

# Comparison of Phage-Based Magnetoelastic Biosensors with Taqman-Based Quantitative Real-Time PCR for the Detection of *Salmonella typhimurium* Directly Grown on Tomato Surfaces

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## Abstract

A phage-based magnetoelastic (ME) biosensor method was compared with a TaqMan-based quantitative real-time PCR (Q-PCR) method for the detection of *Salmonella typhimurium* on tomato surfaces. This ME biosensor method utilizes magnetoelastic resonators coated with E2 filamentous phage to bind with and measure the concentration of *S. typhimurium*. In this study, standard curves, correlations, and limits of detection (LOD) for the ME biosensor and Q-PCR methods were determined by inoculating tomato surfaces with *S. typhimurium* suspensions in concentrations ranging from 1 to 8 log CFU/tomato. The LOD for the ME biosensor method and Q-PCR were 3 and 2 log CFU/tomato, respectively. In a direct comparison of the detection methods, *S. typhimurium* suspensions (3 log CFU/tomato) were inoculated on 65 tomato surfaces, then incubated at 37°C and 100% RH for 24 h. After 24 h, *S. typhimurium* was positively detected by both methods and the quantified concentrations were nearly the same, (6.35 ± 2.03) and (6.34 ± 0.17) log CFU/tomato respectively for the ME biosensor method and the Q-PCR method, which were significantly greater than the concentration determined by the BGS-plate count method (5.33 ± 0.21). Scanning electron microscopy (SEM) was used to confirm the growth of *S. typhimurium* on the tomato surfaces and the binding of *S. typhimurium* on the measurement sensors. This study demonstrated that the ME biosensor method was robust and competitive with Q-PCR for *S. typhimurium* detection on fresh produce.

**Keywords:** *Salmonella typhimurium*; Magnetoelastic biosensor; E2 phage; Quantitative real-time PCR; Probe; Limit of detection

## Introduction

Outbreaks of *Salmonella* infection have recently occurred in a wide variety of fresh fruits and vegetables including lettuce, tomatoes, alfalfa sprouts, alfalfa seeds, artichokes, bean sprouts, cabbage, cilantro, lettuce, parsley and tomatoes, etc. [2]. From 1996 to 2008, eighty-two foodborne illness outbreaks were associated with the consumption of fresh produce (FDA, 2009). Of these outbreaks, 14 outbreaks (17.1%) were associated with the consumption of tomatoes and led to 1,927 illnesses and 3 deaths in the United States (FDA, 2009). Tomatoes have been one of the most common of fresh produce items implicated with *Salmonella* outbreaks [3]. Since contamination can occur at any point along the farm to fork continuum, it would be ideal for fresh tomatoes to be inspected systematically in the field or on the processing line with a real-time and on-site detection methods. Rapid and direct detection of *Salmonella* on fresh produce will decrease the number of illness outbreaks by providing timely data on the identification and quantification of *Salmonella* in contaminated foods [3].

Considerable effort has been directed towards the development of rapid and simple detection methods such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and several types of biosensor methods in order to substitute for the laborious and time-consuming conventional detection methods [5,29,30]. PCR is one of most promising alternative methods of detecting *Salmonella* in foods since many studies have shown that PCR is well suited for rapid, sensitive, specific and accurate bimolecular detection [5,11,16]. In addition, recently emerging PCR assays with fluorogenic probes (dye SYBR green, TaqMan, molecular beacons, and scorpion) have facilitated the development of real-time PCR assays more accurate and sensitive towards the identification and quantification for *Salmonella* [5,8,20]. In a preliminary study, a TaqMan-based quantitative real-time PCR method (Q-PCR) for the detection of *S. typhimurium* on tomato

surfaces was demonstrated. The TaqMan assay system consists of two primers and a probe that binds with a specific site of the PCR amplicon produced during the amplification process. As the PCR process continues, the 5' nuclease activity of DNA polymerase cleaves the probe resulting in separation of the reporter and quencher dyes. The cleavage of the probe increases the fluorescence and its intensity is proportional to the amount of PCR amplicon produced [6,19].

A phage-based magnetoelastic (ME) biosensor has been developed as a novel, wireless, direct detection method for in-field use [10,13,17,18,27]. ME biosensors have been successfully demonstrated to detect pathogens and spores such as *S. typhimurium* and *Bacillus anthracis*. The phage-based ME biosensor is composed of a ME resonator platform coated with genetically engineered filamentous phages for the specific recognition of a target pathogen. The detailed principle of ME biosensor operation has been explained in previous studies [18,23,24]. In brief, the ME biosensor method is based on measuring the change in resonant frequency which is proportional to the number of *S. typhimurium* cells bound to the ME resonator platform. The ME resonator platform is made from a magnetostrictive material that elongates or contracts under an applied external magnetic field. The platform's resonant frequency is measured wirelessly using an

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inductive pick-up coil. On binding with *S. typhimurium*, the mass of the ME biosensor platform increases resulting in a decrease in the platform's resonant frequency. Therefore, *S. typhimurium* can be identified and quantified by the resonant frequency changes in the phage-based ME biosensor. A recent study [23] demonstrated the practical suitability of the ME biosensor method by detecting *S. typhimurium* grown directly on the surface of tomatoes under favorable humidity and temperature conditions.

The comparison with other widely recognized alternative detection methods will be necessary in order to fully validate and evaluate the ME biosensor method. To date, no other investigation is known to have compared the ME biosensor method with a TaqMan-based quantitative real-time PCR (Q-PCR) method. Based on the validated set of primers and advances in Q-PCR assay, the detection limit of the phage-based ME biosensor method was compared with a Q-PCR analysis by developing linear standard curves after controlled growth of *S. typhimurium* on tomato surfaces. *S. typhimurium* was artificially inoculated then directly grown on tomato surfaces, detected, and the concentration quantified using the equations of the standard curves. Finally, the practicability and applicability of the ME biosensor method is compared with Q-PCR assay for use as an on-site and in-field detection method.

## Materials and Methods

### Bacterial strains

*S. typhimurium* (ATCC 13311) was provided from Dr. James M. Barbaree's Laboratory in the Department of Biological Sciences at Auburn University, Auburn, AL.

### Tomatoes and spot-inoculation

Red, ripe tomatoes (*Lycopersicon esculentum* var. *esculentum*) were purchased from a local grocery store (Auburn, AL, USA). Only tomatoes free of visible defects such as bruises, cuts, and abrasions were used after washing with sterilized-distilled water (DW) five times to remove all possible contaminants. A square ( $5 \times 5 \text{ mm}^2$ ) was drawn on the tomato surface with a fine-tip permanent marker using a sterile paper template for spot-inoculation. An aliquot of  $10 \mu\text{l}$  suspension was inoculated in the center of the marked area on the tomato surface. The inoculated tomato was placed in a glass container and was placed under laminar air flow for 90 min to allow the attachment *S. typhimurium* on the tomato surfaces.

### TaqMan-based quantitative real-time PCR (Q-PCR) methodology

**Primers and probe:** PCR primers and probe for the TaqMan assay were originally designed by Park et al. (2008) [22] and the specificity of these primers were evaluated in a preliminary study. The primers were synthesized by IDT (Integrated DNA Technologies Inc., Coralville, IA, USA). The sequences of the primers were as follows: forward primer Sal-F, 5'-GCG CAC CTC AAC ATC TTT C-3' (Tm 54.4°C); and reverse primer Sal-R, 5'-CGG TCA AAT AAC CCA CGT TCA-3' (Tm 55.4°C). The probe was labeled with the fluorescent reporter dye 6-carboxyfluorescein (FAM) on the 5' end and with Minor Groove Binder-NonFluorescent Quencher (MGB-NFQ) on the 3' end. The sequence of the probe was follows: 6FAM ATC ATC GTC GAC ATG CMGBNFQ [22]. Primers and probe concentration were optimized using a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

**TaqMan-based quantitative real-time PCR (Q-PCR) protocol:** Q-PCR was performed using the 7300 Real-Time PCR system. A reaction volume of  $25 \mu\text{l}$  of PCR mixture containing  $12.5 \mu\text{l}$  of TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA),  $10 \mu\text{M}$  each of primers and probe, filtered DW and  $5 \mu\text{l}$  of extracted DNA. The thermal cycling condition was: 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 sec at 95°C, and 1 min at 60°C. All of the experiments were performed in duplicate or triplicate with positive and negative controls. Detection and quantification of amplified DNA was expressed as Ct values. If a Ct value exceeded 40, this reaction contained no target DNA and was considered to be a negative amplification [14,15].

### E2 phage-based magnetoelastic (ME) biosensor methodology

**Preparation of magnetoelastic strip-shaped resonator platforms and E2 phage immobilization:** Magnetoelastic (ME) resonator platforms,  $0.028 \text{ mm} \times 0.2 \text{ mm} \times 1 \text{ mm}$  in size, were fabricated following the procedures described in a previous study [18]. E2 phage was provided in the form of a suspension [ $1.0 \times 10^{12}$  vir/ml in Tris-Buffered Saline (TBS, pH 7.4)] by Dr. Barbaree's Laboratory. The E2 phage suspension was mixed with the equal amounts of TBS buffer to adjust the concentration of E2 phage ( $5.0 \times 10^{11}$  vir/ml in TBS). Each ME resonator platform was placed in an Eppendorf tube containing  $300 \mu\text{l}$  of diluted E2 phage suspension and incubated on a rotary shaker (8 rpm) at 22°C for 1 h. The resonator platforms were then washed three times with the TBS buffer and twice with sterilized DW in order to remove any unbound phage and salt debris. After immobilization of E2 phage, any unbound area of the resonator platform was blocked with  $300 \mu\text{l}$  of 1% bovine serum albumin (BSA, Sigma-Aldrich Co., St. Louis, MO, USA) at 22°C for 1 h. Finally, the resonator platform was washed three times with DW and allowed to air dry. The ME resonator platform was then ready for use as a measurement sensor. Control sensors were prepared using the same procedure, except for the elimination of the E2 phage immobilization step. The control sensors were used to compensate for the effects of environmental changes and non-specific binding. Prior to placement of both measurement and control sensors on an inoculated tomato surface, the initial resonant frequency of both sensors were measured with an HP 8751A network analyzer combined with an S-parameter test set.

**ME biosensor protocol:** The measurement and control sensors were placed on the tomato surfaces within the outlined areas. In order to increase the chance of making contact with *S. typhimurium*, three sets of measurement and control sensors were placed on the tomato surfaces. The tomatoes were placed in a humidity controlled container (RH 100%) and incubated at 30°C for 30 min [24]. After incubation, the measurement and control sensors were placed in the center of an inductive pick-up coil connected to the network analyzer. Finally, the resonant frequencies of the sensors were measured 10 times with the network analyzer which provided an average frequency shift data point. The resonant frequencies of the sensors before and after the placement on the tomato were compared [23,24].

### Determination of limit of detection (LOD)

Tomatoes were divided into two groups containing 27 tomatoes each for testing with ME biosensors and Q-PCR. For the spot-inoculation on the tomato surfaces,  $10 \mu\text{l}$  of *S. typhimurium* suspension, ranging from 3 log CFU/ml to 10 log CFU/ml in 0.03% TSB (Trypticase<sup>®</sup> Soy Broth, Difco Laboratories, Sparks, MD, USA), were inoculated on the defined area following the procedures described in section 2.2. In each group, three tomatoes were inoculated with the same concentration of *S. typhimurium*. After inoculation, one group of tomatoes was analyzed

using the ME biosensor procedures described in section 2.4.2. For the other group, the inoculated area was cut from the tomato with a sterilized knife for Q-PCR analysis. The cuts were transferred to a centrifuge tube containing 1 ml of sterilized phosphate buffered saline (PBS, pH 7.2, Sigma-Aldrich Co., St. Louis, Mo, USA) and glass beads. After mixing vigorously for 3 min, the cut suspensions were centrifuged at 5000 g (7500 rpm) for 10 min and the precipitations were purified using DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Each purified DNA sample was diluted with filtered DW to a total volume of 625  $\mu$ l so that the volume fraction (1/125) of one Q-PCR sample (5  $\mu$ l) corresponded to the same area fraction (1/125) of one ME biosensor (0.2 mm  $\times$  1 mm) over the same inoculation area (5  $\times$  5 mm<sup>2</sup>). In addition, interference by PCR inhibitors possibly existing in the food matrix would be reduced by the dilution [25,29]. The concentration of genomic DNA was measured with a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and Q-PCR was performed in triplicate in order to create the standard curve. The amplification efficiency (AE) of Q-PCR was calculated following the formula  $E = (10^{-1/\text{slope}} - 1) \times 100$  [5]. Lower AE means that the quantity of PCR products generated at each cycle decreases so that the amplification will take longer.

#### Application of ME biosensor and Q-PCR for *S. typhimurium* detection

The viability of bacteria obtained from artificially inoculated samples may differ from that of naturally contaminated samples due to unfavorable growth conditions in nature [21]. Therefore, *S. typhimurium* was inoculated with 0.03% of TSB and grown on tomato surfaces in order to mimic natural environmental conditions. Tomatoes were divided into two groups containing 65 tomatoes in each group and with 5 tomatoes used for controls (inoculated with sterilized DW instead of cell suspension). An aliquot of 10  $\mu$ l of *S. typhimurium* suspension at 5 log CFU/ml in 0.03% TSB medium was inoculated on tomato surfaces following the procedures described in section 2.2. The inoculated tomato samples were incubated at 37°C for 24 h with 100% RH in order to increase the number of *S. typhimurium* bacteria. One group of tomato samples was analyzed by the ME biosensor analysis and the other group by Q-PCR analysis using the procedures described in section 2.5.

For enumeration of viable *S. typhimurium*, plate counting of cultured samples was performed. An aliquot of 10  $\mu$ l of *S. typhimurium* suspension at 5 log CFU/ml in 0.03% TSB was inoculated on the defined 5  $\times$  5 mm<sup>2</sup> areas on 10 tomato surfaces and five tomatoes were inoculated with sterilized DW as a control experiment. All of tomatoes were incubated at the same conditions mentioned above. After incubation, the defined area on the tomato surface was cut with a sterilized knife and the cuts were transferred to a centrifuge tube containing 1 ml of PBS and glass beads. After mixing vigorously for 3 min, 100  $\mu$ l of the mixture was diluted serially with PBS buffer and the serially diluted mixture was plated in triplicate on brilliant green sulfa (BGS) agar (Difco Laboratories, Sparks, MD, USA). Finally, the plates were incubated at 37°C for 24 h and the colonies were counted. The colony count results were divided by 125 to scale to the same fraction as previously employed and the result was expressed as log CFU/tomato.

#### Microscopic analysis

A JEOL-7000F scanning electron microscope (SEM) was used to confirm the attachment and growth of *S. typhimurium* on the tomato surfaces and binding of *S. typhimurium* to measurement and control sensors. A 10  $\mu$ l aliquot of *S. typhimurium* (5 log CFU/ml in 0.03%

TSB) was inoculated on defined areas on ten tomato surfaces following the procedures described in section 2.2. For five of the tomatoes, the inoculated areas were cut from the tomato with a sterilized knife after attachment. The other five tomatoes were incubated at 37°C for 24 h with 100% RH and then the surface was cut for SEM observation. In order to observe the *S. typhimurium*, the cuts were exposed to osmium tetroxide (OsO<sub>4</sub>) vapor for 12 h to fix the bacteria followed by gold coating to a thickness of 140 nm using a PELCO sputter coater (Ted Pella Inc., Redding, CA, USA). In order to confirm the binding of *S. typhimurium* to the sensors, both measurement and control sensors were exposed to OsO<sub>4</sub> vapor for at least 45 min. The tomato segments and sensors were mounted on aluminum stubs for examination by SEM.

#### Statistical analysis

The experiments were replicated at least three times and the experimental results were expressed as arithmetic means (M) and standard deviations (SD). The student's paired t test for two groups and the one-way analysis of variance (ANOVA) among more than two groups were run to compare the means using GraphPad and InStat v.3 programs (GraphPad, San Diego, CA). The significance level was determined as  $P < 0.05$ ,  $P < 0.01$ , or  $P < 0.001$ . As a measure of repeatability, the variation coefficient (CV) was calculated by the formula:  $CV = (SD/M) \times 100$ .

#### Results and Discussion

In this study, we have compared our phage-based ME biosensor method with the well-known Q-PCR method. The specificity of both methods against *S. typhimurium*, one of the most important criteria to detect target cells, was evaluated in previous studies [22,28].

#### Limit of detection (LOD) of Q-PCR

The LOD in this study is defined as the lowest concentration of *S. typhimurium* that can be detected by Q-PCR or ME biosensor methods [7]. In Q-PCR, the threshold cycle (Ct) value is defined as the point at which probe fluorescence rises appreciably above the background [29]. The measured Ct value corresponding to genomic equivalent of the logarithmic CFU is plotted in Figure 1. As shown in Figure 1, the Ct value decreased as the genomic equivalent of the logarithmic CFU increased. A linear relationship was found with a correlation coefficient (R<sup>2</sup>) of 0.977 and a generated slope of -3.456. The amplification efficiency was 95% which was fit to the recommended range of 90 to 110% (Applied Biosystems, 2008). Since the Ct average (Ct = 44.213) at 1 log CFU/tomato was greater than 40, the LOD of Q-PCR assay was determined to be 2 log CFU/tomato for *S. typhimurium* detection. The LOD of Q-PCR was significantly lower than Guo et al.'s [11] study (2000), which showed that the LOD of PCR assay using *hlyA* primers for *S. Montevideo* detection was 5 log CFU /tomato.

#### Limit of detection (LOD) of ME biosensor

Figure 2 shows the resonant frequency changes of ME sensors to different concentrations of *S. typhimurium*. As the concentration of *S. typhimurium* increased, the measured resonant frequency shift increased, as anticipated. SEM microscopy also confirmed that the binding of *S. typhimurium* on the ME resonant platform increased. The LOD of ME biosensor is defined as the point of intersection of two linear lines (measurement and control sensor) where the measurement sensor rises appreciably above the control sensor. As shown in Figure 2, the LOD for the ME biosensor method was determined to be 3 log CFU/tomato. The resonant frequency shift of the measurement sensor could be distinguished from the control sensors at *S. typhimurium*

concentrations of 3 log CFU/tomato and greater. Although the LOD of the Q-PCR was one order lower than that of the ME biosensor, the LOD is a possible limiting factor for either method, especially when *Salmonella* contamination may occur with 1000 cells or less. Nonetheless, the fact that the LOD of the ME biosensor was only one order higher than that of the Q-PCR showed that the ME biosensor method is competitive to Q-PCR, considered to be one of the most sensitive detection methods known to date [5,11,16].

The resonant frequency change of the measurement sensors over the range of *S. typhimurium* concentrations from 3 log CFU/tomato is also plotted in Figure 2. A linear relationship is shown with a correlation coefficient ( $R^2$ ) of 0.944 and a generated slope of 1671, which was lower than that ( $R^2 = 0.977$ ) of Q-PCR. The sensitivity of the ME biosensor method, defined as the slope of the calibration curve [27], was calculated to be 1173 Hz/decade.

### Observation and enumeration of *S. typhimurium* directly grown on the tomato surface

Since the most predominant place for harboring *Salmonella* in or on tomatoes has been found to be the outer surface (82%) [12], the ME biosensor method has been applied directly to the tomato surfaces without any further sample preparation. *S. typhimurium* was inoculated and grown directly on the tomato surfaces in order to compare the ME biosensor method with Q-PCR. Our previous study [24] showed that minimum initial inoculum size for the *S. typhimurium* growth on the tomato surface was determined to be 3 log CFU/tomato surface with diluted nutrients. These findings agreed with Wei et al.'s [31] study (1995) in that *S. Montevideo* could grow with the help of nutrients (for example, in puncture wounds). Without any nutrient sources, the bacteria could only survive for several days on tomato surfaces at 20 and 25°C. Thus, *S. typhimurium* suspension (3 log CFU in 0.03% TSB) was inoculated on each of the tomatoes surfaces and incubated.

The attachment and growth of *S. typhimurium* was confirmed by SEM microscopic analysis (Figure 3). As shown in Figure 3A and 3B, obtained from SEM microscopic analysis, the distribution of *S.*

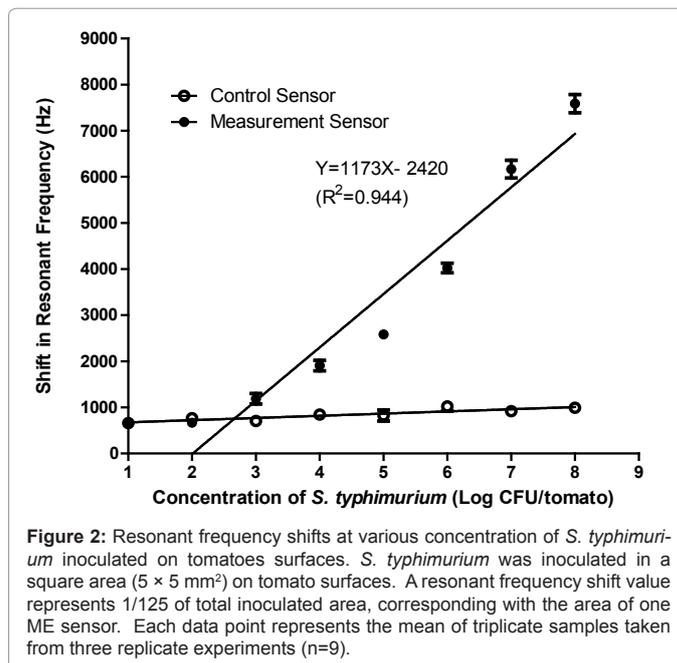


Figure 2: Resonant frequency shifts at various concentration of *S. typhimurium* inoculated on tomatoes surfaces. *S. typhimurium* was inoculated in a square area (5 × 5 mm<sup>2</sup>) on tomato surfaces. A resonant frequency shift value represents 1/125 of total inoculated area, corresponding with the area of one ME sensor. Each data point represents the mean of triplicate samples taken from three replicate experiments (n=9).

*typhimurium* was not uniform before incubation. The number of *S. typhimurium* increased after the 24 h incubation and expanded to adjacent surfaces outside the inoculation area (Figure 3C, D). After incubation, the distribution of *S. typhimurium* was again found to be not uniform. The number of viable *S. typhimurium* was determined to be  $5.33 \pm 0.21$  log CFU/tomato using the BGS-plate count method and this result was compared with the results obtained from the ME biosensor and Q-PCR.

### Detection and quantification of *S. typhimurium* directly grown on the tomato surface using Q-PCR

*S. typhimurium* directly grown on the surface of tomatoes was detected by Q-PCR and the result shown in Figure 4. As a control experiment, five tomatoes inoculated with only sterilized DW showed negative amplification with Ct values exceeding 40 (data not presented). However, all 65 tomatoes inoculated with *S. typhimurium* showed positive amplification with Ct values ranging between 23.2 (6.85 log CFU/tomato) and 26.4 (5.93 log CFU/tomato) (Figure 4). This difference may be attributed to the variation in the attachment and growth of the cells on the tomato surfaces. Since the repeatability represents the precision of the PCR method, the repeatability was determined from the overall mean and standard deviation obtained from Figure 4. The mean and standard deviation were  $24.97 \pm 0.71$  and the variation coefficient (CV) was calculated to be 2.8%. The quantified concentration of *S. typhimurium* was determined to be  $6.34 \pm 0.17$  log CFU/tomato using the averaged Ct value of all data points (24.97) in the equation generated by Figure 1 (Figure 6). The concentration of *S. typhimurium* obtained in this manner was approximately one log higher than the BGS-plate count method  $5.33 \pm 0.21$  log CFU/tomato, which is in good agreement with Jung et al.'s [15] (2005) findings, because DNA was purified from both dead and live cells on the tomato surfaces [26].

### Detection and quantification of *S. typhimurium* directly grown on the tomato surface using ME biosensor

As shown in Figure 3D, the distribution of *S. typhimurium* on the inoculated tomato surfaces was not uniform so that three sets of

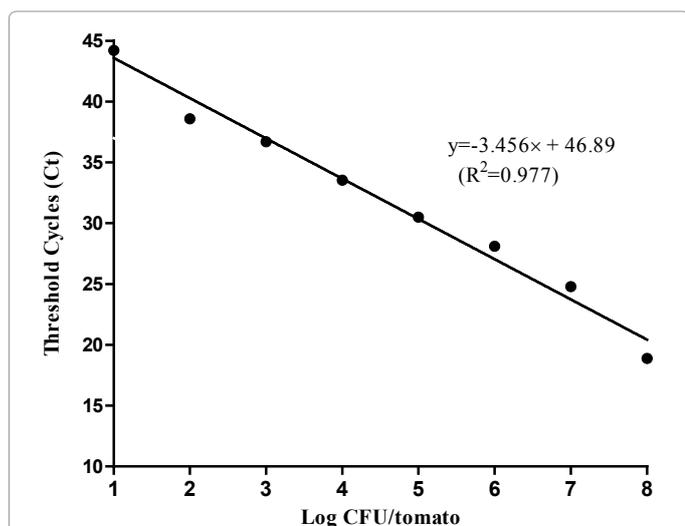


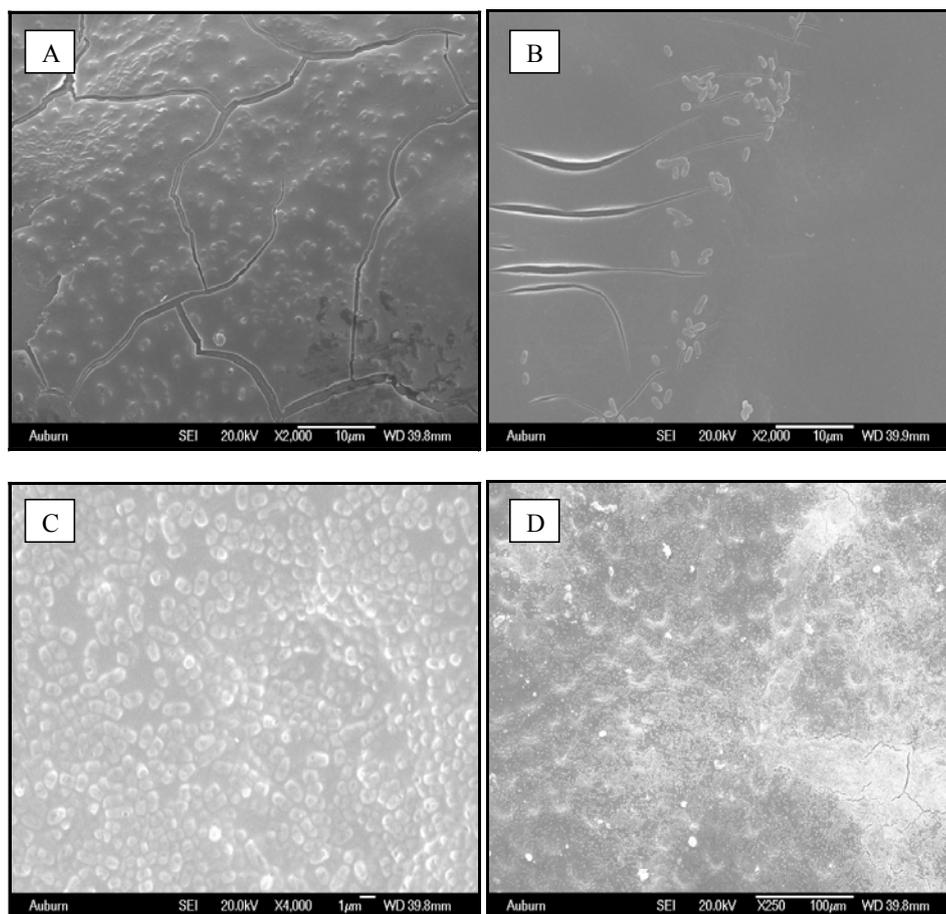
Figure 1: Standard curve for a 10-fold dilution series of *S. typhimurium* on tomato surfaces after Q-PCR performance. Threshold cycle (Ct) is plotted on the axis of ordinates and log CFU genome equivalent is plotted on the axis of abscissae. A Ct value represents 1/125 of the total inoculated area (5 × 5 mm<sup>2</sup>), corresponding with the area of one ME sensor. Each data point represents the mean of triplicate samples taken from three replicate experiments (n=9).

sensors were used in order to increase the chances of bacteria coming into contact with sensors. Figure 5 shows the distribution of measured frequency shifts of numerous measurement and control sensors after exposure to *S. typhimurium* on the tomato surfaces. Significant differences in the resonant frequency shifts between measurement and control sensors ( $P < 0.001$ ) occurred, although some of measurement sensors were not significantly different from control sensors. Due to the uneven distribution of *S. typhimurium* on the tomato surfaces, some of the measurement sensors showed comparatively low resonant frequency shifts. SEM images confirmed that there were obvious differences in the number of *S. typhimurium* bound to control (Figure 5A) and measurement sensors (Figure 5B, 5C). The magnitude of the resonant frequency shift is proportional to the mass of cells bound to the sensors, visually evident in Figure 5B and 5C. All 65 tomato samples inoculated with *S. typhimurium* showed significant increases in the resonant frequency shift based on the average of three measurement sensors per tomato ( $P < 0.05$ ). On the contrary, five tomatoes inoculated with only sterilized DW did not show any significant resonant frequency shift with either measurement or control sensors in the control experiment (data not presented). The repeatability of ME biosensor was determined to be 47.5% from the overall mean and standard deviation obtained from Figure 5. Finally, the quantified concentration of *S. typhimurium* was determined to be  $6.35 \pm 2.03$  log CFU/tomato using the equation

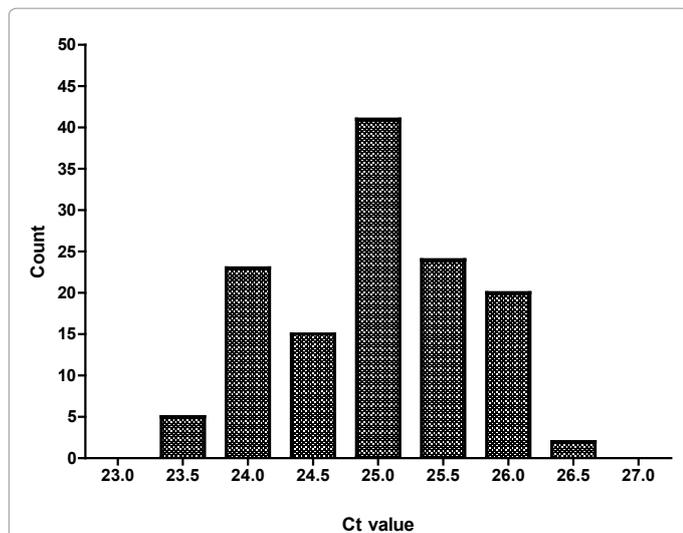
generated by standard curve of Figure 2.

### Comparison with BGS plate count method

Both the ME biosensor and Q-PCR methods were compared and also were compared with the BGS-plate count method to quantify the concentration of *S. typhimurium* grown on tomato surfaces (Figure 6). As shown in Figure 6, the negative control sample inoculated with sterilized DW did not show any growth of *S. typhimurium* on tomato surface. Since the PCR method cannot differentiate between live and dead cells [26], it may be assumed that the quantified concentration determined by Q-PCR may be greater than that obtained from the BGS-plate count method. The quantified concentration of *S. typhimurium* determined by Q-PCR method ( $6.34 \pm 0.17$  log CFU/tomato) was approximately one order higher than the BGS-plate count method ( $5.33 \pm 0.21$ ), which was significantly different ( $P < 0.05$ ). However, the quantified concentrations of *S. typhimurium* determined by the ME biosensor method ( $6.35 \pm 2.03$  log CFU/tomato) was also significantly different from that from BGS-plate count method but there was no significant difference between the Q-PCR and ME biosensor method. The fact that the quantified concentration determined by both methods was very similar, most likely indicates that the E2 phage on the measurement sensor did not differentiate between live or dead cells. While it is not anticipated that the E2 phage can differentiate between live or dead cells, further work is required to demonstrate this. More



**Figure 3:** SEM images of *S. typhimurium* on the tomato surfaces; (A) (B) before and (C) (D) after 24-h incubation at 37 °C with the initial concentration of 3.0 log CFU/tomato ( $5 \times 5$  mm<sup>2</sup>).



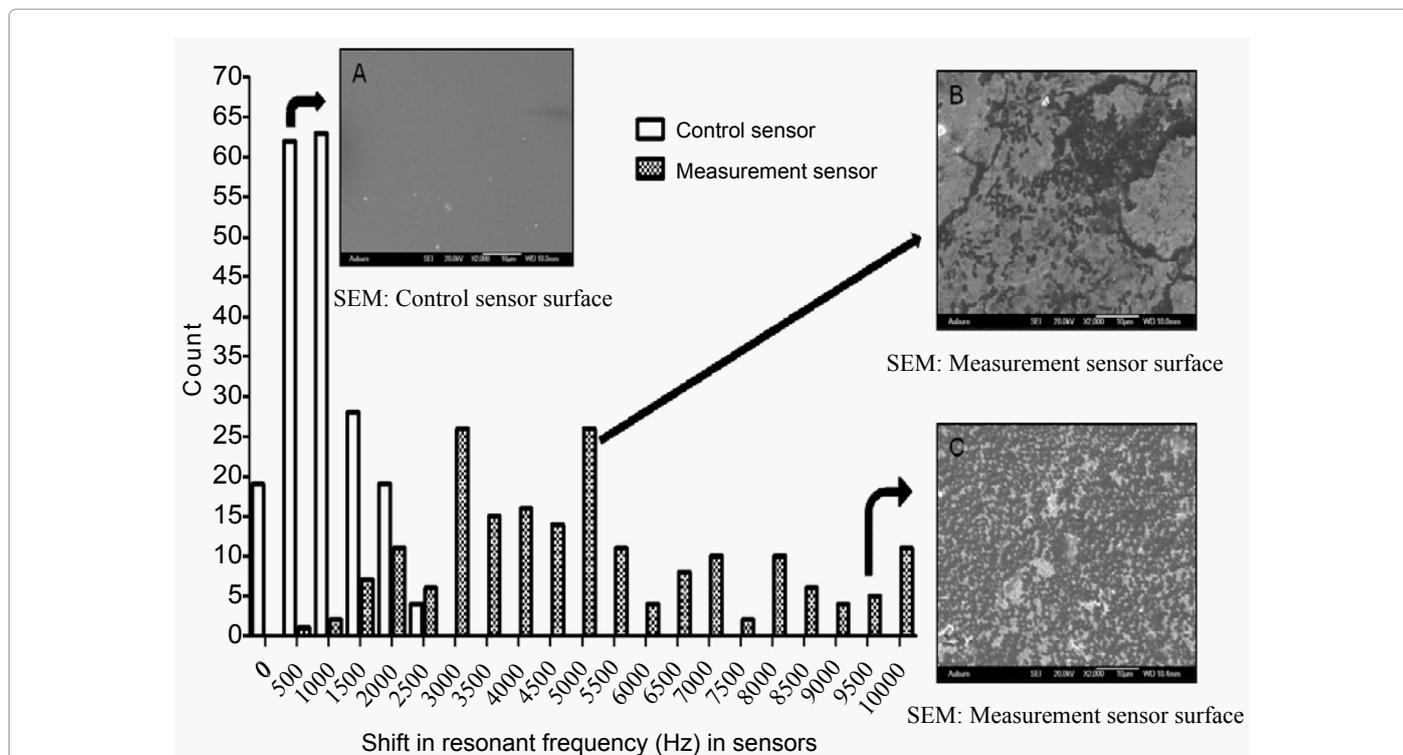
**Figure 4:** Distribution of Ct values for *S. typhimurium* detection using Q-PCR. *S. typhimurium* (3 log CFU in 0.03% TSB medium) was inoculated on tomato surfaces (5 × 5 mm<sup>2</sup>) and grown for 24 h at 37 °C and 100% RH. Q-PCR was performed in duplicate for each tomato sample. A Ct value represents 1/125 of total inoculated area (5 × 5 mm<sup>2</sup>), corresponding with the area of one ME sensor.

importantly, this study showed that our ME biosensor method detected and quantified *S. typhimurium* grown on tomato surfaces with similar accuracy as Q-PCR.

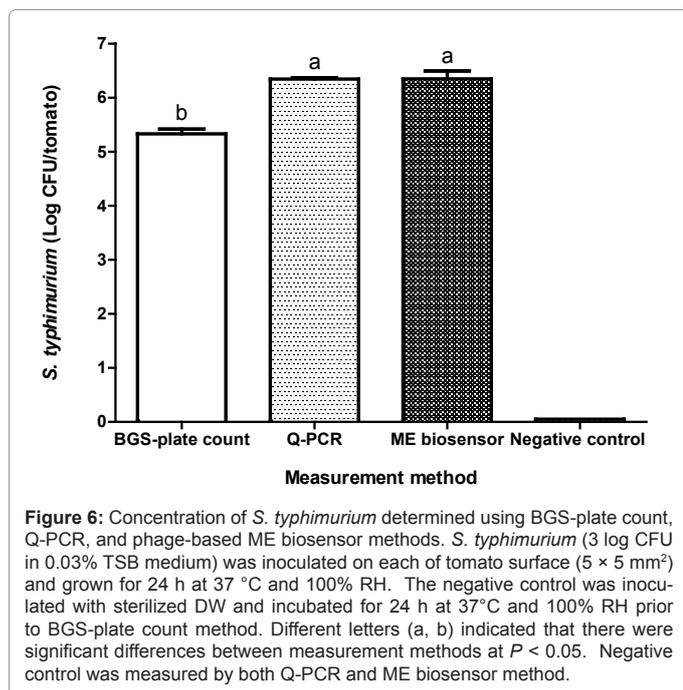
### Comparison of practicability and applicability

The direct detection of *Salmonella* or other bacteria without sample preparation using ME biosensors offers many advantages over competing methods, however, limitations may exist to the effectiveness of this method under certain conditions. Without sample preparation, one limitation to direct detection may be when *S. typhimurium* is found inside of the tomato in the juice or pulp, or when *S. typhimurium* is present under a firmly attached biofilm on the tomato surface. The mode of attachment of *S. typhimurium* to the tomato surface may also affect the accuracy of the ME biosensor method, depending on whether either specific or nonspecific adhesion to the tomato surface occurs which may be mediated by the bacterial cell structures such as pili or flagella. Another consideration is that the repeatability of the ME biosensor measurement (47.5%) was found to be more susceptible to the uneven distribution of *S. typhimurium* on the tomato surfaces (Figure 3) than Q-PCR (2.8%). The reduced opportunity for the bacteria and biosensor to interact at lower bacterial surface densities lessens the comparability of the ME biosensor and Q-PCR. When tomatoes with a more uniform distribution of bacteria were used, achieved by inoculating three tomato surfaces with 8 log CFU/tomato of *S. typhimurium*, the repeatability of the ME biosensor over a three day period was determined to be 5.7% (7732 ± 442). With a uniform distribution of *S. typhimurium* on the tomato surface, the repeatability of the ME biosensor method is both comparable and competitive with Q-PCR at 1.7% (18.882 ± 0.323).

Despite these limitations, there are several advantages of the ME biosensor presented in this study compared to the practicability and applicability of Q-PCR. A previous study [4] showed that the structure



**Figure 5:** Distribution of resonant frequency shifts (left) and SEM images (A, B, C) of *S. typhimurium* detection using a phage-based ME biosensor method. *S. typhimurium* (3 log CFU in 0.03% TSB medium) was inoculated on each of tomato surface (5 × 5 mm<sup>2</sup>) and grown for 24 h at 37 °C and 100% RH. ME biosensor method was performed using three sets of measurement and control sensor per each tomato sample. A shift in resonant frequency was obtained from one sensor, corresponding with 1/125 of total inoculated area (5 × 5 mm<sup>2</sup>).



of E2 phage was very robust, even when exposed to harsh temperature conditions up to 80°C. In another stability study of the ME biosensor method, the binding affinity of E2 phage with *S. typhimurium* was durable for two months even at 65°C, with only a gradual decrease in the binding affinity observed with time [24]. Furthermore, the ME biosensor is a cost-effective detection method due to lack of costly instruments, DNA purification kits, and fluorescence probe and primers. Instead, the ME biosensor method requires only 1 mm-size sensors, phage for a biorecognition probe, and relatively simple instrumentation for detection. In addition, the ME biosensor method is rapid, requiring only minutes of total detection time with no sample preparation involved whereas Q-PCR required 2-3 h detection times after sample preparation time. The level of training or expertise required to perform an analysis using the ME biosensor method is much less than Q-PCR method. Greater sensitivity (lower detection limit) can be obtained by decreasing the size of the ME biosensors [13]. By minimizing the sensor size and directly applying a large number of sensors to the fresh produce, broad coverage of fruits and vegetables can be obtained. This broader coverage is similar to increasing the number of samples taken, providing greater assurance that the produce is free of harmful levels of pathogens. These advantages contribute to the ME biosensor method being an effective on-site and in-field detection method to improve the food safety of fresh fruits and vegetables.

## Conclusions

In this study, a phage-based ME biosensor method was compared with Q-PCR. The correlation coefficient and LOD for these two methods were calculated and determined to be 0.944 and 3 log CFU/tomato for the ME biosensor and 0.977 and 2 log CFU/tomato for Q-PCR. When *S. typhimurium* was directly grown on tomato surfaces, both methods detected positively *S. typhimurium* on all 65 samples. The growth of *S. typhimurium* on the tomato surfaces and the binding of *S. typhimurium* on the measurement sensors for ME biosensor method was confirmed by SEM images. The quantified concentrations of *S. typhimurium* obtained using the equations developed from the standard curves

were nearly the same for both methods. Although the LOD of the ME biosensor method was found to be one order more than that of Q-PCR, the ME biosensor method is robust and competitive with Q-PCR due to several advantages. The practicability and applicability of the ME biosensor method was demonstrated by detecting *S. typhimurium* within minutes without further tedious sample preparation. In addition, the ME biosensor detection method is cost-effective, simple to operate, and suitable for large-scale coverage. Thus, the ME biosensor method is a promising alternative to the Q-PCR method. This study demonstrates that the ME biosensor method has great potential to serve as an on-site and in-field detection method in order to improve the food safety of fresh produce.

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