19.00	-3.16	12.37	-3.16	12.37
0	-11.10	-2.30	5.00	12.40	11.70	-11.10	5.00	17.40	-1.70	4.50	8.40	8.40	-1.70	8.40
0	1.00	0.20	20.60	0.30	3.40	1.00	17.40	-1.70	-1.70	4.50	8.40	8.40	-1.70	8.40
0	-16.20	-10.70	23.40	1.30	-6.90	-16.20	5.90	1.10	1.10	-1.91	12.45	12.45	-1.91	12.45
0	-12.10	-3.30	5.40	5.40	5.40	-12.10	4.00	-0.20	-5.60	-1.46	7.24	7.24	-1.46	7.24
0	0.00	-0.80	16.40	12.40	13.50	0.00	16.40	-18.20	-19.80	2.21	13.94	13.94	2.21	13.94

Figure C.15.b: Normalized values for data in Figure C.15.a.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	117.1	105.9	125.1	130.5	132.6	140.5	138.5	135.6	137.5	129.3	1
*8.3 X 10 ¹	109.6	102.6	105.4	114.1	115.2	115.9	129	118.2	120.1	114.5	0.005695
*8.3 X 10 ²	105.4	94.4	106.7	114.8	123.2	118.9	126.9	126.2	121.8	115.4	0.018571
*8.3 X 10 ³	96.9	98.2	102.5	104.5	107	105.2	106.5	110	109.73	104.5	1.75E-05
*8.3 X 10 ⁴	81.6	79.9	80.1	89.8	90.1	89.8	93	96.1	98.8	88.8	1.01E-07
*8.3 X 10 ⁵	70.4	64.6	70.8	71.9	76.3	77.6	85.7	79.3	88.9	76.2	3.59E-09
*8.3 X 10 ⁶	76.6	65.9	71.1	87.8	91.1	89.1	94	94.1	95.6	85.0	3.07E-07
*8.3 X 10 ⁷	84.3	78.6	89.3	90.4	92.1	87.7	94.2	95.1	96.4	89.8	7.61E-08
*8.3 X 10 ⁸	91.5	86.9	94.6	101	99.2	99.1	105.6	102.2	111.4	99.1	5.4E-06

Figure C.16.a: EHEC biosensor responses with the mixture of *E. coli* O157:H7 (ATCC # 35150), *E. coli* O157:H7 (ATCC # 43895), *S. Thompson*, *S. Typhimurium*, and non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*8.3 X 10 ¹	7.50	3.30	19.70	16.40	17.40	24.60	9.50	17.40	17.40	14.80	6.68	6.68
*8.3 X 10 ²	11.70	11.50	18.40	15.70	9.40	21.60	11.60	11.60	9.40	15.70	13.89	4.22
*8.3 X 10 ³	20.20	7.70	22.60	26.00	25.60	35.30	32.00	25.60	27.77	24.75	7.84	7.84
*8.3 X 10 ⁴	35.50	26.00	45.00	40.70	42.50	50.70	45.50	39.50	38.70	40.46	7.01	7.01
*8.3 X 10 ⁵	46.70	41.30	54.30	58.60	56.30	62.90	52.80	52.80	48.60	53.09	6.60	6.60
*8.3 X 10 ⁶	40.50	40.00	54.00	42.70	41.50	51.40	44.50	41.50	41.90	44.22	5.02	5.02
*8.3 X 10 ⁷	32.80	27.30	35.80	40.10	40.50	52.80	44.30	40.50	41.10	39.47	7.18	7.18
*8.3 X 10 ⁸	25.60	19.00	30.50	29.50	33.40	41.40	32.90	33.40	26.10	30.20	6.30	6.30

Figure C.16.b: Normalized values for data in Figure C.16.a.

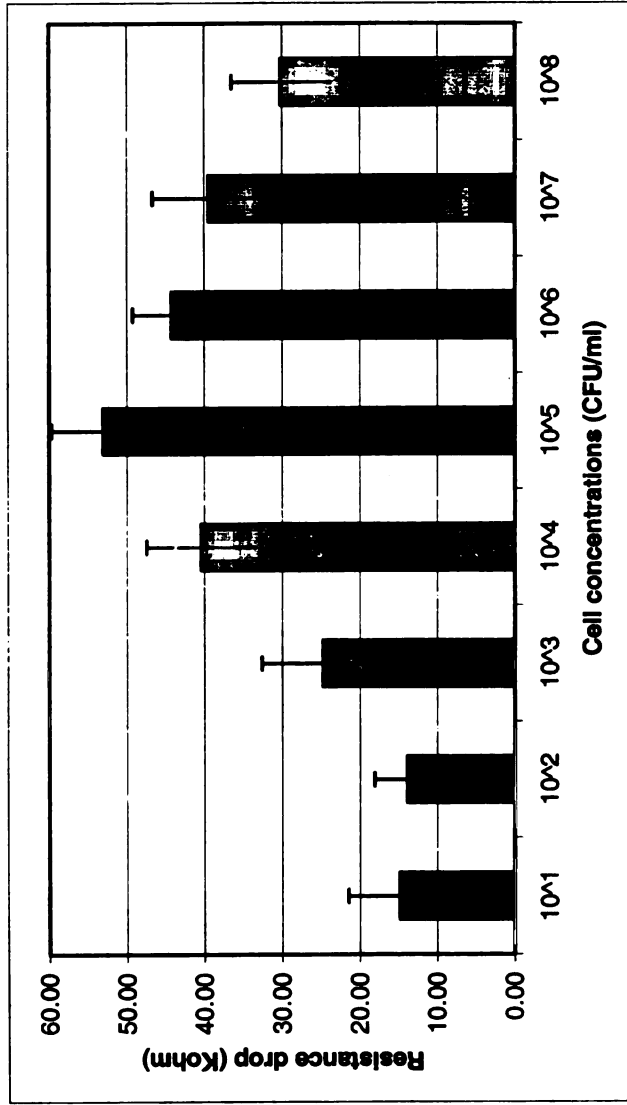


Figure C.16.c: Results of EHEC biosensor responses with the mixture of *E. coli* O157:H7 (ATCC # 35150), *E. coli* O157:H7 (ATCC # 43895), *S. Typhimurium*, *S. Thompson*, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank 1
	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	6min (trial 1)	6min (trial 2)	4min (trial 3)	4min (trial 2)	6min (trial 1)	6min (trial 2)	
	Average	Average	Average	Average	Average	Average	Average	Average	Average	Average	
Blank	113.6	109.1	128.6	114.7	122.4	125.5	123.7	127.9	132.7	122.0	0.00122
*7.2 X 10 ¹	102	102.8	106.9	107.9	115.6	116.7	106.5	110.7	115.5	108.3	0.001269
*7.2 X 10 ²	97.6	97.6	110.2	105.9	113.6	116.7	106.5	110.7	115.5	108.3	0.004087
*7.2 X 10 ³	93.1	95.4	100	100.8	102.5	105.6	120.4	117.5	124.3	106.6	2.12E-09
*7.2 X 10 ⁴	82.6	82.1	81.6	78.1	85.8	78.9	90.7	87.9	92.7	84.5	3.72E-10
*7.2 X 10 ⁵	67	67.8	74.3	68.6	76.3	79.4	77.5	81.7	86.5	75.5	6.78E-12
*7.2 X 10 ⁶	69.4	69.1	74.6	71.5	69.2	72.3	69.6	73.8	78.6	72.0	6.5E-08
*7.2 X 10 ⁷	79	74.8	76.8	82.2	85.9	85	93.5	97.7	96.5	85.7	0.000376
*7.2 X 10 ⁸	89	90.1	98.1	91.5	99.2	92.3	103.4	117.6	122.4	100.4	

Figure C.17 a: EC biosensor responses with the mixture of *E. coli* O157:H7 (ATCC #35150), *E. coli* O157:H7 (ATCC #43895), *S. Typhimurium*, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
	Average	Average	Average	Average	Average	Average	Average	Average	Average	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*7.2 X 10 ¹	11.60	6.30	19.70	6.80	6.80	6.80	19.00	19.00	19.00	12.78	5.91	
*7.2 X 10 ²	16.00	11.50	18.40	8.80	8.80	8.80	17.20	17.20	17.20	13.77	3.96	
*7.2 X 10 ³	20.50	13.70	28.60	13.90	19.90	19.90	3.30	10.40	8.40	15.40	7.20	
*7.2 X 10 ⁴	31.00	27.00	47.00	36.60	36.60	46.60	33.00	40.00	40.00	37.53	6.32	
*7.2 X 10 ⁵	46.60	41.30	54.30	46.10	46.10	46.10	46.20	46.20	46.20	46.57	3.14	
*7.2 X 10 ⁶	44.20	40.00	54.00	43.20	53.20	53.20	54.10	54.10	54.10	50.01	5.44	
*7.2 X 10 ⁷	34.60	34.30	51.80	32.50	36.50	40.50	30.20	30.20	36.20	36.31	6.27	
*7.2 X 10 ⁸	24.60	19.00	30.50	23.20	23.20	33.20	20.30	10.30	10.30	21.62	7.39	

Figure C.17.b: Normalized values for data in Figure C.17.a.

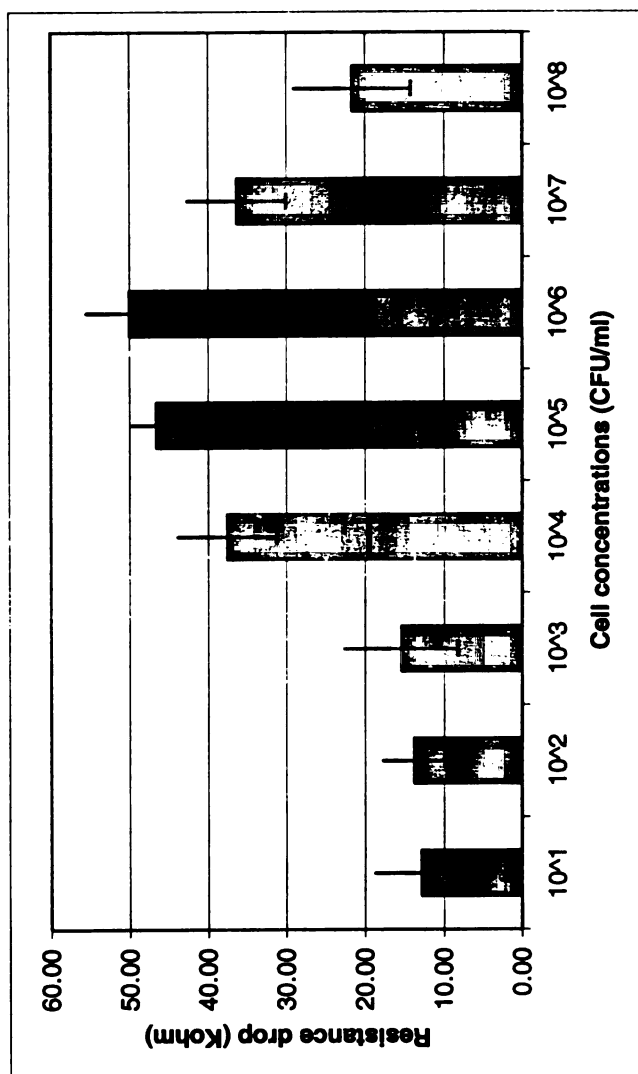


Figure C.17.c: Results of EC biosensor responses with the mixture of *E. coli* O157:H7 (ATCC # 35150), *E. coli* O157:H7 (ATCC # 43895), *S. Thompson*, *S. Typhimurium*, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	99.4	108.6	100.6	127.6	116.11	120	134.64	129.8	131	118.6	1
9 X 10 ¹	90.8	100.5	90.7	95.7	98.5	102.5	119.6	114.7	118.8	103.5	0.019926
*9 X 10 ²	83.8	79.9	80.8	89.8	84.8	90.6	96.8	99	104.6	90.0	5.71E-05
*9 X 10 ³	70.9	69.4	63.8	73.8	78	76.8	86	82.5	90.6	76.9	6.15E-07
*9 X 10 ⁴	54.2	54	51.9	64.2	65	64.1	75.8	77.7	79	65.1	5.85E-08
*9 X 10 ⁵	58.9	61.3	59.9	62.7	63.3	66.8	77.4	77.8	74.6	67.0	2.36E-08
*9 X 10 ⁶	63.9	68.8	73.9	70.6	76.5	80.6	79.6	84.5	82.7	75.7	2.17E-07
*9 X 10 ⁷	73.6	72.7	72.9	83.6	80.4	84.7	91.7	88.5	90.9	82.1	2.36E-06
*9 X 10 ⁸	88.8	89.9	90.9	94.6	96.7	99.6	124.7	116.7	113.4	101.7	0.015426

Figure C.18.a: Sal biosensor responses with the mixture of *E. coli* O157:H7 (ATCC # 35150), *E. coli* O157:H7 (ATCC # 43895), *S. Thompson*, *S. Typhimurium*, and non- pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9 X 10 ¹	8.60	8.10	9.90	31.90	17.61	17.50	15.04	15.10	12.20	15.11	15.11	7.25
*9 X 10 ²	15.60	28.70	19.80	37.80	31.31	29.40	37.84	30.80	26.40	28.63	28.63	7.37
*9 X 10 ³	28.50	39.20	36.80	53.80	38.11	43.20	48.64	47.30	40.40	41.77	41.77	7.48
*9 X 10 ⁴	45.20	54.60	48.70	63.40	51.11	55.90	58.84	52.10	52.00	53.54	53.54	5.42
*9 X 10 ⁵	40.50	47.30	40.70	64.90	52.81	53.20	57.24	52.00	56.40	51.67	51.67	7.88
*9 X 10 ⁶	35.50	39.80	26.70	57.00	39.61	39.40	55.04	45.30	48.30	42.96	42.96	9.56
*9 X 10 ⁷	25.80	35.90	27.70	44.00	35.71	35.30	42.94	41.30	40.10	36.53	36.53	6.40
*9 X 10 ⁸	10.60	18.70	9.70	33.00	19.41	20.40	9.94	13.10	17.60	16.94	16.94	7.38

Figure C.18.b: Normalized values for data in Figure C.18.a.

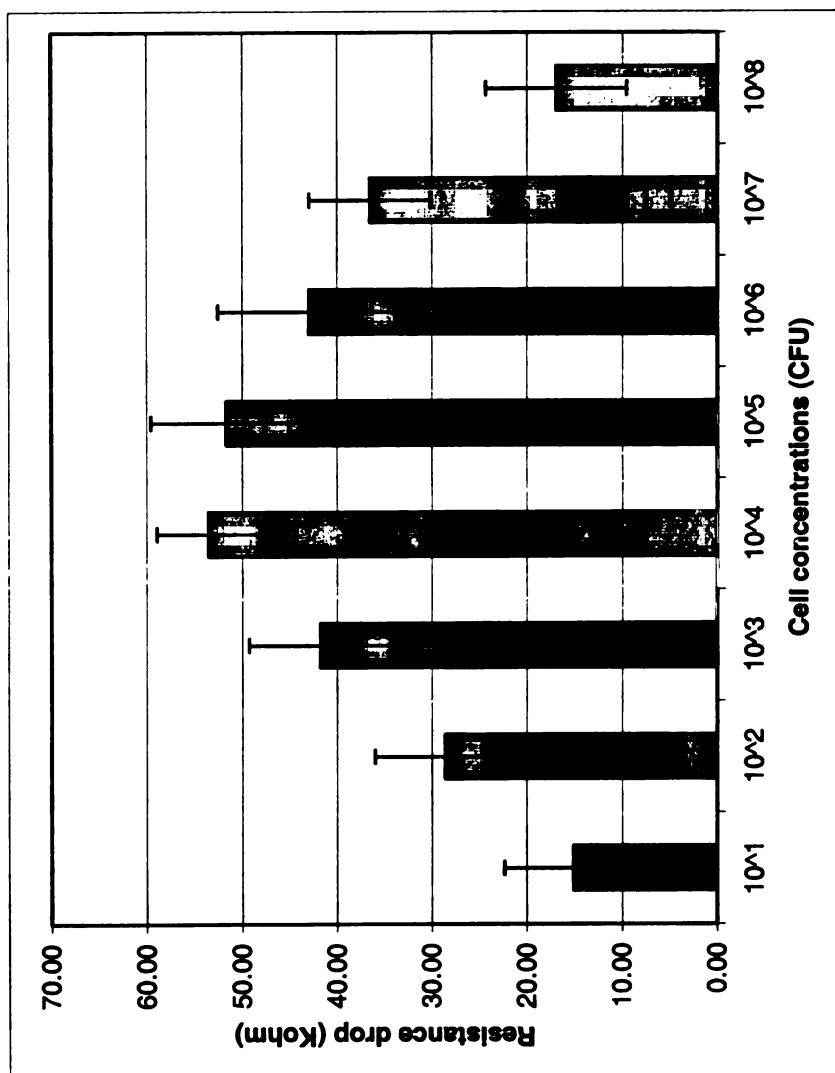


Figure C.18.c: Results of Sal biosensor responses with the mixture of *E. coli* O157:H7 (ATCC # 35150), *E. coli* O157:H7 (ATCC # 43895), *S. Thompson*, *S. Typhimurium*, and non- pathogenic *E. coli*.

APPENDIX D

RESULT FOR CHAPTER 5

PERFORMANCE OF THE ELECTRICAL IMMUNOASSAY BIOSENSOR IN FRESH PRODUCE

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Abbreviations

1. Normalization was calculated by taking the difference between the outputs of the blank and the sample.
2. Plate counts α = Standard plating on differential media according to the type of biosensors used. Plating on Brilliant Green Agar for Sal biosensor, CHROM Agar for EHEC biosensor, and McConkey Agar for EC biosensor.
3. Plate counts α^1 = Standard plating on differential media according to samples tested. Plating on Brilliant Green Agar for *S. Typhimurium* and *S. Thompson*, CHROM Agar for *E. coli* O157:H7, and McConkey Agar for non-pathogenic *E. coli*.
4. STD=Standard deviation
5. *-Estimated bacterial populations by back-counting every step from the last dilution

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	123.1	110.3	118.3	135.2	134.2	120.1	143.2	153.5	124.2	129.1	1
5 X 10 ¹	101.4	99.8	124.5	110.7	105.4	132.1	123.4	115.6	135.6	116.5	0.06E-158
5 X 10 ²	90.2	89.5	86.3	92.1	92.3	88.8	97.4	98.1	92.1	91.9	6.16E-07
5 X 10 ³	72.1	71.5	64.5	74.6	75.6	69.4	78.9	79.1	71.2	73.0	2.77E-09
5 X 10 ⁴	72.3	75.6	85.2	75.6	79.1	86.3	79.9	85.4	87.3	80.7	3.06E-08
5 X 10 ⁵	76.1	79.4	80.2	76.5	84.3	86.3	81.7	85.6	87.2	81.9	2.79E-08
5 X 10 ⁶	75.3	85.6	79.6	79.9	86.8	85.6	85.6	105.6	99.1	87.0	1.07E-06

Figure D.1.a: EHEC biosensor responses on the lettuce samples inoculated with varying concentration of *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance drop(Kohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 X 10 ¹	21.70	10.50	-6.20	24.50	28.80	-12.00	19.80	37.90	-11.40	12.62	18.43	18.43
5 X 10 ²	32.90	20.80	32.00	43.10	41.90	31.30	45.80	55.40	32.10	37.26	10.23	10.23
5 X 10 ³	51.00	38.80	53.80	60.60	58.60	50.70	64.30	74.40	53.00	56.13	9.99	9.99
5 X 10 ⁴	50.80	34.70	33.10	59.60	55.10	33.80	63.30	68.10	36.90	48.38	13.93	13.93
5 X 10 ⁵	47.00	30.90	38.10	58.70	49.90	33.80	61.50	67.90	37.00	47.20	13.25	13.25
5 X 10 ⁶	47.80	24.70	38.70	55.30	47.40	34.50	57.60	47.90	25.10	42.11	12.09	12.09

Figure D.1.b: Normalized values for data in Figure D.1.a

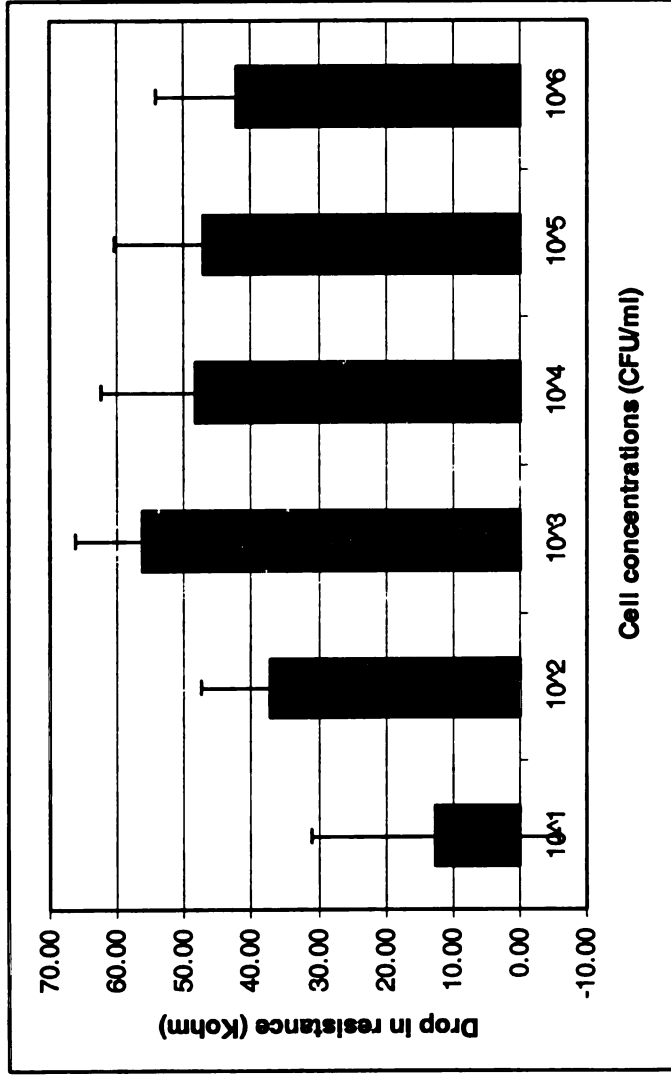


Figure D.1.c: Result of EHEC biosensor responses on the lettuce samples inoculated with varying concentration of *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	
Blank	Blank	121.1	136.8	124.5	111.5	108.7	126.7	124.9	120.8	133.1	123.1
0	5 X 10 ¹	122.2	121.2	132.4	120.1	110.5	107.7	121.1	111.5	132.1	119.9
0	5 X 10 ²	112.1	111.1	129.1	127.3	123.2	111.1	128.3	124.2	130.3	121.9
0	5 X 10 ³	129.3	128.3	126.5	122.4	124.3	111.9	123.4	125.3	126.2	124.2
0	5 X 10 ⁴	102.1	101.1	119.1	104.6	118.4	117.4	105.6	119.4	128.1	112.9
0	5 X 10 ⁵	124.9	140.6	128.3	115.3	112.5	130.5	128.7	124.6	115.7	124.6
0	5 X 10 ⁶	126	125	136.2	123.9	114.3	111.5	124.9	115.3	115.7	121.4
											0.675814

Figure D.2.a: Sal biosensor responses on the lettuce samples inoculated with varying concentration of *E. coli* O157:H7.

Resistance drop (K ohm)												
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD	
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0	1.10	-15.60	7.90	8.60	1.80	-19.00	-3.80	-9.30	-1.00	-3.26	9.69	
0	-9.00	-25.70	4.60	15.80	14.50	-15.60	3.40	3.40	-2.80	-1.27	13.61	
0	8.20	-8.50	2.00	10.90	15.60	-14.80	-1.50	4.50	-6.90	1.06	9.90	
0	-19.00	-35.70	-5.40	-6.90	9.70	-9.30	-19.30	-1.40	-5.00	-10.26	12.97	
0	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	-17.40	1.44	7.07	
0	4.90	-11.80	11.70	12.40	5.60	-15.20	0.00	-5.50	-17.40	-1.70	11.31	

Figure D.2.b: Normalized values for data in Figure D.2.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	125.5	112.7	120.7	137.6	136.6	122.5	145.6	155.9	126.6	132.3	1
6 X 10 ¹	102.6	101	125.7	111.9	106.6	133.3	124.6	116.8	136.8	119.6	0.081347
6 X 10 ²	92.6	91.9	88.7	94.5	94.7	91.2	99.8	100.5	94.5	94.5	4.56E-06
6 X 10 ³	74.5	73.9	66.9	77	78	71.8	81.3	81.5	73.6	75.5	4.4E-08
6 X 10 ⁴	77.3	80.6	81.4	77.7	85.5	87.5	82.9	86.8	88.4	83.9	2.37E-07
6 X 10 ⁵	56.8	96	95.8	101.1	103.4	100.2	109.4	112.1	110.9	103.6	0.000143
6 X 10 ⁶	67.7	98	92	102.3	109.2	108.2	118	128	101.5	107.2	0.001682

Figure D.3.a: EC biosensor responses on the lettuce samples inoculated with varying concentration of *E. coli* O157:H7

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD 0.00
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6 X 10 ¹	22.90	11.70	-5.00	25.70	30.00	-10.80	21.00	39.10	-10.20	13.82	18.43	18.43
6 X 10 ²	32.90	20.80	32.00	43.10	41.90	31.30	45.80	55.40	32.10	37.26	10.23	9.99
6 X 10 ³	51.00	38.80	53.80	60.60	58.60	50.70	64.30	74.40	53.00	56.13	48.40	13.25
6 X 10 ⁴	48.20	32.10	39.30	59.90	51.10	35.00	62.70	69.10	38.20	33.11	16.43	12.80
6 X 10 ⁵	68.70	16.70	24.90	36.50	33.20	22.30	36.20	43.80	15.70	28.76	12.80	12.80
6 X 10 ⁶	57.80	14.70	28.70	35.30	27.40	14.30	27.60	27.90	25.10	28.76	12.80	12.80

Figure D.3.b: Normalized values for data in Figure D.3.a

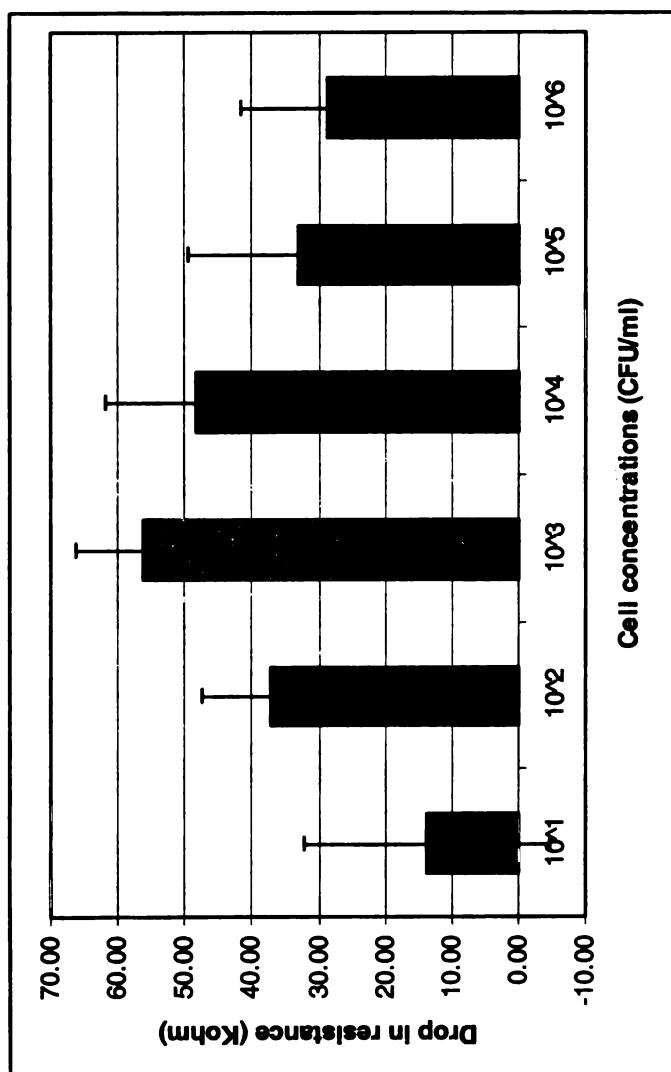


Figure D.3.c: Result of EC biosensor responses on the lettuce samples inoculated with varying concentration of *E. coli* O157:H7.

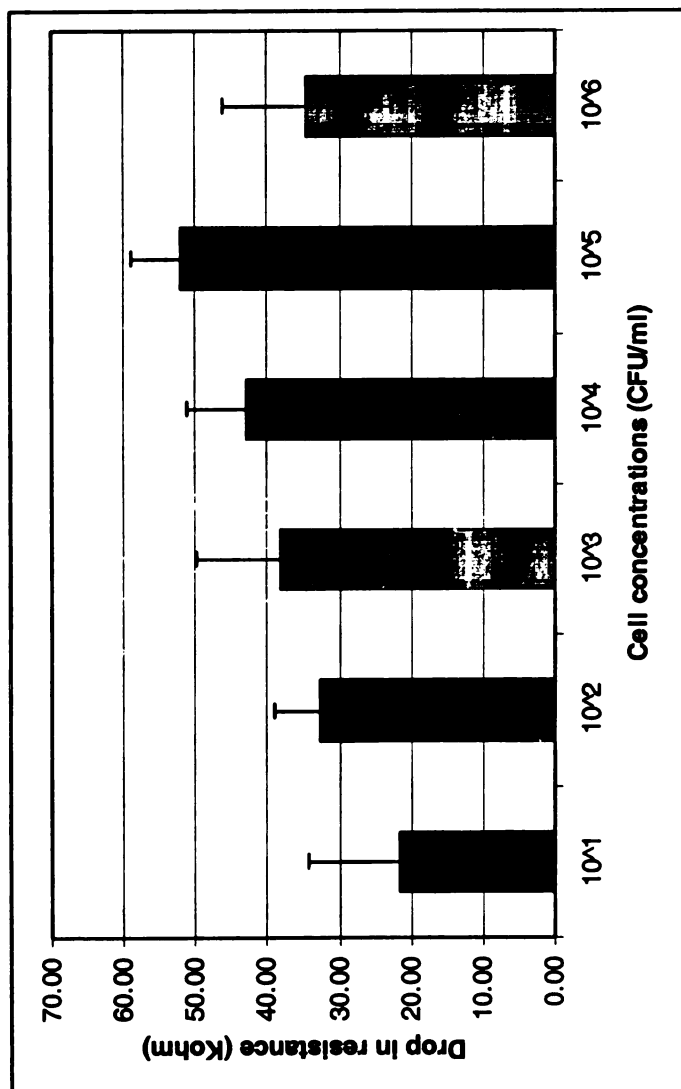


Figure D.4.c: Result of Sal biosensor responses on the lettuce samples inoculated with varying concentration of *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	128.7	120.5	121.6	111.5	120.3	127.2	119	120.1	119.9	1
0	8.2 X 10 ¹	127.7	136.2	120.6	110.5	119.3	126.2	134.7	119.1	109	0.484335
0	8.2 X 10 ²	125.9	123.9	131.8	128.5	133.5	124.4	122.4	130.3	127	0.00592
0	8.2 X 10 ³	121.8	110.9	119.5	126.7	111.6	120.3	109.4	118	125.2	0.564899
0	8.2 X 10 ⁴	123.7	108.1	109.9	122.6	125.4	122.2	106.6	108.4	121.1	0.319259
0	8.2 X 10 ⁵	111.3	126.1	107.1	110.5	124.4	109.8	124.6	105.6	109	0.122014
0	8.2 X 10 ⁶	122.8	124.3	120.5	127.7	112.6	121.3	122.8	119	126.2	0.433071

Figure D.5.a: EC biosensor responses on the lettuce samples inoculated with varying concentration of S. Typhimurium.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	-15.70	1.00	1.00	1.00	1.00	-15.70	1.00	1.00	1.00	-2.71	7.36
0	2.80	-3.40	-10.20	-17.00	-13.20	2.80	-3.40	-10.20	-17.00	-7.64	7.69	
0	6.90	9.50	2.10	-15.20	8.70	6.90	9.60	2.10	-15.20	1.72	10.00	
0	5.00	12.40	11.70	-11.10	-5.10	5.00	12.40	11.70	-11.10	3.43	9.99	
0	17.40	-5.60	14.50	1.00	-4.10	17.40	-5.60	14.50	1.00	5.61	10.15	
0	5.90	-3.80	1.10	-16.20	7.70	5.90	-3.80	1.10	-16.20	-2.03	9.00	

Figure D.5.b: Normalized values for data in Figure D.5.a

Plate counts ^a (CFU/ml)	Plate counts ^{a†} (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
		2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	129.9	128.9	140.1	127.8	118.2	115.4	128.8	119.2	130.6	126.5	1
0	8.2 X 10 ¹	119.8	118.8	136.8	135	130.9	118.8	136	131.9	129.6	128.6	0.569391
0	8.2 X 10 ²	128.6	127.6	141.8	119.9	133.7	132.7	120.9	134.7	140.8	131.2	0.219319
0	8.2 X 10 ³	139.4	138.4	136.6	132.5	134.4	122	133.5	135.4	128.5	133.4	0.042983
0	8.2 X 10 ⁴	131.2	146.9	134.6	121.6	118.8	136.8	135	130.9	118.9	130.5	0.338137
0	8.2 X 10 ⁵	126.9	125.9	140.1	118.2	132	131	119.2	133	116.1	126.9	0.917291
0	8.2 X 10 ⁶	137.7	136.7	134.9	130.8	132.7	120.3	131.8	133.7	129.5	132.0	0.094809

Figure D.6.a: EHEC biosensor responses on the lettuce samples inoculated with varying concentration of *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	10.10	10.10	3.30	-7.20	-12.70	-3.40	-7.20	-12.70	1.00	-2.08	8.77	
0	1.30	1.30	-1.70	7.90	-15.50	-17.30	7.90	-15.50	-10.20	-4.64	10.13	
0	-9.50	-9.50	3.50	-4.70	-16.20	-6.60	-4.70	-16.20	2.10	-6.87	6.94	
0	-1.30	-18.00	5.50	6.20	-0.60	-21.40	-6.20	-11.70	11.70	-3.98	11.31	
0	3.00	3.00	0.00	9.60	-13.80	-15.60	9.60	-13.80	14.50	-0.39	11.38	
0	-7.80	-7.80	5.20	-3.00	-14.50	-4.90	-3.00	-14.50	1.10	-5.47	6.55	

Figure D.6.b: Normalized values for data in Figure D.6.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	132.1	115.3	110.4	135.2	136.7	115.4	143.2	149.8	124.7	129.2	1
6.3 X 10 ¹	101	96.4	124.5	119.2	112.4	132.1	119.2	112.4	132.1	116.6	0.056988
6.3 X 10 ²	94.3	89.5	80.6	92.1	92.3	88.8	97.4	76.4	92.1	89.3	6.47E-07
6.3 X 10 ³	72.1	71.5	64.5	74.6	75.6	69.4	78.9	79.1	71.2	73.0	2.83E-09
6.3 X 10 ⁴	74.1	72.3	80.2	75.5	87.2	86.3	82.7	85.6	86.4	81.1	3.95E-08
6.3 X 10 ⁵	76.5	75.6	87.6	75.6	79.6	86.3	79.9	90.4	85.4	81.9	4.52E-08
6.3 X 10 ⁶	76.4	90.3	79.6	80.4	95.7	85.6	80.4	95.7	85.6	85.5	2.32E-07

Figure D.7.a: EC biosensor responses on the lettuce samples inoculated with varying concentration of non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6.3 X 10 ¹	31.10	18.90	-14.10	16.00	24.30	-16.70	24.00	37.40	-7.40	12.23	18.22	7.25
6.3 X 10 ²	37.80	25.80	29.80	43.10	44.40	26.60	45.80	73.40	32.60	47.70	59.10	11.10
6.3 X 10 ³	60.00	43.80	45.90	60.60	61.10	46.00	64.30	70.70	53.50	59.10	49.37	12.46
6.3 X 10 ⁴	58.00	43.00	30.20	59.70	49.50	29.10	60.50	64.20	38.30	51.00	46.23	13.17
6.3 X 10 ⁵	55.60	39.70	22.80	59.60	57.10	29.10	63.30	59.40	39.30	49.37	46.23	13.17
6.3 X 10 ⁶	55.70	25.00	30.80	54.80	41.00	29.80	62.80	54.10	39.10	46.23	46.23	13.17

Figure D.7.b: Normalized values for data in Figure D.7.a

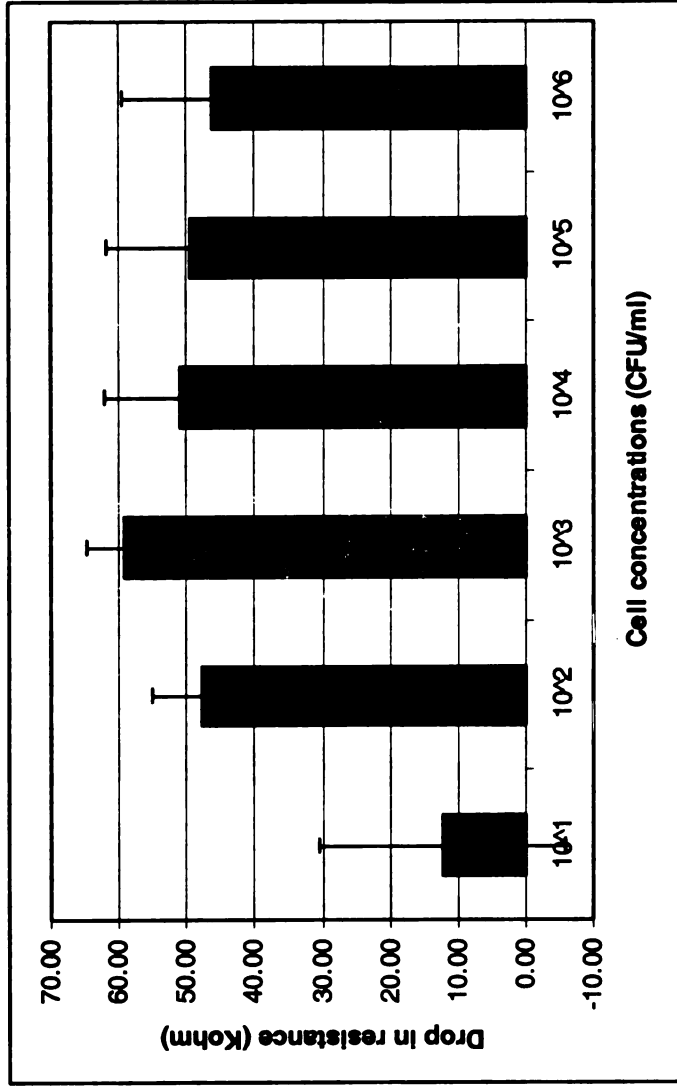


Figure D.4.c: Result of EC biosensor responses on the lettuce samples inoculated with varying concentration of non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	1
0	6.3 X 10 ¹	139.2	123.5	124.6	114.5	131.7	104.5	127.4	119.2	120.3	119.5
0	6.3 X 10 ²	126.9	134.8	131.5	128.9	121.5	124.6	122.6	130.5	127.2	122.3
0	6.3 X 10 ³	113.9	122.5	129.7	124.8	107	120.5	109.6	118.2	125.4	119.1
0	6.3 X 10 ⁴	111.1	112.9	125.6	126.7	120.8	122.4	106.8	118.6	121.3	118.5
0	6.3 X 10 ⁵	129.1	118.1	113.5	114.3	119.8	118	124.8	115.8	109.2	118.1
0	6.3 X 10 ⁶	127.3	123.5	130.7	125.8	108	121.5	123	119.2	126.4	122.8

Figure D.8.a: Sal biosensor responses on the lettuce samples inoculated with varying concentration of non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total		
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-15.70	1.00	1.00	1.00	1.00	1.00	-15.70	1.00	1.00	1.00	9.64	9.64
0	-3.40	-10.20	-17.00	2.80	-17.00	2.80	-3.40	-10.20	-17.00	-1.53	7.85	7.85
0	9.60	2.10	-15.20	6.90	-2.50	6.90	9.60	2.10	-15.20	5.30	12.40	12.40
0	12.40	11.70	-11.10	5.00	-16.30	5.00	12.40	1.70	-11.10	3.90	15.29	15.29
0	-5.60	6.50	1.00	17.40	-15.30	9.40	-5.60	4.50	1.00	10.43	8.20	8.20
0	-3.80	1.10	-16.20	5.90	-3.50	5.90	-3.80	1.10	-16.20	4.30	7.25	7.25

Figure D.8.b: Normalized values for data in Figure D.8

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	120.7	121.8	111.7	120.5	124.7	140.4	128.1	115.1	122	122.8	
0	6.3 X 10 ¹	136.4	120.8	110.7	119.5	125.8	124.8	136	123.7	128.9	0.539696
0	6.3 X 10 ²	124.1	132	128.7	133.7	115.7	114.7	132.7	130.9	120.7	0.406407
0	6.3 X 10 ³	111.1	119.7	126.9	111.8	132.9	131.9	130.1	126	121	0.855762
0	6.3 X 10 ⁴	108.3	110.1	122.8	125.6	105.7	104.7	122.7	108.2	135.2	0.14948
0	6.3 X 10 ⁵	126.3	107.3	110.7	124.6	128.5	129.6	119.5	118.9	113.3	0.456228
0	6.3 X 10 ⁶	124.5	120.7	127.9	112.8	144.2	128.6	118.5	127.5	127.1	0.465233

Figure D.9.a: EHEC biosensor responses on the lettuce samples inoculated with varying concentration of non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-15.70	1.00	1.00	-1.10	15.60	-7.90	-8.60	-6.90	2.67	3.67		
0	-3.40	-10.20	-17.00	-13.20	9.00	25.70	-4.60	-15.80	1.30	-1.10	6.82	
0	9.60	2.10	-15.20	8.70	-8.20	8.50	-2.00	-10.90	1.00	2.10	4.69	
0	12.40	11.70	-11.10	-5.10	19.00	35.70	5.40	6.90	-13.20	12.50	16.16	
0	-5.60	14.50	1.00	-4.10	-3.80	10.80	8.60	-3.80	8.70	0.97	7.19	
0	-3.80	1.10	-16.20	7.70	-19.50	11.80	9.60	-12.40	-5.10	2.37	14.55	

Figure D.9.b: Normalized values for data in Figure D.9.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	80.2	78.5	89.5	75.6	79.5	80.2	80.9	85.4	84.1	81.5	1
8.5 X 10 ¹	59.5	55.8	55.6	66.5	59.8	65.2	60.1	64.5	62	61.0	9.02E-09
8.5 X 10 ²	48.2	45.1	44.2	48.6	45.2	49.2	56.9	60.9	59.6	59.9	2.24E-09
8.5 X 10 ³	45.6	54.2	51.2	50.2	55.4	55.6	54	56.9	55.6	53.2	4.52E-11
8.5 X 10 ⁴	64.2	60.1	69.5	55.6	56.9	56.9	54.1	53.2	49.6	57.8	4.02E-08
8.5 X 10 ⁵	61	60.5	65.2	59.5	59.5	60.2	55.2	55.9	54.6	59.1	9.6E-10
8.5 X 10 ⁶	65.5	66.9	66.5	69.5	65.9	72.1	62.1	60.1	64.5	65.9	2.28E-07

Figure D.10.a: Sal biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.5 X 10 ¹	20.7	22.7	33.9	9.1	19.7	15.0	20.8	20.9	22.1	20.5	6.6	6.6
8.5 X 10 ²	32.0	33.4	45.3	27.0	34.3	31.0	24.0	24.5	24.5	30.7	6.8	6.8
8.5 X 10 ³	34.6	24.3	38.3	25.4	24.1	24.6	26.9	28.5	28.5	28.4	5.0	5.0
8.5 X 10 ⁴	16.0	18.4	20.0	20.0	22.6	23.3	26.8	32.2	34.5	23.8	6.3	6.3
8.5 X 10 ⁵	19.2	18.0	24.3	16.1	20.0	20.0	25.7	29.5	29.5	22.5	5.0	5.0
8.5 X 10 ⁶	14.7	11.6	23.0	6.1	13.6	8.1	18.8	25.3	19.6	15.6	6.5	6.5

Figure D.10.b: Normalized values for data in Figure D.10.a

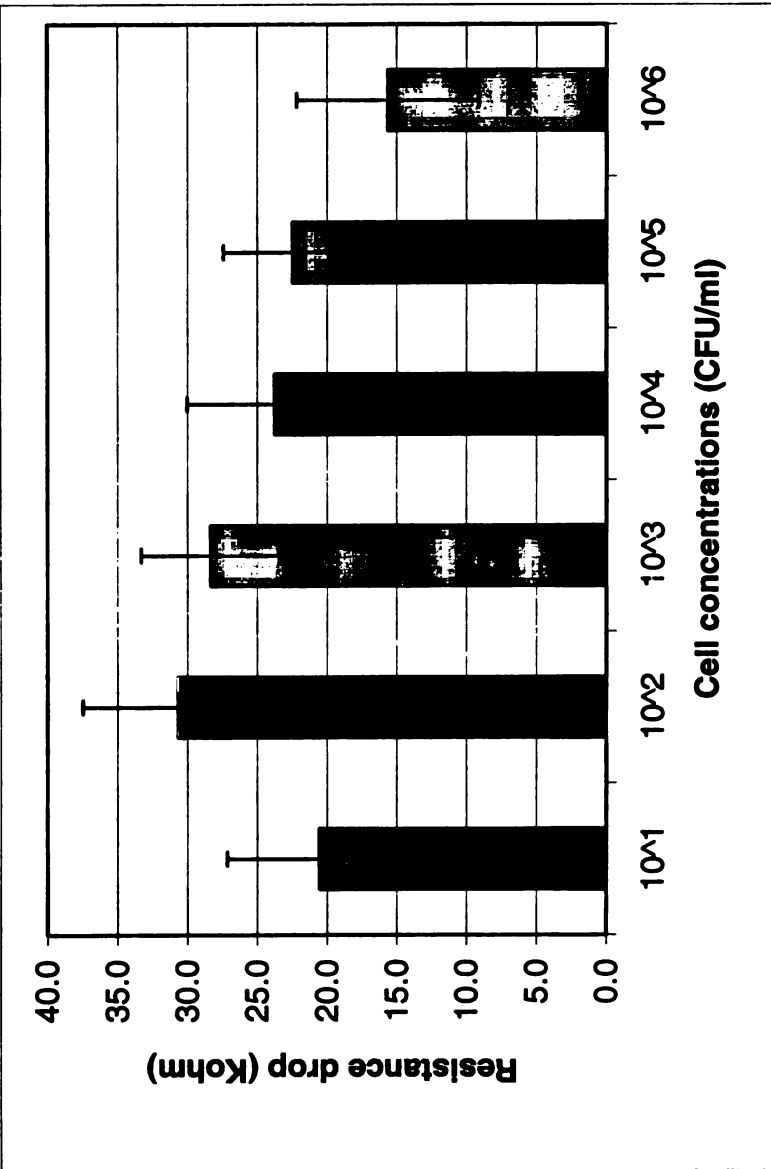


Figure D.10.c: Result of Sal biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	95.4	99.8	98.5	100.1	103.2	100.5	99.6	100.1	100.6	99.8	1
8.9 X 10 ¹	81.1	76.6	80.2	82.2	79.8	85.4	85.4	80.5	79.9	81.2	2.9E-11
8.9 X 10 ²	74.3	70.6	66.9	75.5	70.2	76.9	78.6	75.6	75.6	73.8	4.2E-12
8.9 X 10 ³	69.9	60.5	68.5	69.6	65.5	75.4	75.6	70.5	71.2	69.6	6.09E-12
8.9 X 10 ⁴	76.6	71.2	77.5	69.8	70.5	68.7	60.2	65.9	65.5	69.5	4.05E-11
8.9 X 10 ⁵	75.5	82.6	74.6	68.2	75.5	70.2	68.2	70.5	69.9	72.8	3.63E-11
8.9 X 10 ⁶	85.4	80.5	88.2	79.5	76.8	85.1	73.2	75.4	78.6	80.3	9.89E-09

Figure D.11.a: EC biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.9 X 10 ¹	14.3	23.2	18.3	17.9	23.4	15.1	14.2	19.6	20.7	18.5	3.5	3.5
8.9 X 10 ²	21.1	29.2	31.6	24.6	33.0	23.6	21.0	24.5	25.0	26.0	4.3	4.3
8.9 X 10 ³	25.5	39.3	30.0	30.5	37.7	25.1	24.0	29.6	29.4	30.1	5.3	5.3
8.9 X 10 ⁴	18.8	28.6	21.0	30.3	32.7	31.8	39.4	34.2	35.1	30.2	6.6	6.6
8.9 X 10 ⁵	19.9	17.2	23.9	31.9	27.7	30.3	31.4	29.6	30.7	27.0	5.4	5.4
8.9 X 10 ⁶	10.0	19.3	10.3	20.6	26.4	15.4	26.4	24.7	22.0	19.5	6.3	6.3

Figure D.11.b: Normalized values for data in Figure D.11.a

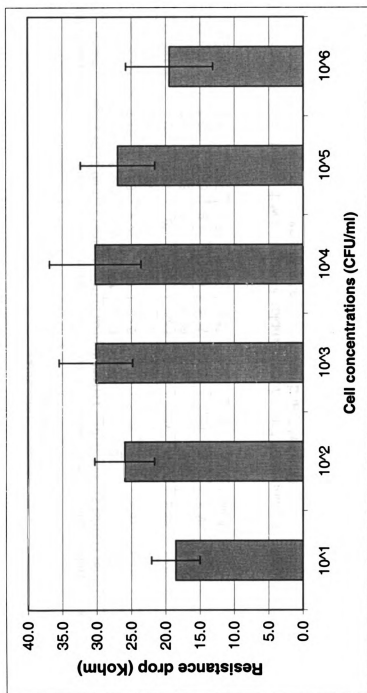


Figure D.11 c: Result of EC biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	100.2	100.6	100.6	98.6	99.8	99.8	100.5	99.8	102.3	100.2	1
7.3 X 10 ¹	83.9	76.5	75.6	85.2	75.5	80.2	92.9	79.9	85.6	81.7	5.62E-08
7.3 X 10 ²	65.9	66.5	62.3	74.3	69.5	74	79.6	72.5	79.8	71.6	2.23E-10
7.3 X 10 ³	69.8	68.2	57.8	75.2	69.5	66.9	78.1	70	75.5	70.1	8.19E-11
7.3 X 10 ⁴	75.9	72.6	78.5	74.9	69.6	68.9	65.4	68.5	66.5	71.2	2.3E-12
7.3 X 10 ⁵	75.3	80.1	81.2	69.2	75.6	75.4	65.4	71.9	69.6	73.7	7.43E-11
7.3 X 10 ⁶	82.3	80.2	86.9	75.6	76.5	76.5	74.8	75.6	75.8	78.2	3.86E-11

Figure D.12.a: EHEC biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 X 10 ¹	16.3	24.1	25.0	13.4	24.3	19.4	7.6	19.9	16.7	18.5	5.7	5.7
7 X 10 ²	34.3	34.1	38.3	24.3	30.3	25.6	20.9	27.3	22.5	28.6	6.0	6.0
7 X 10 ³	30.4	32.4	42.8	23.4	30.3	32.7	22.4	29.8	26.8	30.1	6.0	6.0
7 X 10 ⁴	24.3	28.0	22.1	23.7	30.2	30.7	35.1	31.3	35.8	29.0	4.9	4.9
7 X 10 ⁵	24.9	20.5	19.4	29.4	24.2	24.2	35.1	27.9	32.7	26.5	5.3	5.3
7 X 10 ⁶	17.9	20.4	13.7	23.0	23.3	23.1	25.7	24.2	26.5	22.0	4.0	4.0

Figure D.12.b: Normalized values for data in Figure D.12.a

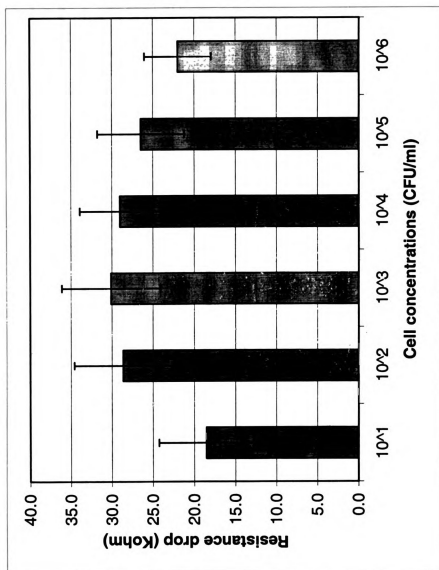


Figure D.12.c: Result of EHEC biosensor responses on the lettuce samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	101.1	103.3	99.1	116.3	113.6	118.7	123.1	121.5	117.6	112.7	1
8 X 10 ¹	89.3	98.3	89.2	94.5	85.8	94.9	99.6	91.9	107.4	94.5	0.000179846
8 X 10 ²	81.3	79.5	79.4	88.4	88.2	87.3	92	94.3	103.2	88.2	1.40252E-05
8 X 10 ³	79.4	77.9	72.3	72.3	90.6	99.7	94.4	96.7	89.1	85.8	2.79757E-05
8 X 10 ⁴	61.5	61.4	59.3	61.6	93	102.1	96.8	99.1	76.4	79.0	0.000167378
8 X 10 ⁵	57.5	59.9	58.3	61.3	95.4	104.5	99.2	101.5	73.2	79.0	0.000389205
8 X 10 ⁶	66.5	67.6	72.4	69.4	97.8	106.9	101.6	103.9	81.3	85.3	0.000614585

Figure D.13.a: Sal biosensor responses on the alfalfa sprout samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8 X 10 ¹	11.80	5.00	9.90	21.80	27.80	23.80	23.50	29.60	10.20	18.16	8.96	5.68
8 X 10 ²	19.80	23.80	19.70	27.90	25.40	31.40	31.10	27.20	14.40	24.52	26.88	7.15
8 X 10 ³	21.70	25.40	26.80	44.00	23.00	19.00	28.70	24.80	28.50	33.68	12.66	14.64
8 X 10 ⁴	39.60	41.90	39.80	54.70	20.60	16.60	26.30	22.40	41.20	33.72	27.43	11.67
8 X 10 ⁵	43.60	43.40	40.80	55.00	18.20	14.20	23.90	20.00	44.40	36.30	27.43	11.67
8 X 10 ⁶	34.60	35.70	26.70	46.90	15.80	11.80	21.50	17.60	36.30	27.43	11.67	11.67

Figure D.13.b: Normalized values for data in Figure D.13.a

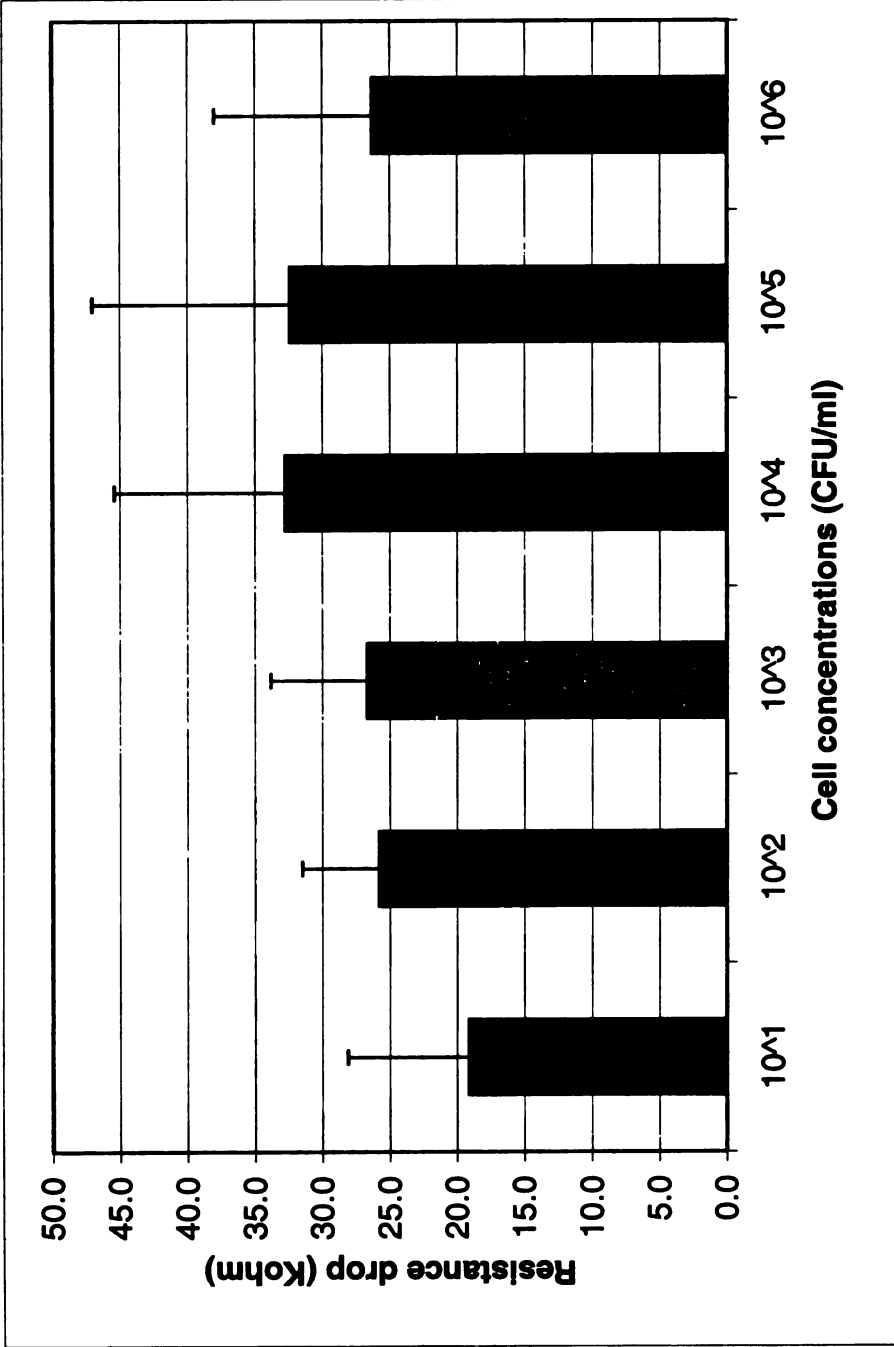


Figure D.13.c: Result of Sal biosensor responses on the alfalfa sprout samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
		2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 1)	6min (trial 2)	Average
Blank	Blank	126.8	135.2	127	128.1	118	126.8	117.7	121.1	125.9	125.2	1
0	8 X 10 ¹	125.8	134.2	142.7	127.1	117	125.8	131.1	138.3	133.1	130.6	0.105577778
0	8 X 10 ²	118.5	132.4	130.4	138.3	135	140	121.5	134.2	129	131.0	0.070129373
0	8 X 10 ³	136.5	128.3	117.4	126	133.2	118.1	118.7	122.1	116.9	124.1	0.735732611
0	8 X 10 ⁴	129.1	130.2	114.6	116.4	129.1	131.9	136.7	140.1	134.9	129.2	0.252313843
0	8 X 10 ⁵	126.6	117.8	132.6	113.6	117	130.9	119.4	122.8	117.6	122.0	0.290158111
0	8 X 10 ⁶	137.5	129.3	130.8	127	134.2	119.1	116.6	120	124.8	126.6	0.64414996

Figure D.14.a: EC biosensor responses on the alfalfa sprout samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)												Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average			
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	-15.70	1.00	1.00	1.00	-13.40	-17.20	-7.20	-5.39	8.04	8.24	8.04
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	-3.80	-13.10	-3.10	-5.86	8.24	8.24	8.24
0	-9.70	6.90	9.60	2.10	-15.20	8.70	-1.00	-1.00	9.00	1.04	8.79	8.79	8.79
0	-2.30	5.00	12.40	11.70	-11.10	-5.10	-19.00	-19.00	-9.00	-4.04	11.86	11.86	11.86
0	0.20	17.40	-5.60	14.50	1.00	-4.10	-1.70	-1.70	8.30	3.14	8.28	8.28	8.28
0	-10.70	5.90	-3.80	1.10	-16.20	7.70	1.10	1.10	1.10	-1.41	7.68	7.68	7.68

Figure D.14.b: Normalized values for data in Figure D.14.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average
Blank	Blank	117.7	121.1	125.9	125.5	133.9	125.7	126.8	116.7	135.5	125.4
0	8 X 10 ¹	131.1	138.3	133.1	124.5	132.9	141.4	125.8	115.7	124.5	129.7
0	8 X 10 ²	121.5	134.2	129	117.2	131.1	129.1	137	133.7	138.7	130.2
0	8 X 10 ³	118.7	122.1	116.9	135.2	127	116.1	124.7	131.9	126.8	124.4
0	8 X 10 ⁴	136.7	140.1	134.9	127.8	128.9	113.3	115.1	127.8	130.6	128.4
0	8 X 10 ⁵	119.4	122.8	117.6	125.3	116.5	131.3	112.3	115.7	129.6	121.2
0	8 X 10 ⁶	116.6	120	124.8	136.2	128	129.5	125.7	132.9	117.8	125.7

Figure D.15.a: EHEC biosensor responses on the alfalfa sprout samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-13.40	-17.20	-7.20	1.00	1.00	-15.70	1.00	1.00	1.00	11.00	-4.28	9.58
0	-3.80	-13.10	-3.10	8.30	2.80	-3.40	-10.20	-17.00	-3.20	-4.74	7.78	7.78
0	-1.00	-1.00	9.00	-9.70	6.90	9.60	2.10	-15.20	8.70	1.04	8.79	8.79
0	-19.00	-19.00	-9.00	-2.30	5.00	12.40	11.70	-11.10	4.90	-2.93	12.22	12.22
0	-1.70	-1.70	8.30	0.20	17.40	-5.60	14.50	1.00	5.90	4.26	7.84	7.84
0	1.10	1.10	1.10	-10.70	5.90	-3.80	1.10	-16.20	17.70	-0.30	9.64	9.64

Figure D.15.b: Normalized values for data in Figure D.15.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	103.7	123.2	109.3	114.7	120.1	118.3	122.5	126.7	103.6	115.8	1
7.7 X 10 ¹	97.4	103.5	102.5	107.9	113.3	99.3	103.5	107.7	92	103.0	0.002338
7.7 X 10 ²	92.2	104.8	100.5	105.9	111.3	101.1	105.3	109.5	87.6	102.0	0.002506
7.7 X 10 ³	90	94.6	95.4	94.8	100.2	115	112.1	118.3	83.1	100.4	0.006545
7.7 X 10 ⁴	76.7	76.2	72.7	78.1	73.5	85.3	82.5	86.7	72.6	78.3	5.77E-09
7.7 X 10 ⁵	62.4	68.9	63.2	68.6	74	72.1	76.3	80.5	57	69.2	1.35E-09
7.7 X 10 ⁶	63.7	69.2	66.1	61.5	66.9	64.2	68.4	72.6	59.4	65.8	3.24E-11

Figure D.16.a: EHEC biosensor responses on the alfalfa sprout samples inoculated with *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.7 X 10 ¹	6.30	19.70	6.80	6.80	6.80	19.00	19.00	19.00	19.00	11.60	12.78	6.27
7.7 X 10 ²	11.50	18.40	8.80	8.80	8.80	17.20	17.20	17.20	16.00	13.77	4.20	4.20
7.7 X 10 ³	13.70	28.60	13.90	19.90	19.90	3.30	10.40	8.40	20.50	15.40	7.63	7.63
7.7 X 10 ⁴	27.00	47.00	36.60	46.60	46.60	33.00	40.00	40.00	40.00	31.00	37.53	6.71
7.7 X 10 ⁵	41.30	54.30	46.10	46.10	46.10	46.20	46.20	46.20	46.20	46.60	46.57	3.33
7.7 X 10 ⁶	40.00	54.00	43.20	53.20	53.20	54.10	54.10	54.10	54.10	44.20	50.01	5.77

Figure D.16.b: Normalized values for data in Figure D.16.a

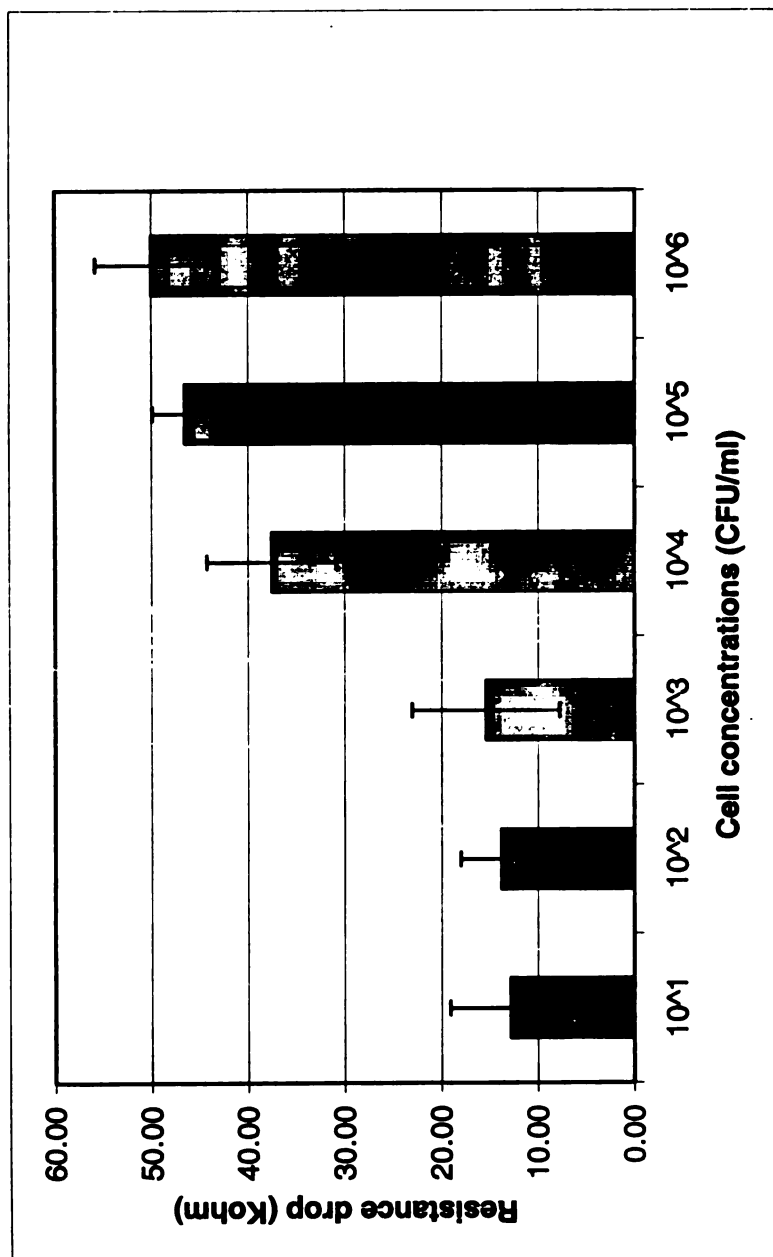


Figure D.13.c: Result of EHEC biosensor responses on the alfalfa sprout samples inoculated with *E. coli* O157:H7

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average
Blank	Blank	128.1	136.5	128.3	129.4	119.3	128.1	138.9	130.7	131.8	130.1
0	7.7 X 10 ¹	127.1	135.5	144	128.4	118.3	127.1	137.9	146.4	130.8	132.8
0	7.7 X 10 ²	119.8	133.7	131.7	139.6	136.3	141.3	136.1	134.1	142	135.0
0	7.7 X 10 ³	137.8	129.6	118.7	127.3	134.5	119.4	132	121.1	129.7	127.8
0	7.7 X 10 ⁴	130.4	131.5	115.9	117.7	130.4	133.2	133.9	118.3	120.1	125.7
0	7.7 X 10 ⁵	127.9	119.1	133.9	114.9	118.3	132.2	121.5	136.3	117.3	124.6
0	7.7 X 10 ⁶	138.8	130.6	132.1	128.3	135.5	120.4	133	134.5	130.7	131.5

Figure D.17.a: Sal biosensor responses on the alfalfa sprout samples inoculated with *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total		
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	-15.70	1.00	1.00	1.00	1.00	-15.70	1.00	-2.71	7.36	7.36
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	2.80	-3.40	-10.20	-4.83	8.43	8.43
0	-9.70	6.90	9.60	2.10	-15.20	8.70	6.90	9.60	2.10	2.33	8.95	8.95
0	-2.30	5.00	12.40	11.70	-11.10	-5.10	5.00	12.40	11.70	4.41	8.74	8.74
0	0.20	17.40	-5.60	14.50	1.00	-4.10	17.40	-5.60	14.50	5.52	10.20	10.20
0	-10.70	5.90	-3.80	1.10	-16.20	7.70	5.90	-3.80	1.10	-1.42	8.05	8.05

Figure D.17.b: Normalized values for data in Figure D.17.a

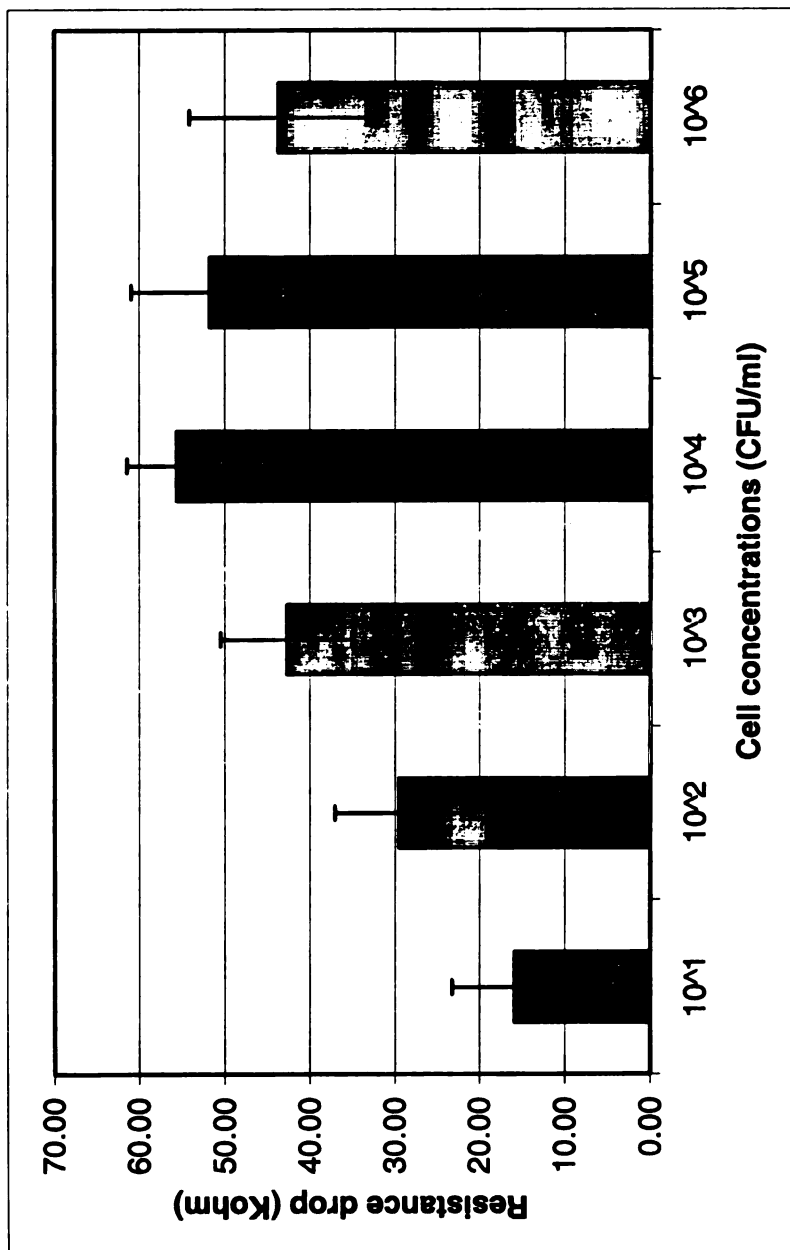


Figure D.18.c: Result of EC biosensor responses on the alfalfa sprout samples inoculated with *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	115.7	104.2	123.7	129.1	130.8	139.1	137.1	138.1	136.1	128.2	1
8.2 X 10 ¹	108.2	97.9	104	112.7	113.4	114.5	127.6	124	125.5	114.2	0.015584
8.2 X 10 ²	104	92.7	105.3	113.4	111.4	117.5	125.5	127.2	130.4	114.2	0.025439
8.2 X 10 ³	95.5	90.5	101.1	103.1	105.2	103.8	105.1	107.2	108.33	102.2	2.11E-05
8.2 X 10 ⁴	75.2	77.2	76.7	81.4	83.3	86.4	91.6	92.9	93.4	84.2	5.35E-08
8.2 X 10 ⁵	69	62.9	69.4	70.5	74.5	76.2	84.3	86.4	87.5	75.6	9.99E-09
8.2 X 10 ⁶	75.2	64.2	69.7	86.4	89.3	87.7	92.6	93.2	94.2	83.6	3.68E-07

Figure D.19.a: EC biosensor responses on the alfalfa sprout samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.2 X 10 ¹	7.50	6.30	19.70	16.40	17.40	24.60	9.50	14.10	10.60	14.01	6.08	6.08
8.2 X 10 ²	11.70	11.50	18.40	15.70	19.40	21.60	11.60	10.90	5.70	14.06	5.06	5.06
8.2 X 10 ³	20.20	13.70	22.60	26.00	25.60	35.30	32.00	30.90	27.77	26.01	6.58	7.22
8.2 X 10 ⁴	40.50	27.00	47.00	47.70	47.50	52.70	45.50	45.20	42.70	43.98	6.50	6.50
8.2 X 10 ⁵	46.70	41.30	54.30	58.60	56.30	62.90	52.80	51.70	48.60	52.58	4.91	4.91
8.2 X 10 ⁶	40.50	40.00	54.00	42.70	41.50	51.40	44.50	44.90	41.90	44.60	4.91	4.91

Figure D.19.b: Normalized values for data in Figure D.19.a

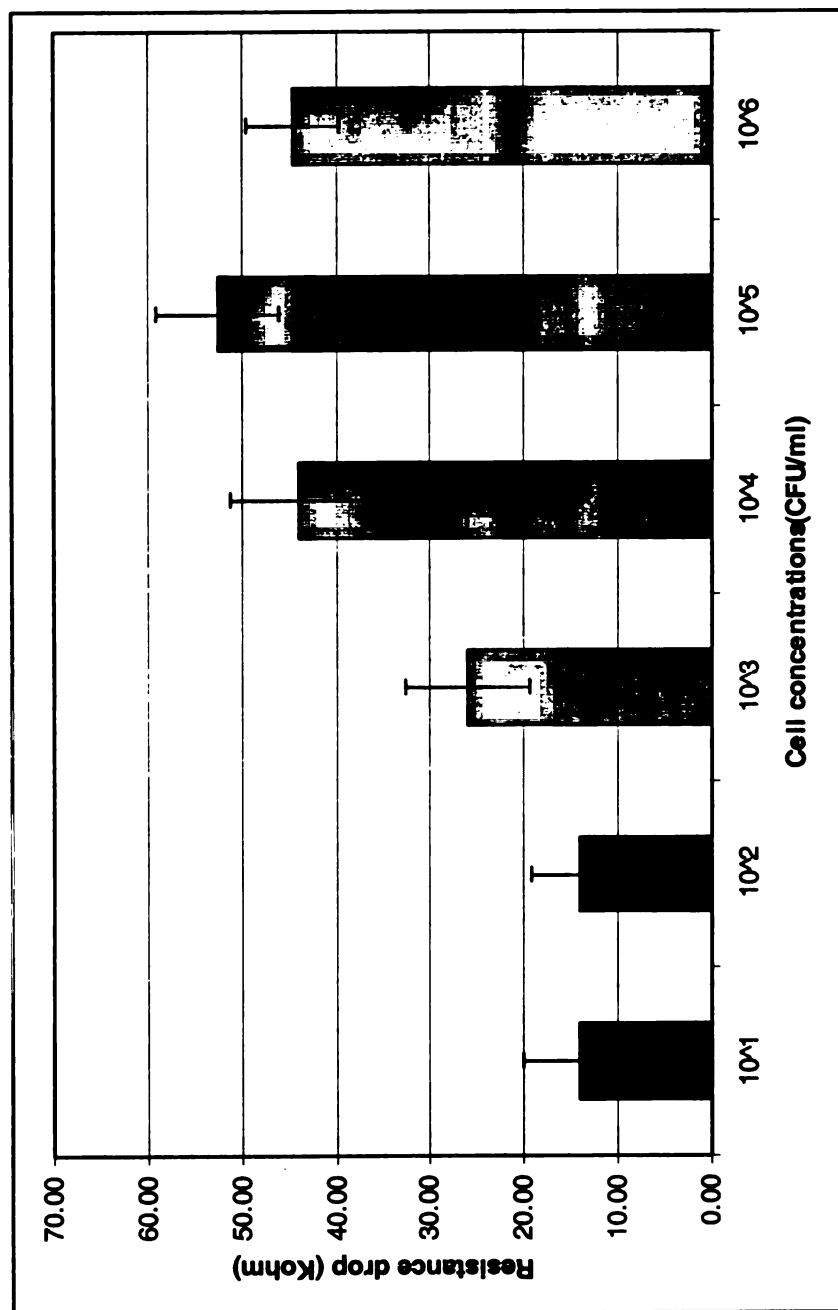


Figure D.19.c: Result of EC biosensor responses on the alfalfa sprout samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	128.9	120.7	121.8	111.7	120.5	123.8	124.9	114.8	132	122.1	1
0	8.2 X 10 ¹	127.9	136.4	120.8	110.7	119.5	139.5	123.9	113.8	131	0.494848
0	8.2 X 10 ²	126.1	124.1	132	128.7	133.7	127.2	135.1	131.8	129.2	0.006372
0	8.2 X 10 ³	122	111.1	119.7	126.9	111.8	114.2	122.8	130	125.1	0.584396
0	8.2 X 10 ⁴	123.9	108.3	110.1	122.8	125.6	111.4	113.2	125.9	127	0.318003
0	8.2 X 10 ⁵	111.5	126.3	107.3	110.7	124.6	129.4	110.4	113.8	114.6	0.120035
0	8.2 X 10 ⁶	123	124.5	120.7	127.9	112.8	127.6	123.8	131	126.1	0.468572

Figure D.20.a: EHEC biosensor responses on the alfalfa sprout samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
BLANK	0	0	0	0	0	0	0	0	0	0	0	0
0	1.00	-15.70	1.00	1.00	1.00	-15.70	1.00	1.00	1.00	-2.71	7.36	
0	2.80	-3.40	-10.20	-17.00	-13.20	-3.40	-10.20	-17.00	2.80	-7.64	7.69	
0	6.90	9.60	2.10	-15.20	8.70	9.60	2.10	-15.20	6.90	1.72	10.00	
0	5.00	12.40	11.70	-11.10	-5.10	12.40	11.70	-11.10	5.00	3.43	9.99	
0	17.40	-5.60	14.50	1.00	-4.10	-5.60	14.50	1.00	17.40	5.61	10.15	
0	5.90	-3.80	1.10	-16.20	7.70	-3.80	1.10	-16.20	5.90	-2.03	9.00	

Figure D.20.b: Normalized values for data in Figure D.20.a

Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	Resistance measured (K ohm)										p-value to the blank
		2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	112.1	120.5	112.3	113.4	103.3	112.1	103	106.4	122	111.7	1
0	8.2×10^1	111.1	119.5	128	112.4	102.3	111.1	116.4	123.6	129.9	117.1	0.162915
0	8.2×10^2	103.8	117.7	115.7	123.6	120.3	125.3	106.8	119.5	115.8	116.5	0.159902
0	8.2×10^3	116	105.1	113.7	120.9	105.8	106.4	109.8	115.8	113.4	111.9	0.945535
0	8.2×10^4	117.9	112.3	104.1	116.8	119.6	124.4	127.8	134.1	131.7	121.0	0.030033
0	8.2×10^5	105.5	120.3	101.3	104.7	118.6	107.1	110.5	116.8	114.4	111.0	0.839522
0	8.2×10^6	117	118.5	114.7	121.9	106.8	104.3	107.7	125	122.6	115.4	0.286838

Figure D.21.a: Sal biosensor responses on the alfalfa sprout samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	-15.70	1.00	1.00	1.00	-13.40	-17.20	-7.90	-5.47	8.06	8.06
0	8.30	2.80	-3.40	-10.20	-17.00	-13.20	-3.80	-13.10	6.20	-4.82	9.16	9.16
0	-3.90	15.40	-1.40	-7.50	-2.50	5.70	-6.80	-9.40	8.60	-0.20	8.35	8.35
0	-5.80	8.20	8.20	-3.40	-16.30	-12.30	-24.80	-27.70	-9.70	-9.29	12.73	12.73
0	6.60	0.20	11.00	8.70	-15.30	5.00	-7.50	-10.40	7.60	0.66	9.47	9.47
0	-4.90	2.00	-2.40	-8.50	-3.50	7.80	-4.70	-18.60	-0.60	-3.71	7.28	7.28

Figure D.21.b: Normalized values for data in Figure D.21.a

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	P-value to the blank
Blank	89.5	85.4	78.5	80.2	80.1	79.5	84.1	79.9	85.4	1
7.6 X 10 ¹	55.6	59.6	55.8	65.2	62.1	59.8	62	66.9	64.5	2.95E-09
7.6 X 10 ²	44.2	59.2	45.1	49.2	65.2	45.2	59.6	65.9	60.9	2.39E-07
7.6 X 10 ³	51.2	55.6	54.2	55.6	60.2	55.4	55.6	64.9	56.9	1.4E-10
7.6 X 10 ⁴	59.5	60.1	60.1	56.9	57.8	56.9	49.6	55.6	53.2	5.95E-11
7.6 X 10 ⁵	60.2	63.2	60.5	60.2	66.9	59.5	54.6	59.6	55.9	6.64E-10
7.6 X 10 ⁶	66.5	75.6	66.9	72.1	69.5	65.9	64.5	65.4	60.1	8.31E-07

Figure D.22.a: Sal biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average Total STD
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.6 X 10 ¹	33.9	25.8	22.7	15.0	18.0	19.7	22.1	13.0	20.9	21.3
7.6 X 10 ²	45.3	26.2	33.4	31.0	14.9	34.3	24.5	14.0	24.5	28.0
7.6 X 10 ³	38.3	29.8	24.3	24.6	19.9	24.1	28.5	15.0	28.5	25.6
7.6 X 10 ⁴	30.0	25.3	18.4	23.3	22.3	22.6	34.5	24.3	32.2	25.1
7.6 X 10 ⁵	29.3	22.2	18.0	20.0	13.2	20.0	29.5	20.3	29.5	21.6
7.6 X 10 ⁶	23.0	9.8	11.6	8.1	10.6	13.6	19.6	14.5	25.3	13.9

Figure D.22.b: Normalized values for data in Figure D.22.a

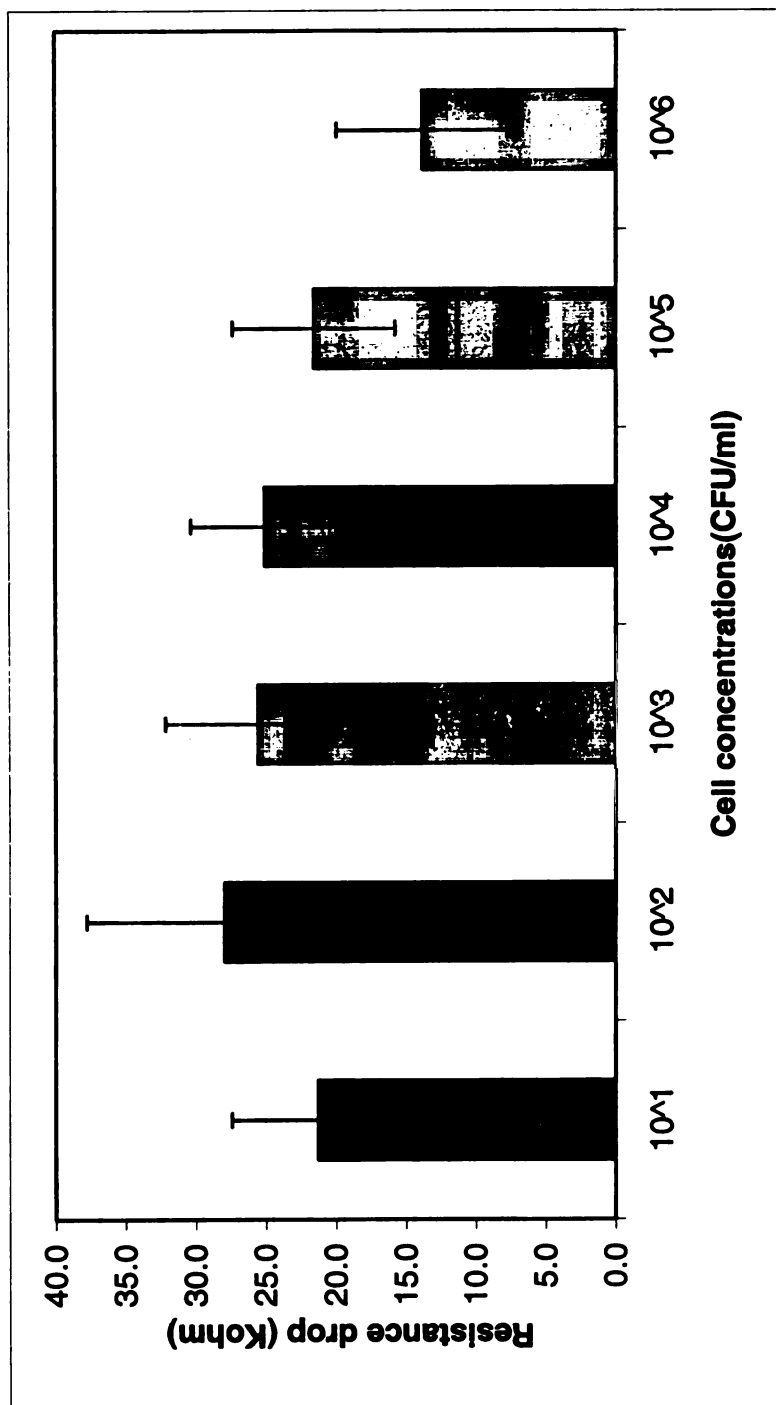


Figure D.22.c: Result of Sal biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	100.6	100.9	100.6	99.6	99.6	99.8	100.6	98.7	100.1	100.1	1
8.9 X 10 ¹	55.6	59.6	55.8	85.4	80.2	79.8	78.6	70.1	75.4	71.2	1.13E-06
8.9 X 10 ²	44.2	59.2	45.1	76.9	75.4	70.2	69.9	65.5	70.5	64.1	1.48E-07
8.9 X 10 ³	51.2	55.6	54.2	65.4	66.5	65.5	65.5	64.1	65.9	61.5	2.14E-12
8.9 X 10 ⁴	59.5	60.1	60.1	68.7	69.5	70.5	71.2	60.2	70.5	65.6	1.98E-12
8.9 X 10 ⁵	66.9	78.5	70.6	70.2	66.9	75.5	75.6	70.2	75.6	72.2	1.25E-12
8.9 X 10 ⁶	80.2	85.5	76.6	75.1	71.6	76.8	79.9	75.4	80.5	78.0	2.45E-11

Figure D.23.a: Result of EC biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.9 X 10 ¹	45.0	41.3	44.8	14.2	19.4	20.0	22.0	28.6	24.7	29.4	11.8	
8.9 X 10 ²	56.4	41.7	55.5	22.7	24.2	29.6	30.7	33.2	29.6	36.8	12.6	
8.9 X 10 ³	49.4	45.3	46.4	34.2	33.1	34.3	35.1	34.6	34.2	39.1	6.5	
8.9 X 10 ⁴	41.1	40.8	40.5	30.9	30.1	29.3	29.4	38.5	29.6	35.1	5.5	
8.9 X 10 ⁵	33.7	22.4	30.0	29.4	32.7	24.3	25.0	28.5	24.5	28.3	4.0	
8.9 X 10 ⁶	20.4	15.4	24.0	24.5	28.0	23.0	20.7	23.3	19.6	22.4	3.6	

Figure D.23.b: Normalized values for data in Figure D.23.a

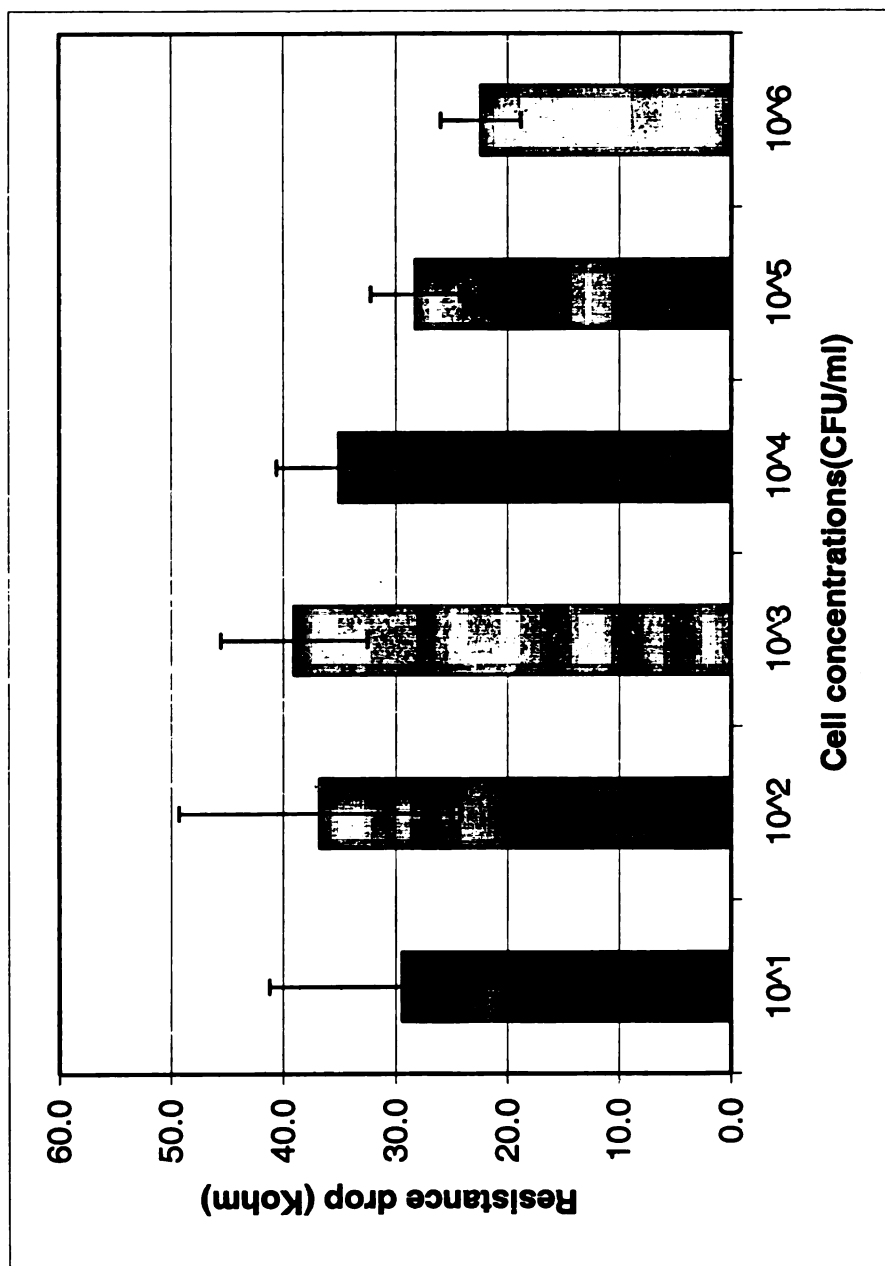


Figure D.23.c: Result of EC biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	111.9	102.2	110.9	110.9	120.9	115.1	113.6	124.8	124.1	113.8	1
7.8 X 10 ¹	78.6	69.5	76.5	80.2	71.5	79.5	85.6	80.5	79.9	77.7	2.68E-10
7.8 X 10 ²	63.6	62.5	67.8	75.3	68.2	70.8	81.1	76.9	73.8	70.8	1.13E-10
7.8 X 10 ³	57.8	64.3	68.2	66.9	66.8	69.5	75.5	68.5	78	67.2	8.33E-13
7.8 X 10 ⁴	79.8	81.1	73.9	70.2	77.2	70.9	67.8	71.5	69.8	74.1	8.37E-12
7.8 X 10 ⁵	81.2	84.9	80.1	75.4	79.9	75.6	69.6	75.5	71.9	77.8	1.78E-10
7.8 X 10 ⁶	86.5	75.8	88.5	75.6	88.9	88.5	80.2	76.5	80.2	82.6	1.02E-09

Figure D.24.a: EHEC biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.8 X 10 ¹	33.3	32.7	34.4	30.7	49.4	35.6	28.0	44.3	44.2	36.1	7.2	7.2
7.8 X 10 ²	48.3	39.7	43.1	35.6	52.7	44.3	32.5	47.9	50.3	43.0	6.8	6.8
7.8 X 10 ³	54.1	37.9	42.7	44.0	54.1	45.6	38.1	56.3	46.1	46.6	6.9	6.9
7.8 X 10 ⁴	32.1	21.1	37.0	40.7	43.7	44.2	45.8	53.3	54.3	39.7	10.4	10.4
7.8 X 10 ⁵	30.7	17.3	30.8	35.5	41.0	39.5	44.0	49.3	52.2	36.0	10.7	10.7
7.8 X 10 ⁶	25.4	26.4	22.4	35.3	32.0	26.6	33.4	48.3	43.9	31.2	8.8	8.8

Figure D.24.b: Normalized values for data in Figure D.24.a

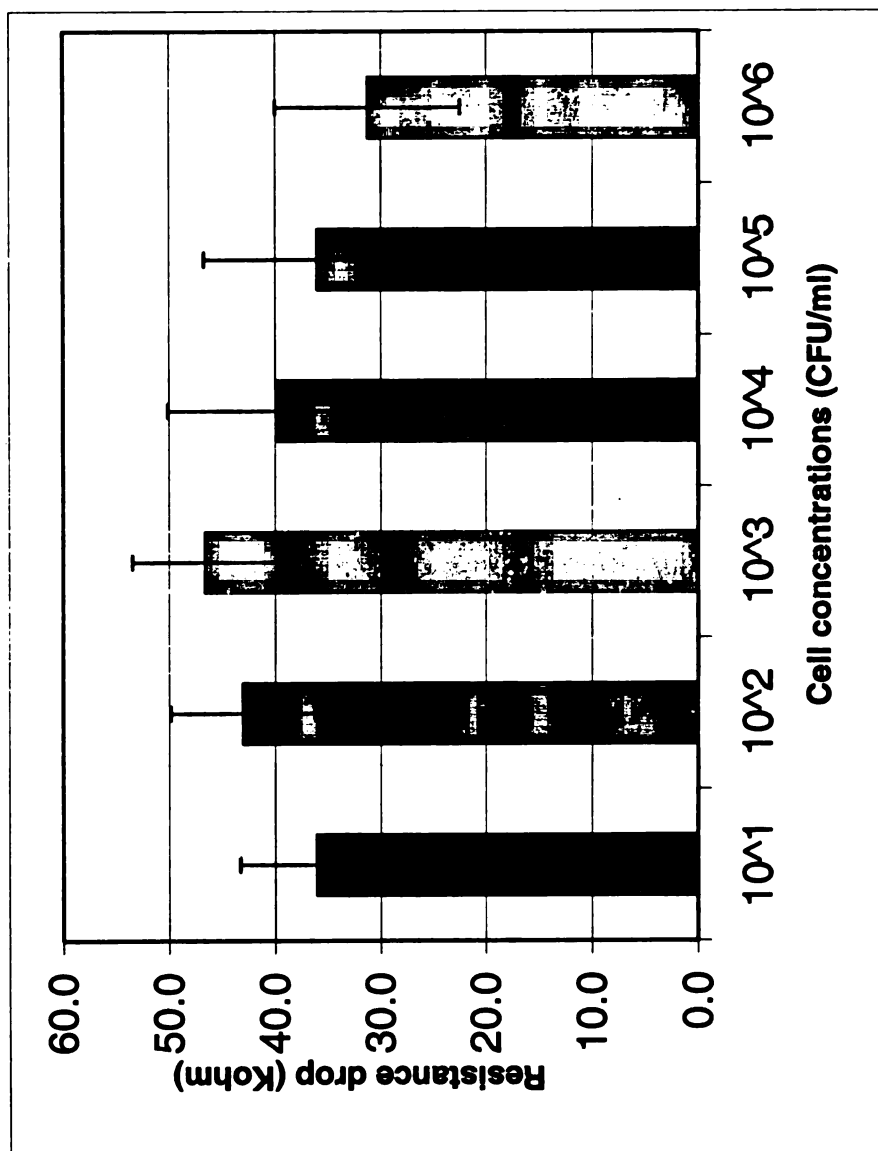


Figure D.24.c: Result of EHEC biosensor responses on the alfalfa sprout samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)								P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	
Blank	116.6	105.1	124.6	130	131.7	140	138	139	1
7.9 X 10 ¹	104.9	93.6	106.2	114.3	112.3	118.4	126.4	128.1	0.025439
7.9 X 10 ²	109.1	98.8	104.9	113.6	114.3	115.4	118.5	124.9	0.015584
7.9 X 10 ³	96.4	91.4	102	114	106.1	104.7	106	108.1	2.11E-05
7.9 X 10 ⁴	69.9	63.8	70.3	71.4	75.4	80.1	85.2	87.3	9.99E-09
7.9 X 10 ⁵	76.1	78.1	77.6	82.3	88.2	87.3	94.5	93.8	5.35E-08
7.9 X 10 ⁶	76.1	65.1	70.6	87.3	90.2	90.6	93.5	94.1	3.68E-07

Figure D.25.a: EC biosensor responses on the strawberry samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)								Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.9 X 10 ¹	11.70	11.50	18.40	15.70	19.40	21.60	11.60	10.90	2.70	13.72
7.9 X 10 ²	7.50	6.30	19.70	16.40	17.40	24.60	19.50	14.10	10.60	15.12
7.9 X 10 ³	20.20	13.70	22.60	16.00	25.60	35.30	32.00	30.90	27.77	24.90
7.9 X 10 ⁴	40.50	27.00	47.00	47.70	43.50	52.70	43.50	45.20	42.70	43.31
7.9 X 10 ⁵	46.70	41.30	54.30	58.60	56.30	59.90	52.80	51.70	48.60	52.24
7.9 X 10 ⁶	40.50	40.00	54.00	42.70	41.50	49.40	44.50	44.90	41.90	44.38

Figure D.25.b: Normalized values for data in Figure D.25.a

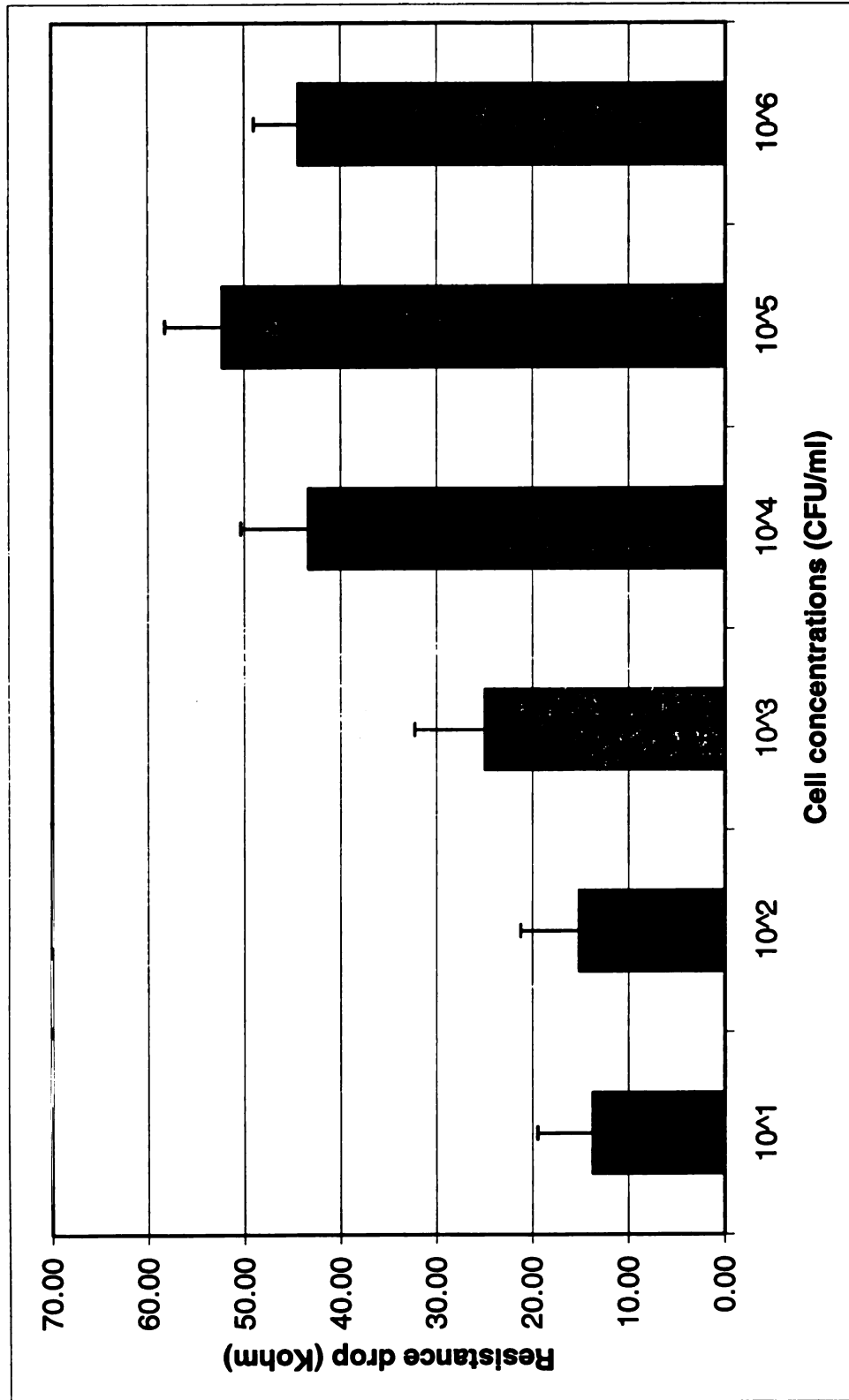


Figure D.25.c: Result of EC biosensor responses on the strawberry samples inoculated with non-pathogenic *E. coli*.

		Resistance measured (K ohm)											
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	P-value to the blank	
Blank	Blank	135.5	122.3	123.4	123.3	133.4	124.3	122.3	130.2	126.9	126.8		
0	7.9 X 10 ¹	129.5	138	122.4	122.3	121.5	120.2	129.3	127.9	128.1	126.6	0.484335	
0	7.9 X 10 ²	127.7	125.7	133.6	130.3	125.3	122.1	106.5	138.3	121	125.6	0.592015	
0	7.9 X 10 ³	123.6	122.7	121.3	128.5	124.3	123.7	124.5	122.5	117.9	123.2	0.564899	
0	7.9 X 10 ⁴	125.5	127.9	121.7	124.4	118.5	121.2	122.7	118.9	126.1	123.0	0.319259	
0	7.9 X 10 ⁵	120.3	129	128.1	136.7	123.9	121.5	123	129.2	126.4	126.5	0.122014	
0	7.9 X 10 ⁶	124.1	120.9	125.3	127.1	129.8	125.5	123.5	131.4	128.1	126.2	0.433071	

Figure D.26.a: EHEC biosensor responses on the strawberry samples inoculated with non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	Resistance drop (K ohm)								Total Average	Total STD
				4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 1)	6min (trial 2)		
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	6.00	-15.70	1.00	1.00	11.90	4.10	-7.00	2.30	-1.20	0.27	7.89		
0	7.80	-3.40	-10.20	-7.00	8.10	2.20	15.80	-8.10	5.90	1.23	8.89		
0	11.90	-0.40	2.10	-5.20	9.10	0.60	-2.20	7.70	9.00	3.62	5.95		
0	10.00	-5.60	1.70	-1.10	14.90	3.10	-0.40	11.30	0.80	3.86	6.72		
0	15.20	-6.70	-4.70	-13.40	9.50	2.80	-0.70	1.00	0.50	0.39	8.49		
0	11.40	1.40	-1.90	-3.80	3.60	-1.20	-1.20	-1.20	-1.20	0.66	4.54		

Figure D.26.b: Normalized values for data in Figure D.26.a

Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
		2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	Blank	122.3	123.4	124.3	130.5	133.3	126.1	127.2	117.1	134.3	126.5	
0	7.9×10^1	138	122.4	112.3	129.5	102.3	141.8	126.2	116.1	133.3	124.7	0.615934
0	7.9×10^2	125.7	133.6	130.3	127.7	120.3	129.5	137.4	134.1	131.5	130.0	0.03872
0	7.9×10^3	112.7	121.3	128.5	123.6	129.8	116.5	125.1	132.3	127.4	124.1	0.911383
0	7.9×10^4	119.9	121.7	124.4	125.5	124.6	123.9	125.5	128.2	129.3	124.8	0.591874
0	7.9×10^5	127.9	118.9	123.3	121.1	128.6	121.7	112.7	136.1	126.9	124.1	0.295847
0	7.9×10^6	126.1	122.3	129.5	124.6	116.8	129.9	126.1	133.3	128.4	126.3	0.430809

Figure D.27 a: Sal biosensor responses on the strawberry samples inoculated with non-pathogenic *E. coli*

Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	Resistance drop (K ohm)						6min (trial 3)	Total Average	Total STD
				4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-15.70	1.00	12.00	1.00	31.00	-15.70	1.00	1.00	1.00	1.84	14.02	
0	-3.40	-10.20	-6.00	2.80	13.00	-3.40	-10.20	-17.00	2.80	-3.51	8.87	
0	9.60	2.10	-4.20	6.90	3.50	9.60	2.10	-15.20	6.90	2.37	7.90	
0	2.40	1.70	-0.10	5.00	8.70	2.20	1.70	-11.10	5.00	1.72	5.46	
0	-5.60	4.50	1.00	9.40	4.70	4.40	14.50	-19.00	7.40	2.37	9.73	
0	-3.80	1.10	-5.20	5.90	16.50	-3.80	1.10	-16.20	5.90	0.17	9.10	

Figure D.27.b: Normalized values for data in Figure D.27.a

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	P-value to the blank
Blank	106.8	126.3	112.4	116.8	122.2	120.4	124.6	127.5	113.6	1
8.1 X 10 ¹	100.5	106.6	105.6	110	115.4	101.4	105.6	107.8	106.8	0.000404
8.1 X 10 ²	95.3	107.9	103.6	108	113.4	103.2	107.4	109.1	104.8	0.000333
8.1 X 10 ³	93.1	97.7	98.5	96.9	102.3	117.1	114.2	98.9	99.7	0.00023
8.1 X 10 ⁴	79.8	79.3	75.8	80.2	75.6	87.4	84.6	80.5	77	1.25E-10
8.1 X 10 ⁵	65.5	72	66.3	70.7	76.1	74.2	78.4	73.2	67.5	1.07E-11
8.1 X 10 ⁶	66.8	72.3	69.2	63.6	69	66.3	70.5	73.5	70.4	1.43E-12

Figure D.28.a: EHEC biosensor responses on the strawberry samples inoculated with *E. coli* O157:H7.

Resistance drop (K ohm)										
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.1 X 10 ¹	6.30	19.70	6.80	6.80	6.80	19.00	19.00	19.70	6.80	12.32
8.1 X 10 ²	11.50	18.40	8.80	8.80	8.80	17.20	17.20	18.40	8.80	13.10
8.1 X 10 ³	13.70	28.60	13.90	19.90	19.90	3.30	10.40	28.60	13.90	16.91
8.1 X 10 ⁴	27.00	47.00	36.60	36.60	46.60	33.00	40.00	47.00	36.60	8.27
8.1 X 10 ⁵	41.30	54.30	46.10	46.10	46.10	46.20	46.20	54.30	46.10	47.41
8.1 X 10 ⁶	40.00	54.00	43.20	53.20	53.20	54.10	54.10	54.00	43.20	49.89
										5.90

Figure D.28.b: Normalized values for data in Figure D.28.a

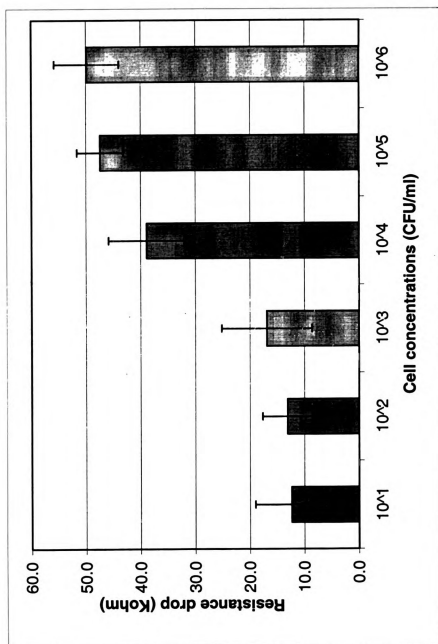


Figure D.28.c: EHEC biosensor responses on the strawberry samples inoculated with *E. coli* O157:H7.

Resistance measured (K ohm)										
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	P-value to the blank
Blank	Blank	128	117.9	126.7	137.5	129.3	125	135.8	128.7	1
0	8.1×10^1	127	116.9	125.7	136.5	145	124	134.8	127.7	0.468165
0	8.1×10^2	138.2	134.9	139.9	134.7	132.7	138.2	133	138.9	0.004493
0	8.1×10^3	125.9	133.1	118	130.6	119.7	116.3	128.9	118	0.14067
0	8.1×10^4	116.3	129	131.8	132.5	116.9	130.1	130.8	115.2	0.219209
0	8.1×10^5	113.5	116.9	130.8	120.1	134.9	129.1	118.4	133.2	0.159167
0	8.1×10^6	126.9	134.1	119	131.6	133.1	117.3	129.9	131.4	0.825035

Figure D.29.a: Sal biosensor responses on the strawberry samples inoculated with *E. coli* O157:H7.

Resistance drop (K ohm)									
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	1.00	1.00	1.00	-15.70	1.00	1.00	-15.70	1.00	-2.71
0	-10.20	-17.00	2.80	-3.40	-13.20	2.80	-3.40	-10.20	-7.22
0	2.10	-15.20	8.70	9.60	8.70	6.90	9.60	2.10	4.38
0	11.70	-11.10	5.00	12.40	-5.10	5.00	12.40	11.70	4.10
0	14.50	1.00	-4.10	-5.60	-4.10	17.40	-5.60	14.50	5.04
0	1.10	-16.20	7.70	-3.80	7.70	5.90	-3.80	1.10	0.62
									7.73

Figure D.29.b: Normalized values for data in Figure D.29.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	130.7	119	123.1	137.5	132.9	142	139.7	105.8	130.7	129.0	1
8.2 X 10 ¹	98.9	101.7	105.6	122.8	117.8	121.8	119.5	95.9	98.9	109.2	0.00181
8.2 X 10 ²	92.8	87.8	93.6	99.8	102	107.6	105.3	86.1	93.9	96.5	2.47E-06
8.2 X 10 ³	76.7	80.9	79.7	88.9	85.6	93.5	91.2	69	76.7	82.5	2.69E-08
8.2 X 10 ⁴	66	66.8	65.9	77.6	79.5	80.8	78.5	56	66.4	70.8	1.57E-09
8.2 X 10 ⁵	65.7	73.3	69.8	80.4	80.9	77.6	75.3	65.1	65.7	72.6	6.99E-10
8.2 X 10 ⁶	73.8	79.7	83.7	82.8	87.6	85.7	83.4	79.1	74.8	81.2	3.38E-09

Figure D.30.a: EC biosensor responses on the strawberry samples inoculated with *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	6min (trial 3)		
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.2 X 10 ¹	31.80	17.30	17.50	14.70	15.10	20.20	20.20	9.90	31.80	19.83	7.47	7.47
8.2 X 10 ²	37.90	31.20	29.50	37.70	30.90	34.40	34.40	19.70	36.80	32.50	5.69	5.69
8.2 X 10 ³	54.00	38.10	43.40	48.60	47.30	48.50	48.50	36.80	54.00	46.58	6.12	6.12
8.2 X 10 ⁴	64.70	52.20	57.20	59.90	53.40	61.20	61.20	49.80	64.30	58.21	5.37	5.37
8.2 X 10 ⁵	65.00	45.70	53.30	57.10	52.00	64.40	64.40	40.70	65.00	56.40	9.13	9.13
8.2 X 10 ⁶	56.90	39.30	39.40	54.70	45.30	56.30	56.30	26.70	55.90	47.87	10.81	10.81

Figure D.30.b: Normalized values for data in Figure D.30.a

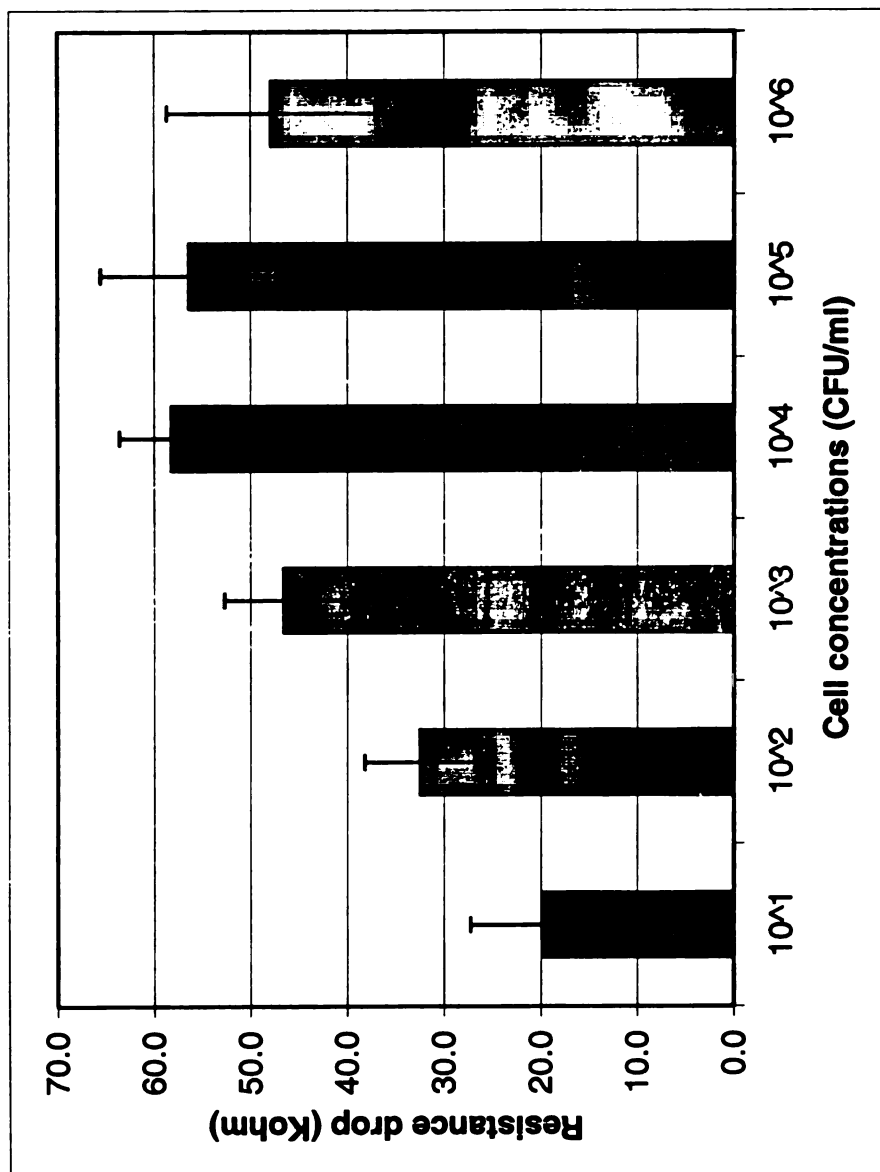


Figure D.30.c: Results of EC biosensor responses on the strawberry samples inoculated with *E. coli* O157:H7.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	109.1	105.2	100.8	98.1	103.2	107.6	110.5	115.6	106	106.2	1
8.3 X 10 ¹	79.5	95	79	70.3	79.4	84.1	82.7	91.8	76.4	82.0	6.91E-07
8.3 X 10 ²	81.9	90.8	72.9	72.7	71.8	76.5	85.1	84.2	78.8	79.4	5.16E-08
8.3 X 10 ³	84.3	76.7	56.8	75.1	84.2	78.9	87.5	96.6	81.2	80.1	7.54E-06
8.3 X 10 ⁴	86.7	64	46.1	77.5	86.6	81.3	89.9	99	83.6	79.4	0.000174
8.3 X 10 ⁵	89.1	60.8	45.8	79.9	89	83.7	92.3	101.4	86	80.9	0.000628
8.3 X 10 ⁶	91.5	68.9	53.9	82.3	91.4	86.1	94.7	103.8	88.4	84.6	0.000809

Figure D.31.a: Sal biosensor responses on the strawberry samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)			
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.3 X 10 ¹	29.6	10.2	21.8	27.8	23.8	23.5	27.8	23.8	29.6	24.2	6.0	6.0
8.3 X 10 ²	27.2	14.4	27.9	25.4	31.4	31.1	25.4	31.4	27.2	26.8	5.2	5.2
8.3 X 10 ³	24.8	28.5	44.0	23.0	19.0	28.7	23.0	19.0	24.8	26.1	7.6	7.6
8.3 X 10 ⁴	22.4	41.2	54.7	20.6	16.6	26.3	20.6	16.6	22.4	26.8	12.8	12.8
8.3 X 10 ⁵	20.0	44.4	55.0	18.2	14.2	23.9	18.2	14.2	20.0	25.3	14.4	14.4
8.3 X 10 ⁶	17.6	36.3	46.9	15.8	11.8	21.5	15.8	11.8	17.6	21.7	12.0	12.0

Figure D.31.b: Normalized values for data in Figure D.31.a

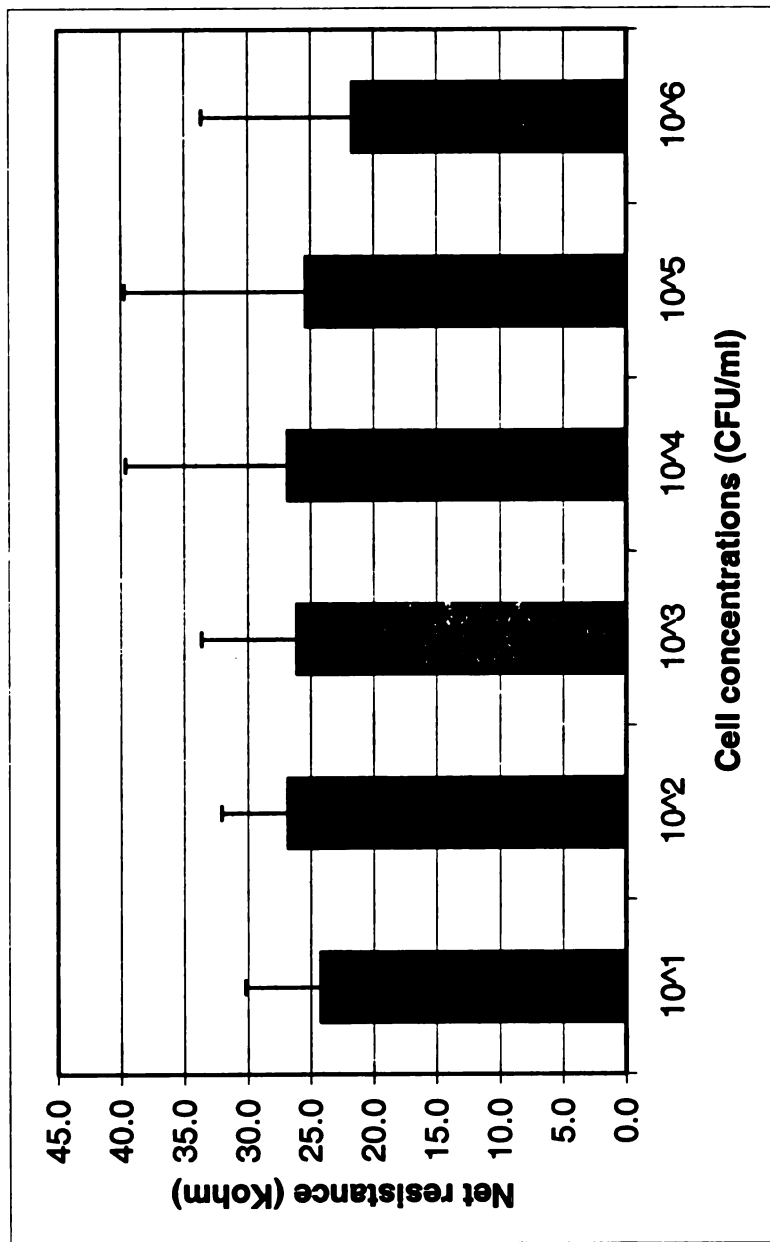


Figure D.31.c: Results of Sal biosensor responses on the strawberry samples inoculated with *S. Typhimurium*.

Resistance measured (K ohm)											
Plate counts ^a (CFU/ml)	Plate counts ^{a1} (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	P-value to the blank
Blank	Blank	128	127.9	126.7	130.1	129.3	125	135.8	127.6	128.7	128.8
0	8.3 X 10 ¹	127	116.9	125.7	136.5	145	124	134.8	143.3	127.7	131.2
0	8.3 X 10 ²	138.2	134.9	139.9	134.7	132.7	138.2	133	131	138.9	135.7
0	8.3 X 10 ³	125.9	133.1	118	130.6	119.7	116.3	128.9	118	126.6	124.1
0	8.3 X 10 ⁴	116.3	129	131.8	132.5	116.9	130.1	130.8	115.2	117	124.4
0	8.3 X 10 ⁵	113.5	116.9	130.8	120.1	134.9	129.1	118.4	133.2	114.2	123.5
0	8.3 X 10 ⁶	126.9	134.1	119	131.6	133.1	117.3	129.9	131.4	127.6	127.9
											0.825035

Figure D.32.a: EC biosensor responses on the strawberry samples inoculated with *S. Typhimurium*.

Resistance drop (K ohm)											
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BLANK											
0	1.00	11.00	1.00	-6.40	-15.70	1.00	1.00	-15.70	1.00	-2.42	8.71
0	-10.20	-7.00	-13.20	-4.60	-3.40	-13.20	2.80	-3.40	-10.20	-6.93	5.30
0	2.10	-5.20	8.70	-0.50	9.60	8.70	6.90	9.60	2.10	4.67	5.28
0	11.70	-1.10	-5.10	-2.40	12.40	-5.10	5.00	12.40	11.70	4.39	7.84
0	14.50	11.00	-4.10	10.00	-5.60	-4.10	17.40	-5.60	14.50	5.33	9.90
0	1.10	-6.20	7.70	-1.50	-3.80	7.70	5.90	-3.80	1.10	0.91	5.22

Figure D.32.b: Normalized values for data in Figure D.32.a

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	
Blank	Blank	119.5	118.5	136.5	134.7	130.6	118.5	135.7	125.7	129.2	127.7
0	8.3 X 10 ¹	128.3	127.3	141.5	119.6	133.4	132.4	120.6	134.5	130.3	129.8
0	8.3 X 10 ²	139.1	138.1	136.3	132.2	134.1	121.7	133.2	145.3	125.7	134.0
0	8.3 X 10 ³	130.9	146.6	134.3	121.3	118.5	136.5	134.7	137.1	144.9	133.9
0	8.3 X 10 ⁴	126.6	125.6	139.8	117.9	131.7	130.7	118.9	132.8	129.3	128.1
0	8.3 X 10 ⁵	137.4	136.4	134.6	130.5	132.4	120	131.5	143.6	124.7	132.3
0	8.3 X 10 ⁶	129.2	144.9	132.6	119.6	116.8	134.8	133	135.4	132.6	131.0

Figure D.33.a: EHEC biosensor responses on the strawberry samples inoculated with *S. Typhimurium*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	Blank	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)		
Blank	Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	-8.80	-8.80	-5.00	15.10	-2.80	-13.90	15.10	-8.80	-1.10	-2.11	-2.11	10.46
0	-19.60	-19.60	0.20	2.50	-3.50	-3.20	2.50	-19.60	3.50	-6.31	-6.31	10.25
0	-11.40	-28.10	2.20	13.40	12.10	-18.00	1.00	-11.40	-15.70	-6.21	-6.21	14.16
0	-7.10	-7.10	-3.30	16.80	-1.10	-12.20	16.80	-7.10	-0.10	-0.49	-0.49	10.46
0	-17.90	-17.90	1.90	4.20	-1.80	-1.50	4.20	-17.90	4.50	-4.69	-4.69	10.17
0	-9.70	-26.40	3.90	15.10	13.80	-16.30	2.70	-9.70	-3.40	-3.33	-3.33	13.71

Figure D.33.b: Normalized values for data in Figure D.33.a

Resistance measured (K ohm)											
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	P-value to the blank
Blank	80.9	82.6	82.5	81.9	86.5	82.3	97.8	94.3	90.2	86.6	1
7 X 10 ¹	58.2	67.6	64.5	62.2	64.4	69.3	66.9	60.4	64.4	64.2	6.05E-08
7 X 10 ²	47.5	51.6	67.6	47.6	62	68.3	63.3	49	64	57.9	5.43E-07
7 X 10 ³	56.6	58.3	62.6	57.8	58	67.3	59.3	56	56.4	59.1	3.85E-09
7 X 10 ⁴	62.5	59.3	60.2	59.3	52	58	55.6	64.3	64.9	59.6	7.86E-09
7 X 10 ⁵	62.9	62.6	69.3	61.9	57	62	58.3	65	68	63.0	4.77E-08
7 X 10 ⁶	69.3	74.5	71.9	68.3	66.9	67.8	62.5	71.3	80.4	70.3	1.54E-05

Figure D.34.a: Sal biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non- pathogenic *E. coli*.

Resistance drop (K ohm)											
Plate counts ^a (CFU/ml)	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total Average	Total STD
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 X 10 ¹	22.7	15.0	18.0	19.7	22.1	13.0	30.9	33.9	25.8	22.3	6.9
7 X 10 ²	33.4	31.0	14.9	34.3	24.5	14.0	34.5	45.3	26.2	28.7	10.0
7 X 10 ³	24.3	24.3	19.9	24.1	28.5	15.0	38.5	38.3	33.8	27.4	8.1
7 X 10 ⁴	18.4	23.3	22.3	22.6	34.5	24.3	42.2	30.0	25.3	27.0	7.4
7 X 10 ⁵	18.0	20.0	13.2	20.0	29.5	20.3	39.5	29.3	22.2	23.6	7.9
7 X 10 ⁶	11.6	8.1	10.6	13.6	19.6	14.5	35.3	23.0	9.8	16.2	8.6

Figure D.34.b: Normalized values for data in Figure D.34.a

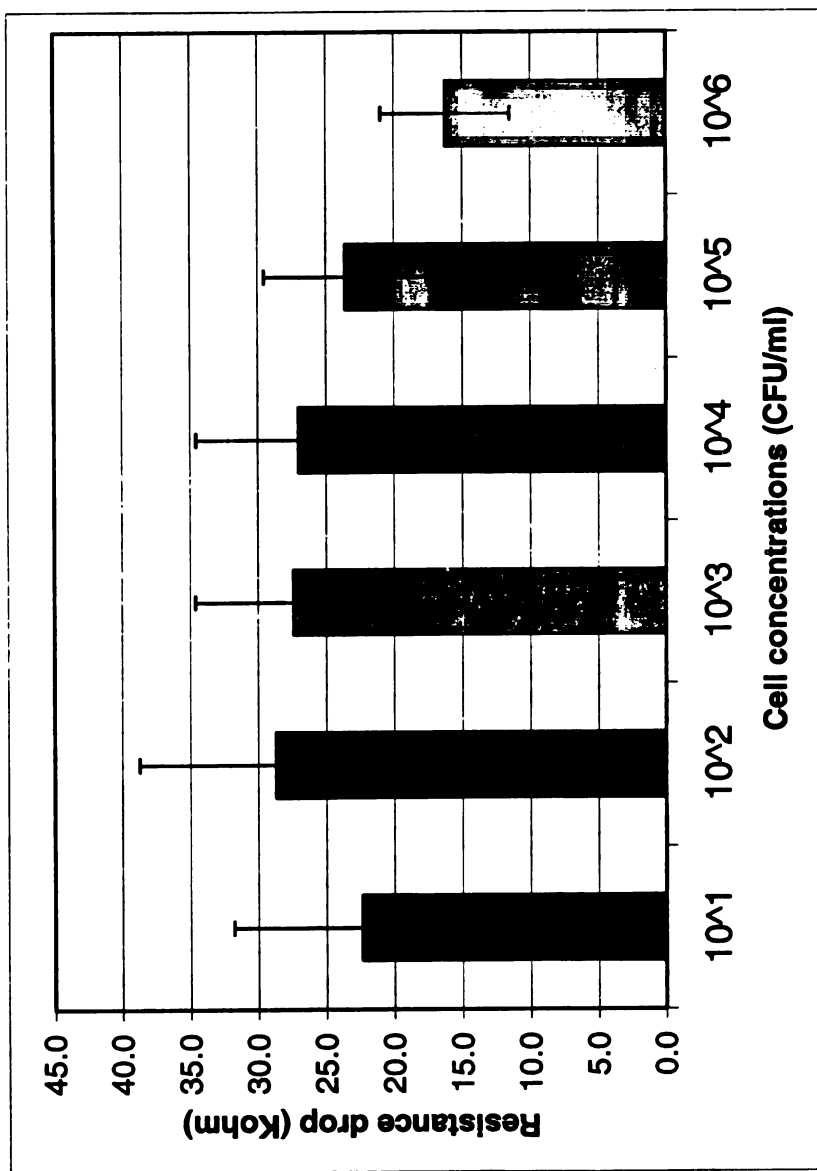


Figure D.34.c: Results of Sal biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non- pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	101.3	101.6	101.3	100.3	102.2	103.6	106.4	106.7	102	102.8	1
8.3 X 10 ¹	76.3	70.2	77.2	80.9	73.6	78.9	61.4	65.4	85.3	74.4	9.69E-09
8.3 X 10 ²	63	61.9	67.2	74.7	69	74	50	65	79.5	67.1	2.28E-09
8.3 X 10 ³	58.5	65	68.9	67.6	67.6	69.4	57	61.4	75.2	65.6	5.11E-12
8.3 X 10 ⁴	79.2	80.5	73.3	69.6	63.7	74	65.3	65.9	66.2	70.9	1.28E-10
8.3 X 10 ⁵	81.9	85.6	80.8	76.1	73.7	79.1	72.7	84.3	69.3	78.2	1.44E-09
8.3 X 10 ⁶	87.6	89.2	80.9	77.2	78.9	84	86	91.3	75.5	83.4	4.34E-08

Figure D.35.a: EHEC biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Total		
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 X 10 ¹	25.0	31.4	24.1	19.4	28.6	24.7	45.0	41.3	16.7	28.5	9.4	9.4
7 X 10 ²	38.3	39.7	34.1	25.6	33.2	29.6	56.4	41.7	22.5	35.7	10.1	10.1
7 X 10 ³	42.8	36.6	32.4	32.7	34.6	34.2	49.4	45.3	26.8	37.2	7.2	7.2
7 X 10 ⁴	22.1	21.1	28.0	30.7	38.5	29.6	41.1	40.8	35.8	32.0	7.6	7.6
7 X 10 ⁵	19.4	16.0	20.5	24.2	28.5	24.5	33.7	22.4	32.7	24.7	6.0	6.0
7 X 10 ⁶	13.7	12.4	20.4	23.1	23.3	19.6	20.4	15.4	26.5	19.4	4.7	4.7

Figure D.35.b: Normalized values for data in Figure D.35.a

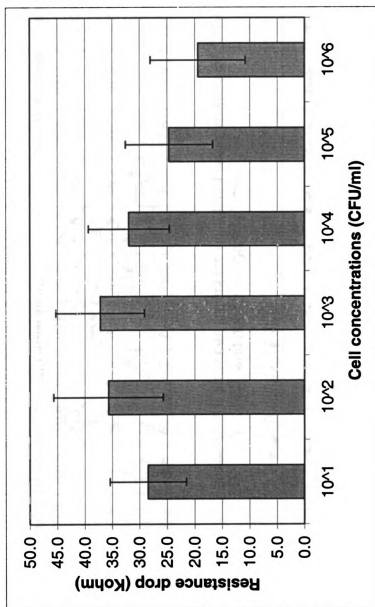


Figure D.35.c: Results of EHEC biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance measured (K ohm)										P-value to the blank
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average	
Blank	100.6	100.9	100.6	99.6	99.6	99.8	102.3	103.5	99.8	100.7	1
8.9 X 10 ¹	75.6	69.5	76.5	80.2	71.5	75.5	85.6	80.5	79.9	77.2	2.68E-10
8.9 X 10 ²	62.3	61.2	66.5	74	66.9	69.5	79.8	75.6	72.5	69.8	1.13E-10
8.9 X 10 ³	57.8	64.3	68.2	66.9	66.8	69.5	75.5	68.5	70	67.5	8.33E-13
8.9 X 10 ⁴	78.5	79.8	72.6	68.9	75.9	69.6	66.5	70.2	68.5	72.3	8.37E-12
8.9 X 10 ⁵	81.2	84.9	80.1	75.4	79.9	75.6	69.6	75.5	71.9	77.1	1.78E-10
8.9 X 10 ⁶	86.9	88.5	80.2	76.5	80.2	76.5	75.8	78.5	75.6	79.9	1.02E-09

Figure D.36.a: EC biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*.

Plate counts ^a (CFU/ml)	Resistance drop (K ohm)										Total Average	Total STD
	2min (trial 1)	2min (trial 2)	2min (trial 3)	4min (trial 1)	4min (trial 2)	4min (trial 3)	6min (trial 1)	6min (trial 2)	6min (trial 3)	Average		
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 X 10 ¹	25.0	31.4	24.1	19.4	28.1	24.3	16.7	23.0	19.9	23.5	4.5	4.5
7 X 10 ²	38.3	39.7	34.1	25.6	32.7	30.3	22.5	27.9	27.3	30.9	5.8	5.8
7 X 10 ³	42.8	36.6	32.4	32.7	32.8	30.3	26.8	35.0	29.8	33.2	4.6	4.6
7 X 10 ⁴	22.1	21.1	28.0	30.7	23.7	30.2	35.8	33.3	31.3	28.5	5.1	5.1
7 X 10 ⁵	19.4	16.0	20.5	24.2	19.7	24.2	32.7	28.0	27.9	23.6	5.3	5.3
7 X 10 ⁶	13.7	12.4	20.4	23.1	19.4	23.3	26.5	25.0	24.2	20.9	5.0	5.0

Figure D.36.b: Normalized values for data in Figure D.36.a

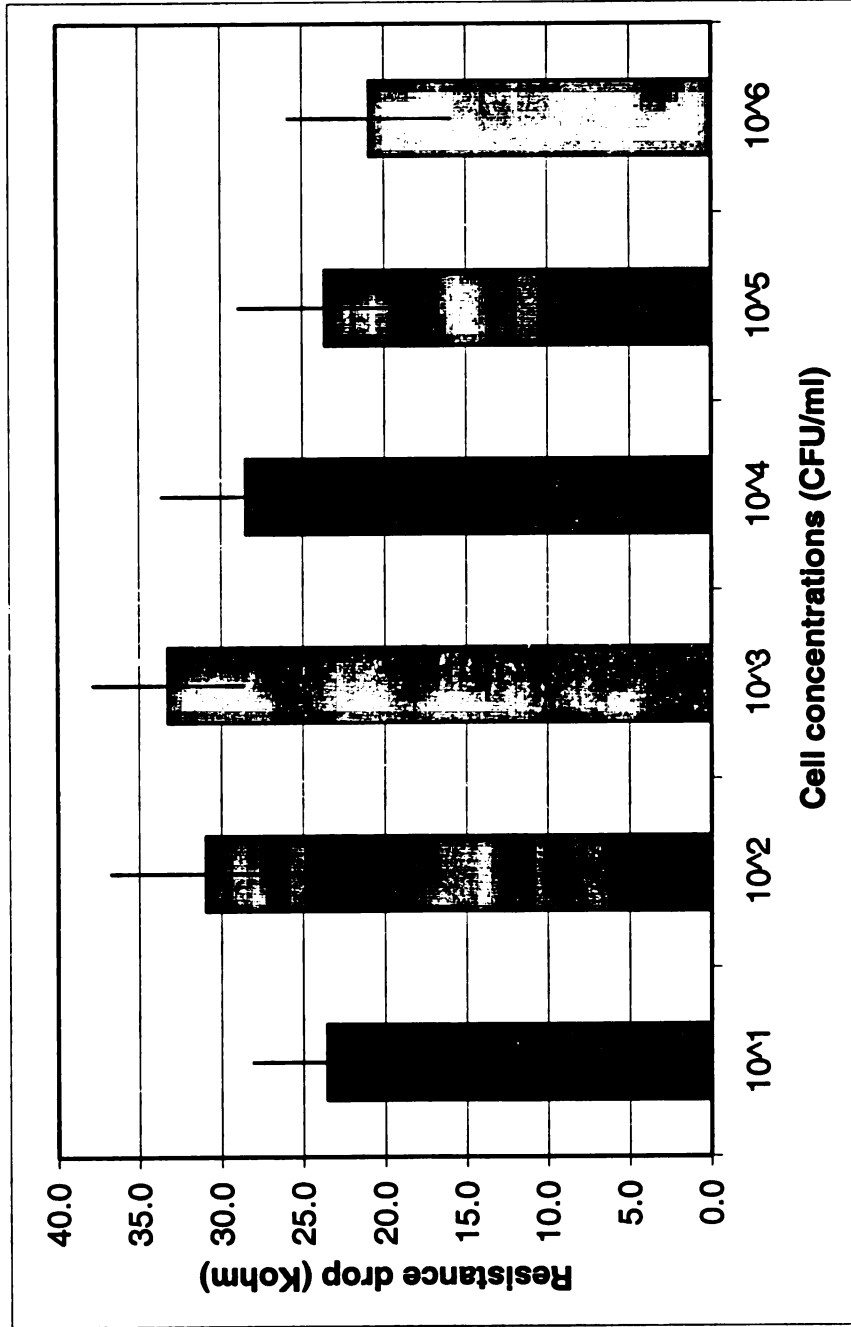


Figure D.36.c: Results of EC biosensor responses on the strawberry samples inoculated with a mixture of *S. Typhimurium*, *E. coli* O157:H7, and non-pathogenic *E. coli*

Appendix E

In this study, the biosensor was further tested using water samples obtained from the Red Cedar river and the MSU dairy farm. The biosensor performance was compared with the results obtained from the Michigan Department of Community Health (MDCH).

Materials and Methods

Water sample preparation

The first batch of water samples consisted of distilled water inoculated with *E. coli* O157:H7. The culture was serially diluted in eight 15-ml culture tubes filled with 9 ml of distilled water.

The second batch of the samples of water samples were collected daily over a period of five days from cattle-drinking pails at the Michigan State University. The last batch of water samples, which was collected weekly for five weeks, came from four sites along Red cedar river: Hagadorn, Williamston, Farm Lane, and Kalamazoo Streets. The samples from the dairy farm and the river were tested directly with the biosensor without artificial inoculation. One hundred ml of each sample was filtered through a bio-filter membrane (BIOPath, Florida). The membranes filter was transferred to a test tube containing 10 ml of 0.1% peptone water. The test tube was vortexed to release the trapped cells from the membrane. Then, 0.1 of each water sample was pipetted onto the application pad of the biosensor. Three replicates were done for each treatment. Uninoculated distilled water was used as the blank (negative control).

Confirmation

Duplicate water samples were sent to the Michigan Department of Community Health (MDCH) for *E. coli* analysis. Results were compared with the data found using the biosensor.

RESULTS

Result on the inoculated distilled water samples

In water samples inoculated with *E. coli* O157:H7, the signal was proportional to the cell concentration between 10^1 and 10^4 CFU/ml. The signal was observed to decrease at concentrations higher than 10^4 CFU/ml (Figure F.1). The response pattern gave a parabolic curve, which was similar to previous experiments (Results section in Chapters 3, 4, and 5). ANOVA reveals that the sensitivity limit of the biosensor was at $8.3 \pm 0.1 \times 10^1$ CFU/ml.

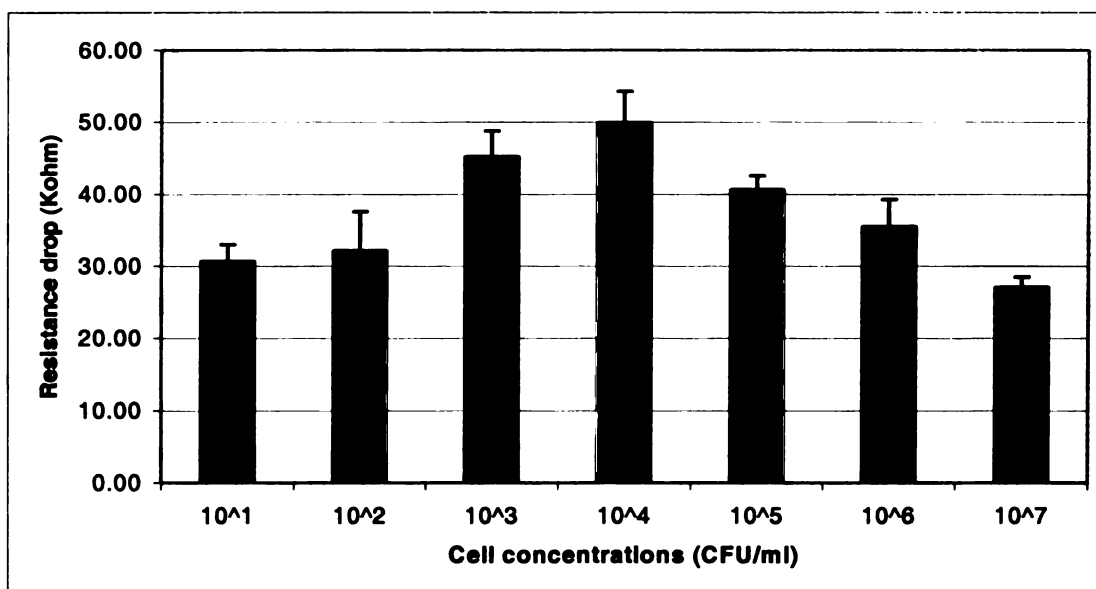


Figure F.1 Resistance drop of the *E. coli* O157:H7 biosensor tested in distilled water samples inoculated with *E. coli* O157:H7.

Results on samples taken from the dairy farm

The biosensor revealed one false-positive result in one of five samples taken from the dairy farm giving a false-positive rate of 20%. The signal generated by the biosensor was statistically different from the blank. Therefore, the sample was presumptively identified to be contaminated with *E. coli* O157:H7. The results from MDCH, however, reported only the presence of non-O157:H7 *E. coli*. The biosensor also showed the absence of *E. coli* O157:H7 in the other four samples and these results were also verified by MHDC.

Results on samples taken from the Red Cedar river

Signals generated from the 20 samples were all above or not statistically different from the blank. This indicated the absence of *E. coli* O57:H7. These data were verified by MDHC.

DISCUSSION

Data on the inoculated distilled water indicated that the biosensor was sensitive to the presence of the analyte at a concentration of 8.3×10^1 CFU/ml. The response pattern was observed similar to the shape from the previous experiments. A parabolic curve was generated when plotting the resistance drop against the bacterial concentration.

The biosensor was further tested using water samples obtained from the dairy farm and various sites along the Red Cedar river. One sample yielded a positive result. However, results from MDHC, indicated the presence of non-O157:H7 *E. coli*. The Stx1 and Stx2 toxins produced by serotype O157:H7 were

not found. The specificity of the antibody used may be partly responsible for these findings. According to the manufacturer of the antibody (KPL 2002), cross-reactivity of the antibody to non-O157:H7 *E. coli* is still possible. Unspecific binding of the antibody may be responsible for the decrease in the biosensor specificity. Grif et al. (1998) found that the number of ubiquitous organisms other than *E. coli* O157:H7 adhered to the antibodies used in the experiment.

As for samples taken from the Red Cedar river, all results were confirmed negative for the presence of *E. coli* O157:H7. Since these water samples were not contaminated with *E. coli* O157:H7, the success of the biosensor could not be adequately evaluated.

APPENDIX F

Objective: To evaluate the minimal vortexing-time required to release trapped cells from a membrane filter.

Instrument/Media: A 47mm Analytical Filter Funnel (pore size of 0.45 μ m), CHROM agar, a pump, and *E. coli* O157:H7 culture.

Procedure:

1. Two 10-ml *E. coli* O157:H7 culture were grown in nutrient broth at 37^o C for 24 hours. Each test tube was labeled "A" or "B"
2. The A labeled culture was serially diluted and plate counted.
3. The B labeled culture was transferred into 100 ml of distilled water.
4. The inoculated water was transferred into a funnel attached to a pump.
5. The membrane was removed from the funnel and transferred to a 50-ml test tube containing 10 ml of 0.1 % peptone water.
6. The solution was vortexed for 15 sec.
7. The membrane was removed and transferred on a plating agar.
8. The suspended solution was plated.
9. The above procedure was repeated 3 times.
10. Steps 1-9 were repeated for 30 sec, 1 min, 2 min, 4min, 6 min, 8 min, and 10 min of vortexing times (VT).

Results:

VT	Estimated cell concentration of A (CFU/ml)	Average Plate count of a membrane (CFU/ML)	Average cell concentration of suspended solution (CFU/ML)
15 sec	$8.9 \times 10^8 \pm 0.05$	∞^1	$5.3 \times 10^4 \pm 0.4$
30 sec	$8.3 \times 10^8 \pm 0.06$	∞^2	$3.7 \times 10^5 \pm 0.1$
1 min	$8.9 \times 10^8 \pm 0.05$	∞^3	$7.5 \times 10^5 \pm 0.08$
2 min	$8.9 \times 10^8 \pm 0.05$	158 ± 0.3	$8.7 \times 10^6 \pm 0.03$
4 min	$9.0 \times 10^8 \pm 0.04$	13 ± 0.05	$6.9 \times 10^7 \pm 0.05$
6 min	$8.7 \times 10^8 \pm 0.05$	10 ± 0.08	$7.3 \times 10^7 \pm 0.09$
8 min	$8.9 \times 10^8 \pm 0.06$	8 ± 0.05	$7.2 \times 10^7 \pm 0.1$
10 min	$9.0 \times 10^8 \pm 0.04$	6 ± 0.06	$7.0 \times 10^7 \pm 0.2$

∞ - Unable to count the cell population. The cells were clumped together at the edge of the membrane. ∞¹ was observed more than ∞² and ∞³ (∞¹ > ∞² > ∞³)

Discussion:

A pump was used to expedite the flow of liquid through the membrane filter. The filtration was completed in less than a minute.

As the VT increased from 15 sec to 4min, the numbers of cell released from the membrane were shown to increase as well. The cell concentrations of 5.3×10^4 and 6.9×10^7 CFU/ml were found in the solution after vortexing the membrane for 30 sec and 4 min, respectively. The numbers of cell released from the membrane however, show minimal differences when VT was increased to 6, 8 and 10 minutes.

Conclusion: The experiment shows that a VT of 4 min was the minimum time required to release the trapped cell from the membrane.

BIBLIOGRAPHY

Abu-Elyazeed, R., Wiezbra, T. F., Mourad, A.S., Peruski, L.F., Kay, B.A., Rao, M., Churilla, A.M. 1999. Epidemiology of enterotoxigenic *Escherichia coli* diarrhea in a pediatric cohort in an urban area of lower Egypt. Journal of Infectious Disease **179**: 382-389.

Acheson, D., Gerald, T.K. 1996. Which Shiga toxin-producing types of *E. coli* are important? ASM News **62**: 302-306.

Adcock, P., Yang, S., Kamimoto, L., Smith, S., Scott, K., Greene, K., Friedman, C. 1998. The largest foodborne outbreak of enterotoxigenic *Escherichia coli* in the United States. Clinical Infectious Disease **27**: 1022-1027

Adeloju, S.B., Shaw, S.J., Wallace, G.G. 1997. Pulse-amperometric detection of urea in blood samples on a conducting polypyrrole-urease biosensor. Analytical Chimica Acta **341**:155-160.

Alocilja, E.C. 2000. E-Nose Detection of *E. coli* O157:H7 in Environmental Samples. In Proceedings of the Institute of Biological Engineering 2000, Annual International Meeting, Seattle, Washington. October, 2000.

Altekruse, S. F., Hyman, F.H., Klontz, K.C. 1994. Foodborne bacterial infections in individuals with immunodeficiency virus. Southern Medical Journal **87**: 169-173.

Altekruse, S. F., Swerdlow, D.L., Wells, S.J. 1998. Factors in the emergence of foodborne disease. In The Veterinary Clinics of North America: Food Animal Practice. Microbial foodborne pathogens, J. Vassallo, ed. W.B. Saunders Company: Pennsylvania: 1-5

Angelopoulos, M., Dipietro, R., Zheng, W.G., MacDiarmid, A.G. 1997. Effect of selected processing parameters on solution properties and morphology of polyaniline and impact on conductivity. Synthetic Metal **84**: 35-39.

Barbour W. M., George, T. 1997. Genetic and immunologic techniques for detecting foodborne pathogens and toxins. Food microbiology. Fundamentals and Frontiers. B. M. Doyle, L., Montville, T.J, ed. ASM Press. Washington DC.

Barnett, D., Laing, D.G., Skopec, S., Sadik, O., Wallace, G.G. 1994. Determination of P-Cresol using a conducting polymer based electro-immunological sensing system. Analytical Letters. **27**: 2417-2429.

Bean, N. H., Goulding, J.S., Lao, C. 1996. Surveillance for Foodborne Disease Outbreaks - United States, 1988-1991. CDC Surveillance Summaries. **45**(5): 1-66.

Beesley, J. E., Ed. 1993. Immunocytochemistry: A practical Approach, Oxford University Press, Oxford.

Bernard, D. T. 1997. Hazard Analysis and Critical Control Point System. Use in controlling microbiological hazards. In Food Microbiology: Fundamentals and Frontiers. M. P. Doyle, Beuchat, L.R., Montville, T.J., ed.: American Society for Microbiology Press. Washington, DC.

Besser, T. E., Goldoft, M., Pritchett, L.C., Khakhria, R., Hancock, D.D., Rice, D.H. 2000. Multiresistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiology Infection **124**: 193-200.

Beuchat, L., Jee-Hoon, R. 1996. Produce Handling and Processing Practices, Emerging Infectious Disease. Journal of Food Protection **59**:204-216.

Bhan, M. K., Raj, P., Levine, M.K., kaper, J. B., Bhandari, N., Srivastava, R., Kumar, R., Sazawal, S. 1989. Enteraggregative *Escherichia coli* associated with persistence diarrhea in cohort rural children in India. Journal of Infectious Disease **159**: 1061-1064.

Black, R. E. 1990. Epidemiology of travelers' diarrhea and relative importance of various pathogens. Rev. Infectious Disease **12**(suppl. 1): S73-79.

Black, R. E., Merson, M.H., Huq, I. 1981. Incidence and severity of rotavirus and *E. coli* diarrhea in rural Bangladesh. Lancet **1**: 141-113.

Bokete, T. N., O'Callahan, C.M., Clausen, C.R., 1993. Shiga-like toxin producing *Escherichia coli* in Seattle children: A prospective study. Gastroenterology **105**: 1724-1731.

Bowersox, J. 1992. Bowesox, J: Top July stores are % a day, new cancer risks. Journal of National Cancer Institute **84**: 1149-1150.

Brunelle, S. 2001. "Electroimmunoassay technology for Foodborne Pathogen Detection." Detection Technology **55-62**.

Buchanan, R. L., Doyle 1997. Foodborne Disease Significance of *Escherichia coli* O157:H7 and other Enterohemorrhagic *E. coli*. Food technology **51**: 69-76.

Buzby, J., Roberts T. 1996. Microbial Foodborne Illnesses. Choices: First Quarter: 14-17.

Cahn, T. M. 1993. Biosensors. London, UK, Chapman and Hall.

CAST 1999. Foodborne pathogens: Risk and Consequences. Council for Agriculture Science and Technology. Washington, DC: 11-15.

Cavelier, G. 1995. Possible role of surface electrochemical electron-transfer and semiconductor charge transport processes in ion channel function. Bioelectronics and Bioengineering **40**: 197-213.

CDC. 1996. Multidrug-resistant *Salmonella* serotype Typhimurium - United States. Morbidity and Mortality Weekly Report. 46: 308-310.

CDC. 2000. CDC surveillance summaries; March 17, 2000. Morbidity and Mortality Weekly Report. 49: 1-51.

Chard, T. 1987. An introduction to radioimmunoassay and related techniques. Amsterdam, Elsevier.

Chiang, J. C., MacDiarmid, A.G. 1986. Polyaniline: protonic acid doping of the emeraldine form to metallic regime. Synthetic Metal **13**: 193-205.

Cravioto, A., Reyes, R.E., Trujillo, F., Uribe, F., Navarro, A.1990. Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. American Journal of Epidemiology **131**: 886-904.

Cravioto, A., Tello, A., Villafan, J. 1991. Inhibition of localized adhesion of enteropathogenic *Escherichia coli* to Hep-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk. Journal of Infectious Disease **163**: 1247-1255.

Davies, P. 1997. Food safety and its impact on domestic and export markets. Swine Health Product **5**: 13-20.

Davis, M. A., Hancock, D.D., Besser, T.E., Rice, D.H., Gay, J.M., Gay, C. 1999. Changes in antimicrobial resistance among *Salmonella* enterica serovar Typhimurium isolates from humans and cattle in the Northwestern United States. Emerging Infectious Disease **5**: 802-806.

Deshpande, M.V., Hall, E.A.H. 1990. An electrochemically-grown polymer as an immobilization matrix for whole cells-Application in an amperometric dopamine sensor. Biosensors & Bioelectronics **5**: 431-448

Doyle, M. P., Beuchat, L.R., Montville, T.J., ed. 1997. Food Microbiology: Fundamentals and Frontiers. American Society for Microbiology Press. Washington, DC.

D'Souza, S. F. 2001. Microbial Biosensors (Review). Biosensor & Bioelectronics **16**: 337-353.

Echeverria, P., Blacklow, N.R., Smith, N.R. 1975. Role of heat labile toxigenic *Escherichia coli* and virus-like agent in diarrhea in Boston children. Lancet 2: 1113-1116.

Echeverria, P., Taylor, D. N., Leksomboon, U., Bhaibulaya, M., Blacklow, N., Tamura, K., Sakazaki, K. 1989. Case control study of endemic diarrheal disease in Thai children. Journal of Infectious Disease 159: 543-548.

Elkind, J. L., Stimpson, D.I., Strong, A., Bartholomew, D.U., Melendez, J.L. 1998. Integrated Analytical Sensors: The Use of TISPR-1 as a Biosensor. Sensors and Actuators 11: 98.

Epstein, A. J. 1987. Insulator to metal transition in polyaniline. Synthetic Metal 18: 303-309.

Fang, G. D., Lima, A.A.M., Martins, C.V., Nataro, J.P., Guerrant, R. L. 1995. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: A hospital based, prospective, case-control study. Journal of Pediatric Gastroenterology Nutrition 21: 137-144.

Fratamico, P. M., Strobaugh, T.P., Medina, M.B., Gehring, A.G. 1998. Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor. Biotechnology Technique 12: 517-576.

Freedman, D. J., Tacket, C. O., Delehanty, A., Maneval, D. R., Nataro, J., Crabb, J. H. 1998. Milk immunoglobulin with specific activity against purified colonization factor antigens can protect against oral challenge with enterotoxigenic *Escherichia coli*. Journal of Infectious Disease 177: 662-667.

Gang, G., Mathan, M.M., Mathan, V.I. 1995. Evaluation of a simplified HEp-2 cell adherence assay for *Escherichia coli* isolated from south Indian children with acute diarrhea and controls. Journal of Clinical Microbiology 33: 2204-2205.

Glynn, M. K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar, M., Angulo, F.J. 1998. Emergence of multidrug-resistant *Salmonella* enterica serotype Typhimurium DT104 infections in the United States. New England Journal of Medicine 338: 1333-1338.

Gordillo, M. E., Reeve, G.R., Pappas, J., Mathewson, J., DuPont, H. L., Murrays, B. 1992. Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. Journal of Clinical Microbiology 30: 889-893.

Granholm, P., Paloheimo, J., Stubb, H. 1997. "Conducting Langmuir-Blodgett films of polyaniline: Fabrication and charge transfer properties." Synthetic Metal **84**: 783-784.

Grif, K., Dierich, M.P., Allerberger, F. 1998. DynaBeads plus 3Mpetrifilm HEC versus Vitex Immunodiagnostic Assay system for detection of *E. coli* O157:H7 in minced meat. Letters in Applied microbiology. **26**:199-204

Griffin, P. M., Tauxe, R.V 1991. The Epidemiology of Infections Caused by *Escherichia coli* O157:H7, other Enterohemorrhagic *E. coli*, and the Associated Hemolytic Uremic Syndrome. Epidemiology Review **13**: 60-98.

Guerrant, R. L., Kirchhoff, L.V., Schields, D. F. 1983. Prospective study of diarrheal illnesses in Northeastern Brazil: Pattern of disease, nutritional impact, etiologies and risks factors. Journal of Infectious Disease **148**: 986-997.

Gurwith, M. J., Hinde, D., Gross, R., Rowe, B. 1978. A prospective study of enteropathogenic *Escherichia coli* in endemic diarrheal disease. Journal of Infectious Disease **137**: 292-297.

Gurwith, M. J., Williams, T.W. 1997. Gastroenteritis in children. A two-year review in Manitoba. Journal of Infectious Disease **136**: 239-247.

Hale, T. L., Sansonetti, P.J., Schad, P.A., Austin, S, Formal, S.B. 1983. Characteristics of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. Infection and Immunity **40**: 340-350.

Harris, J. R., Marieno, J., Wella, J.G., Payne, B.J., Donelle, H.D., Cohen, M. L. 1985. Person to person transmission in an outbreak of enteroinvasive *Escherichia coli*. American Journal of Epidemiology **122**: 245-242.

Hatch, A., Weigl,B., Zebert, D.,Yager, P. 1999. Microfluidic approaches toward immunoassays. Microfluidic devices and systems II, Santa Clara, CA, Proceedings of SPIE.

Hedberg C.W., Osterholm M.T. 1994. "Changing epidemiology of food-borne disease: a Minnesota perspective." Clin Infect Dis **18**: 671-82.

Hedberg, C. W., Savarino, S. J., Besser, J. M. 1997. An outbreak of foodborne illness caused by *Escherichia coli* O39: NM: An agent that does not fit into the existing scheme for classifying diarrheagenic *E. coli*. Journal of Infectious Disease **176**: 1625-1628.

Heller, A. 1990. "Electrical wiring of redox enzymes. Acc. Chem. Res **23**: 128-134.

Hoa, D. T., Suresh Kumar, T.N., Punekar, N.S., Srinivasa, R.S., Lal, R., 1992. Contractor, Biosensor based on conducting polymers. Analytical Chemistry **64**: 2645-2646.

Hobbs, B. C., Thomas, M, Taylor, J. 1949. School outbreak of gastroenteritis associated with pathogenic paracolon bacillus. Lancet ii: 530-532.

Hoffmann, S. L. 1993. Shiga-Like Toxins in Hemolytic-Uremic Syndrome and Thrombotic Thrombocytopenic Purpura. American Journal of Medicine Science **306**: 398-406.

Hui, Y. H., Gorham, J.R., Murell, K.D., 1994. Foodborne Disease Handbook, Volume 1: Disease caused by bacteria. Marcel Dekker, Inc. New York.

Hyams, K. C., Bourgeois, A.L., Merrell, B. R. 1991. Diarrheal disease during operation Desert Shield. New England Journal of Medicine **325**: 1423-1428.

Imisides, M. D., John, R., Wallace, G.G. 1996. Microsensors based on conducting polymers. Chemtech **261**(5): 9-25.

Inoue, K., Miki, K., Tamuru, K., Sakazaki, R. 1996. Evaluation of L-pyrrolidonyl peptidase paper strip test for differentiation of members of the family Enterobacteriaceae, particularly *Salmonella* spp. Journal of Clinical Microbiology **34**: 1811-1812.

Ivnitski, D., Abdel-Hamid, I., Atanasov, P., Wilkins, E. 1999. Biosensor for detection of pathogenic bacteria. Biosensor & Bioelectronics **14**: 599-624.

Jay, J.M. 2000. Modern Food Microbiology. Gaithersburg, MD, Aspen Publishers, Inc.

John, R., Spencer, M., Wallace, G.G., Smith, M. 1991. Development of a polypyrrole-based human serum-albumin sensor. Analytical Chimica Acta **249**:381-385

Killalea, D., Ward, L.R., Roberts, D. 1996. An outbreak of *Salmonella* infection in England and the United States caused by ready to eat savoury snack. British Medical Journal **313**: 1105-1107.

Killard, A.J., Smith, M.R., Grennan, K., Micheli, L., Palleshi, G. 2000. Rapid antibody biosensor assays for environmental analysis. Biochemical Society Transactions **28**: 81-84.

Kim, J. H., Cho, J.H., Cha, G.S 2000. Conductimetric membrane strip immunosensor with polyaniline-bound gold colloids as signal generator. Biosensor & Bioelectronics **14**: 907-915.

KPL, Kirkegaard & Perry Laboratories, Inc. 2002. Online catalog for affinity purified antibody to *E. coli* O157:H7. Available from URL:<http://kpl.com/ordering/onlinecat2.html>

Le Monor, L., Popoff, M.Y. 1987. Designation of *Salmonella enterica* sp. nov., nom.rev., as the type and only species of genus *Salmonella*. International Journal of Systematic Bacteriology **37**: 465-468.

Lee, L. A., Puh, N.D., maloney, E.K. 1994. Increase in antimicrobial resistant *Salmonella* infections in the United States. Journal of Infectious Disease **170**: 18-34.

Levine, M. M., Ferreccio, C., Prado, V., Cayazzo, M., Abrego, P., Martinez, J., Maggi, L., Baldini, M.M., Martin, W., Maneval, D., Kay, B., Guers, L., Lior, H., Wasserman, S.S., Nataro, J.P. 1993. Epidemiologic studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri- urban community in Santiago, Chile. American Journal of Epidemiology **131**: 886-904.

Lin, J. S., Tsen, H.Y. 1999. Development and the Use of Polymerase Chain Reaction for the Specific Detection of *Salmonella* Typhimurium in Stool and Food Samples. Journal of Food Protection **62**(10): 1103-1110.

Macdiarmid, A. G., Chiang, J.C., Richter, A.F. 1987. Polyaniline: A new concept in conducting polymer. Synthetic Metal **18**: 285-290.

MacDonald, K. D., Edison, M., Strohmeyer, C. 1985. A multistate outbreak of gastrointestinal illnesses caused by enterotoxigenic *Escherichia coli* in imported semi soft cheese. Journal of Infectious Disease **151**: 716-720.

Manchester, A., Clauson, A. 1995. 1994 Spending for food away from home. Food review **18**: 12-15.

Marier, R., Wells, J.G., Swanson, R.C., Callahan, W., Melhman, I.J. 1973. An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. Lancet **ii**: 1376-1378.

Martin, D. L., MacDonald, K.L., White, K.E., Soler, J.T., Osterholm, M.T. 1990. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. New England Journal of Medicine **323**: 1161-1167.

Mayer, H. B., Wanke, C.A. 1995. Enteroaggregative *Escherichia coli* as a possible cause of diarrhea in an HIV-infected patient. New England Journal of Medicine **332**: 273-274.

Mead, P. S., Slutsker, L., Dietz, V., McGaig, L., Bresee, J., Shapiro, P., Tauxe, R. 1999. Food-Related Illnesses and Death in the United States.

Meeusen, C. 2000. Evaluation of a surface resonance biosensor for the identification of *Salmonella* Typhimurium and *Escherichia coli* O157: H7 in the pork production chain. Master of Science Thesis. Department of Agricultural Engineering, Michigan State University, USA.

Millipore .1999. A short guide: Developing Immunochromatographic Test Strips.

Mishu, B., Koehler, J., Lee, L.A. 1994. Outbreaks of *Salmonella* enteritidis infections in the United States, 1991- 1994. Journal of Infectious Disease **169**: 547-552.

Moss, J., Vaughan, M. 1980. Mechanism of activation of adenylate cyclase by cholera toxin and *E. coli* heat-labile enterotoxins. In Secretory Diarrhea, 107-126. M. Field, ed. Bethesda, MD: American Physiological Society.

Neill, M. A., Tarr, P. I., Taylor, D. N., Wolf, M. 2001. *Escherichia coli*: Second Edition, revised and expanded. In Foodborne Disease Handbook, 169-212. Y. H.

NFSI. 1997. National Food Safety Initiative: Food Safety from Farm to Table. Washington, DC.

Nisbet, D. J., Ziprin, R.L. 2001. Salmonellosis in Animals. Foodborne Disease handbook, Second Edition, revised and expanded, 265-275. Y. H. Hui, ed. New York, Basel: Marcel Dekker, Inc.

O' Brien, A. D., Lively, T.A., Chen, M.E., Rothman, S.W., Formal, S.B. 1983. *Escherichia coli* O157:H7 strains associated with hemorrhagic colitis in the United States. Lancet **i**: 702.

Osama, T., Kimura, O., Kabata, T. 1992. A solid electrolytic paper battery containing electroconductive polymers. Electronics and communications in Japan **75** :88-96

Ostroff, S. M., Kobayashi, J.M., Lewis, J.H. 1989. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. JAMA **262**: 355-359.

Paek, S.H., Lee, C.H., Yook, S.H., Kwon, O.H., park, Y.N. 1999. Performance control strategies of one step immunochromatographic assay for *Salmonella* Typhimurium Analytical letters **32**:335-360

Quadrat, O., Stejskal, J., Kraochvil, P., Klason, C., McQueen, D., Kubat, J., Saha, P. 1998. Electrical properties of polyaniline suspensions. Synthetic Metal **97**: 37-42.

Ramsey, P. G., Neill, M.A. 1986. Thrombotic thrombocytopenic purpura associated with *Escherichia coli* O157:H7. Morbidity and Mortality Weekly Report **35**: 549-551.

Riemann, H. P., Cliver, D.O. 1998. *Escherichia coli* O157:H7. The Veterinary Clinics of North America: Food Animal Practice. Microbial foodborne pathogens, 41-48. J. Vassallo, ed. Pennsylvania: W.B Saunders Company.

Riley, L. W., Remis, R.S., Helgerson, S. D., 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine **308**: 681-685.

Rowe, P. C., Orrbine, E., Wells, J.G., McLaine, P.N. 1991. Epidemiology of hemolytic uremic syndrome in Canadian children from 1986- 1988. Journal of Pediatrics **119**: 218-224.

Ryder, K. S., Morris, D.G., Cooper, J.M. 1997. Role of conducting polymeric interfaces in promoting biological electron transfer. Biosensor & Bioelectronics **12**: 721-727.

Sadik, O. 1995. Analytical Applications of conducting polymers. Analytical Methods and Instrumentation **2**: 293-301.

Salaneck, W. R., Lundstrom, I. 1987. Electronics properties of some polyaniline. Synthetic Metal **18**: 291-296.

Salyers, A. A., Whitt, D.D. 1994. Bacterial Pathogenesis, a Molecular Approach. Washington, DC: ASM Press.

Schroeder, S. A., Caldwell, J.R., Vernon, J.M. 1968. A waterborne outbreak of gastroenteritis in adults associated with *Escherichia coli*. Lancet **i**: 737-740.

Sergeyeva, T. A., Lavrik, N.V., Rachkov, A.E. 1996. Polyaniline label-based Conductimetric Sensor for IgG detection. Sensors and Actuators B **34**: 283-288.

Sergeyeva, T. A., Pilletskii, S. A., Rachkov, A. E., El' skaya, A. V. 1994. Synthesis and Examination of Polyaniline as labels in Immunosensor Analysis. Journal of Analytical Chemistry **51**(4): 394-396.

Skotheim, A. 1987. A Handbook of Conducting Polymers. New York: Marcel Dekker.

Slutsker, L., Ries, A.A., Greene, K.D. 1997. *Escherichia coli* O157:H7 diarrhea in the United States: Clinical and epidemiologic features. Ann. Intern. Med 126: 505-513.

Smith, H. W., Gyles, C. L. 1970. The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *Escherichia coli* of porcine origin. Journal of Medicine and Microbiology 3: 387-401.

Syed, A. A and Dinesan, M.K .1991. Polyaniline-novel polymeric material. Talanta 38: 815-837.

Tamiya, E., Karube, I., Hattori, S., Suzuki, M., Yokoyama, K. 1989. Micro glucose sensors using electron mediators immobilized on a polypyrrole-modified electrode. Sensors and Actuators 18: 297-307.

Taylor, D. N., Echeverria, P. 1986. Etiology and epidemiology of travelers' diarrhea in Asia. Rev. Infectious Disease 8 (suppl. 2): 136-141.

Taylor, D. N., Echeverria, P., Sethabutr, O. 1988. Clinical and Microbiological features of Shigella and enteroinvasive *Escherichia coli* infections detected by DNA hybridization. Journal of Clinical Microbiology 26: 1362-1366.

Thayer, D. W., and Raykowski, K.T. 1999. Developments in Irradiation of Fresh Produce and Vegetables. Food Technology 53: 62-65.

Toledo, M., Alvariza, M., Murahovschi, J., Ramos, S.R.T., Trabulsi, L.R. 1983. Enteropathogenic *Escherichia coli* serotypes and endemic diarrhea in infants. Infection and Immunity 39: 586-589.

Turner, A. P., Newman, J.D .1998. An Introduction to Biosensor. Biosensor for Food Analysis. UK, Athenaeum Press Ltd: 13-27.

USBC. 1990. 1990 Census of Population General population characteristics. Washington D.C., U.S. Department of Commerce Bureau of Census: CP 1-1.

USBC. 1993. Population projections of the US by age, race, sex and hispanic origin: 1993-2050. Washington, DC, U.S department of Commerce, Bureau of Census: 5-1104.

USDA 1996. Vegetables and specialties. USDA Economic Research Service. Washington, DC. Pg. 22.

USDA-FSIS. 1998. Foodnet: An active surveillance system for bacterial foodborne disease in the United States. Washington, DC, United State Department of Agriculture.

USEPA. 1986. Parker, L. Health and Ecological Criteria Division, U.S. Environmental Protection Agency (USEPA), Office of Water, Washington, DC. This manual was prepared under EPA Contract No. 68-C-98-141 by The CDM Group, Inc., Chevy Chase, Maryland.

USEPA. 2002. U.S. Environmental Protection Agency (USEPA). Ground water & Drinking water. Available from URL: <http://www.epa.gov/safewater/ecoli.html>.

Wanger, A. R., Murra, B. E., Echeverria, P., Mathewson, J.J. 1988. Enteroinvasive *Escherichia coli* in travelers with diarrhea. Journal of Infectious Disease **158**: 640-642.

Watts, H., Lowe, C., Pollard-Knight, D. 1994. Optical Biosensor for Monitoring Microbial Cells. Analytical Chemistry **66**: 2465-2470.

Weil, A. J., Saphra, I. 1953. Salmonella and Shigellae. Springfield, IL: Charles C. Thomas.

WHO. 1997. Multidrug resistant *Salmonella* Typhimurium, World Health Organization. 2002. Available from URL: <http://www.who.int/inf-fs/en/fact139.html>

Yamamura, K., Sumi, N., Egashira, Y., Fukuoka, I., Motomura, S., Tsuchida, R. 1992. Food poisoning caused by enteroinvasive *Escherichia coli* (O164:H7) -a case in which the causative agent was identified. Kansenshogaku Zasshi **66**: 761-768.

Yen Wei, X. t., Yan Sun. 1989. A study of the mechanism of aniline polymerization. Journal of polymer science: Part A: Polymer chemistry **27**: 2385-2396.

Yin, W., Angelopoulos, M., Epstein, A.J., MacDiarmid, A.G. 1997. Experimental evidence for hydrogen bonding in polyaniline: Mechanism of aggregate formation and dependency on oxidation state. Macromolecules **30**: 2953-2955.

Younts, S. 1999. Development and evaluation of a gas sensor based instrument for the detection and differentiation of *E. coli* O157H7 from non-O157: H7 *E. coli*. Master of Science Thesis. Department of Animal Science. Michigan State University, USA.

Zhang, S, Wright, G., Yang, Y. (2000). Materials and techniques for electrochemical biosensor design and construction. Biosensors & Bioelectronics 15:273-282

Ziprin, R. L. 1994. *Salmonella*. Foodborne Disease Handbook, Volume 1: Disease Caused by bacteria, 253-318. Y. H. Hui, Gorham, J.R., Murell, K.D., Cliver, D.O., ed. Marcel Dekker. New York.

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