# Conductivity and pH Dual Detection of Growth Profile of Healthy and Stressed *Listeria monocytogenes*

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**Abstract:** In this study, growth of *Listeria monocytogenes* in a low conductivity growth medium (LCGM) was simultaneously monitored by conductivity and pH measurements. Detection times obtained from the conductivity and pH growth curves were inversely related to the initial concentration of *L. monocytogenes* in the medium. Linear responses were found by plotting detection times obtained from both conductivity and pH growth curves as a function of initial cell concentration in the range of $10^2$ to $10^7$ cfu/mL. The detection time was approximately 12 and 2 h for $10^2$ and $10^7$ cfu/mL of viable *L. monocytogenes*, respectively, using the conductivity growth curves, whereas it was approximately 1 h less using the pH growth curves. This dual detection system was used for evaluating the growth of acid-, temperature-, and salt-treated *L. monocytogenes* in the medium. Acid stress at pH 2 and 3 for 3 h caused approximately 12 and 4 h delay in the detection time on pH growth curves, while stress at pH 5 for 3 h did not cause a significant delay in detection time. Delay in detection times was also observed for *L. monocytogenes* cells exposed to 45°C for more than 1 h (2 and 6 h). Exposure to 10% NaCl for 3 h did not cause visible delay in the detection time. These observations on detection times for stressed *L. monocytogenes* had a consistent trend with the cell number decrease determined by surface plating method.

**Keywords:** pH; conductivity; detection; *Listeria monocytogenes*; stressed bacteria

## INTRODUCTION

*Listeria monocytogenes* has emerged as one of the most hazardous, potentially life-threatening, human food-borne pathogens. It is found widely throughout the environment and can contaminate many food products, such as milk, cheese, ice cream, raw vegetables, poultry, and meats. According to the Centers for Disease Control and Prevention (CDC), an estimated 2,500 illnesses with 500 deaths occur with listeriosis in the United States each year. Development of rapid, sensitive, simple, and cost effective methods for detecting this pathogen is extremely important in implementing an effective response to prevention of foodborne diseases.

The conventional microbiological methods for detection of *L. monocytogenes* based on culture enrichment and plate count techniques are generally time consuming and labor intensive, usually requiring 3–7 days for a presumptive result. As a result, over the past several years, a variety of rapid methods have been introduced for detecting *Listeria* in an effort to reduce the total assay time. Many of these rapid methods are based on typical or derived immunological assays (Koubova et al., 2001; Sewell et al., 2003; Vaughan et al., 2001) and nucleic acid-based tests (Amagliani et al., 2004; Choi and Hong, 2003; Ingianni et al., 2001), which reduce the assay time within several hours to 1 day and achieve detection limits varying from $10^1$ to $10^6$ cfu/mL. However, these methods are not capable of differentiating dead and live cells. In most cases, detection of live *Listeria* cells in samples, especially in food samples, is more appropriate as screening tests to ensure food safety since dead *Listeria* cells are usually not pathogenic.

One category of rapid methods for detection of live cells is by monitoring the growth of the organism (Nobel, 1999; Ur and Brown, 1975). Attempts in this area to detect the growth of *Listeria* have focused on culturing the organisms in medium and detecting the release of ionic metabolites from live *Listeria* cells electrochemically by impedance, conductivity, and capacitance (Gomez et al., 2002, 2005), the consumption of oxygen by cyclic voltammetric current (Ruan et al., 2003), or the cell density by optically measuring turbidity (Augustin et al., 1999). The major advantages of these growth based methods lie in the capability of detecting a few live pathogenic cells, the great reduction in the total assay time, and the

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continuous measurement without any operator. However, each of the growth based techniques individually may be open to interference or interpretation. This limitation can be overcome if dual or multiple detection techniques are used simultaneously. For example, Madrid et al. (1999) developed an automatic analyzer using simultaneous measurements of impedance and turbidity for monitoring microbial growth. Kounaves et al. (2001) proposed a life detection system using various sensors in array to detect many chemical parameters resulting from microbial metabolism and excretion to study the nature of extraterrestrial microbial life.

pH is a very important parameter related to the growth of bacteria that can provide much information about the bacteria. For example, organisms that can produce acid from carbohydrates produce sufficient acid to exceed the buffering capacity of the substrate medium and cause a pH change in the medium, which can produce a color change as detected by a pH indicator. Patterns of acid production from carbohydrates can be used for identifying microorganisms, which usually require 24–48 h incubation before the reaction patterns can be determined (CDC, 2004). The influence of pH on the lag phase of *L. monocytogenes* was studied by McKellar et al. (2002).

It is also very important to note that in food products, *L. monocytogenes* is often affected by stresses caused by a variety of processing treatments, including heating, freezing, drying, exposure to acids, salts, antimicrobials and to disinfectants. Sub-lethally injured or stressed bacteria may recover in food and regain their pathogenicity (McCarthy, 1991). Therefore, detection of stressed *L. monocytogenes* is of great importance in food safety and food hygiene. The development of better strategies for detection and enumeration of stressed *Listeria* in food have been reported by Kang and Fung (1999) and Wood et al. (1996).

In this study, we present a detection system for monitoring the growth profile of *L. monocytogenes* in a low conductivity growth medium (LCGM) using conductivity and pH dual measurements. The pH and conductivity of the medium were monitored simultaneously during the growth of *L. monocytogenes*. Enumeration of bacteria was carried out based on the observation of detection time on both pH-growth curve and conductivity-growth curve, that is the time for a cell population to reach a detectable level of signal. This growth-based method could detect only live *Listeria* cells in the medium instead of both live and dead *Listeria* in immunological and DNA-based methods. Besides, the dual detection system could provide a more reliable analytical tool for bacterial detection. In this work, this dual detection system was also used for evaluating the growth of *L. monocytogenes* that were stressed by acid, temperature, and salt.

## MATERIALS AND METHODS

### *Listeria* Culture, Media, and Growth Condition

Culture of *Listeria monocytogenes* V7 (serovar 1/2a) was grown in brain heart infusion broth (BHI) at 37°C, 140 rpm, for 16 h. The cells were pelleted by centrifuging (Eppendorf) at 6,000*g* for 5 min and resuspended in sterilized deionized water. Serial dilutions were prepared in deionized water for further applications. The viable cell number of *L. monocytogenes* was determined by surface plating 0.1 mL of appropriate dilutions onto BHI agar. Colonies were counted after incubation at 37°C for 24 h. The number of cells usually reached $10^9$ colony forming units per milliliter (cfu/mL).

The BioV LCGM$^{TM}$ growth medium consisting of tryptone, yeast extract, glucose, and bovine serum albumin (BSA) (BioVitesse, Inc., Sunnyvale, CA) was used in all the experiments for growth and recovery of stressed *Listeria* cells. It has an initial pH of $6.8 \pm 0.2$ and an initial conductivity of $\sim 1.2 \pm 0.2$ ms/cm.

### Stress Conditions

For acid stress, *L. monocytogenes* cells from log phase (16 h) of growth were centrifuged (6,000*g*, 10 min) and resuspended in BHI broth, which were previously adjusted to pH 5, 3, and 2 with 1 M HCl, and was incubated at room temperature for 3 h. For temperature stress, *L. monocytogenes* V7 was grown in BHI broth at 37°C for 16 h under constant agitation. The culture was shifted to 45°C and was held for 1, 2, and 6 h, respectively. Salt stress was performed by resuspending the centrifuged cells into BHI broth added with 10% (w/v) NaCl for 3 h. *L. monocytogenes* cells were also stressed under a combined condition as 10% NaCl, pH 5 and 45°C for 1 h. Before and after treatments, the cultures were serially-diluted and plated in triplicate on BHI agar plates to determine viable cell counts because both injured and uninjured *L. monocytogenes* are able to grow on BHI agar plates.

### Conductivity and pH Measurements

The setup of the conductivity and pH measurements is shown in Figure 1. Conductivity and pH measurements were performed using a pH/conductivity meter (Jenco, San Diego, CA). A 15 mL of the tested medium was held in a 50 mL tube (Becton Dickinson Labware, Frankin Lakes, NJ) and inoculated with the dilutions of *L. monocytogenes* to obtain a desired initial cell number in the medium. The tube was

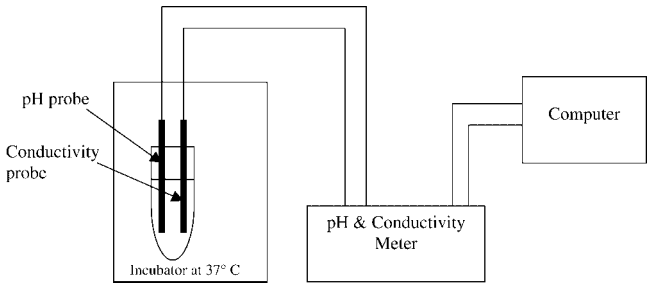


**Figure 1.** The experimental setup of the conductivity and pH dual detection system. A 15 mL of low conductivity growth medium (LCGM) inoculated with 150 μL culture dilutions to obtain desired initial concentrations.

placed in an incubator (Lab-Line Instruments, Inc., Melrose Park, IL) to keep the medium temperature at $37 \pm 0.5^{\circ}$C. A pH probe (Serial No. JC05708) and a conductivity probe (Serial No. JC01452) (Jenco, San Diego, CA) were immersed into the medium and were linked to the pH/conductivity meter. pH and conductivity data were collected every 5 min during the growth of *L. monocytogenes* within a total testing time of 20 h. pH and conductivity growth curves were obtained by plotting the absolute values of pH and conductivity as a function of growth time. Before and after each test, the pH sensor was calibrated with pH 4.0 and 7.0 standard solutions, and conductivity probe was calibrated with a standard solution with 0.7 ms/cm. At the end of each test, the pH and conductivity probes were sterilized in 70% ethanol for 30 min, and rinsed thoroughly with deionized water.

## RESULTS AND DISCUSSION

### Conductivity and pH Growth Curves of *L. monocytogenes*

Figure 2 shows the typical (A) conductivity and (B) pH growth curves, absolute conductivity or pH value as a function of growth time, obtained using the conductivity/pH dual detection system for an initial cell number of $2.66 \times 10^5$ cfu/mL. In the conductivity growth curve, there are three regions, an initial region where the conductivity changes very little, the second region where the conductivity increases sharply, and the third region where the conductivity become relatively constant again. The conductivity of the medium is 1.15 ms/cm at initial time, while it increases slowly to 1.24 ms/cm during the first 7 h, it increases rapidly during 7–10 h period, raising the conductivity to 1.35 ms/cm, with an increase of 17.4% of the initial conductivity. After the fast increase period, the conductivity start to slowly increase again, it reaches approximately 1.45 ms/cm after 20 h growth of *Listeria* in the medium, which increases 26.1% from the initial value. The pH growth curve shows a similar pattern with three regions except that the pH value decreases over the time while the conductivity value has an increasing trend during the whole growth period. As shown in Figure 2, pH of the medium changes a little during the first 6 h. It decreases from 6.7 to approximately 5.6 during the next 4 h with a 16.4% decrease of the initial pH, and decreases slowly to 4.9 at the end of test (20 h) with a decrease of 26.9% from the initial pH. Repeated tests on samples with initial cell numbers of $2.55 \times 10^6$, $2.39 \times 10^6$, and $2.66 \times 10^6$ cfu/mL indicated that the pattern of both the conductivity and pH growth curve were reproducible. The counts for cfu/mL were obtained from triplicate culture plate measurements. The percentage

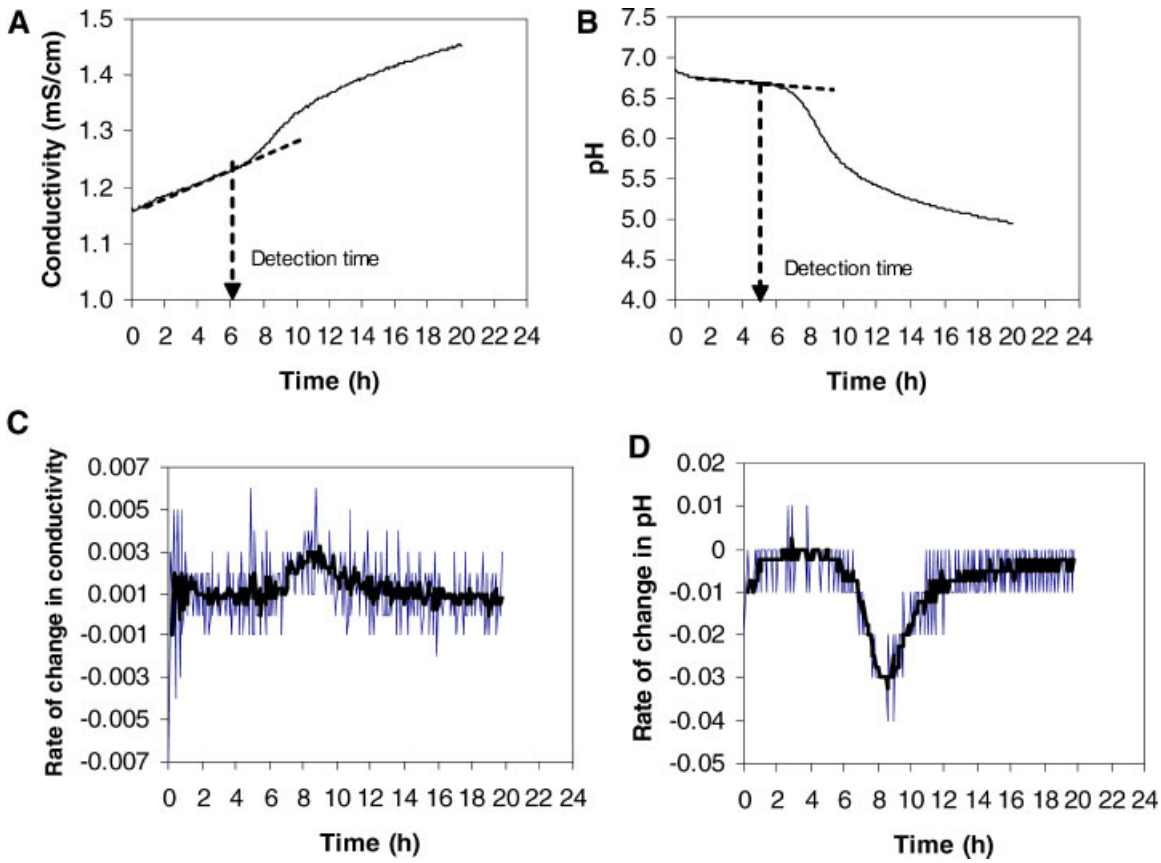


**Figure 2.** Typical conductivity (**A**) and pH (**B**) growth curves and the rates of changes in conductivity (**C**) and pH (**D**) during the growth of *Listeria monocytogenes* in LCGM medium. Initial cells number: $2.66 \times 10^5$ cfu/mL. Data were recorded every 5 min. Rates of changes in conductivity or pH refer to Δconductivity of ΔpH per 5 min. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

changes in pH and conductivity at 20 h were $26.6 \pm 0.5\%$ and $25.8 \pm 2.2\%$, respectively. These results indicate that both the curve pattern and the percentage change in pH measurement are consistent with those of conductivity measurement. As conductivity method has been widely accepted as one of the automatic methods for monitoring bacterial growth, our results suggest that pH measurement could also provide information about bacterial growth characteristics during the growth of bacteria, if the calibration of the pH sensor is not significantly altered by biofouling. It can be used together with conductivity measurement or serve as an alternative tool in some cases when conductivity measurement is not applicable.

When *L. monocytogenes* cells grow in the medium, they metabolize the substrates that are usually non-charged or weakly charged molecules into small and highly charged molecules, resulting in an increase in ionic strength and thus an increase in conductivity. When there are carbohydrates in the medium that can be metabolized by *L. monocytogens* cells, $H^+$ are released into the medium. While conductivity measurement monitors the total ionic composition change, which includes the release of $H^+$ and other ions, the pH measurement detects only the release of $H^+$. In the conductivity or pH measurement, the total ionic species or $H^+$ produced by cell growth must significantly change the total ionic strength or pH to obtain a reliable measurement.

Figure 2 also shows the rates of changes in (C) conductivity and (D) pH per 5 min during the growth of *L. monocytogenes* derived from conductivity and pH growth curves. It can be seen that the rates of changes in pH and conductivity have a quite similar trend. They are relatively constant in the first period, then increase and reach a maximum rate, and decease to a relatively constant value, which is the same as that of the first period. As shown in Figure 2, for the sample containing $2.66 \times 10^5$ cfu/mL *L. monocytogenes*, the rate of change in conductivity in the first period is at an average of 0.001 ms/cm, and reaches a maximum average value at 0.003 ms/cm. The rate of change in pH is at an average of $-0.01$ units in the first 5–6 h, and has a maximum of $-0.04$ units. It is believed that the changes in pH and conductivity during the first period are due to the dissolution of $CO_2$ into the medium or temperature variations, as the initial slow changes in pH and conductivity were also observed with no cell in the medium. In this period, the cell number is too low to generate enough amounts of $H^+$ or other ions to significantly change pH or conductivity. These results indicate that conductivity measurement requires a threshold of 0.001 ms/cm per 5 min and pH measurement requires a threshold of 0.01 units per 5 min for detection of the growth of *Listeria* in this study.

## Effects of Bacterial Cell Population on Changes in pH and Conductivity

The shapes of the pH and conductivity curves match very well with the typical shape of a bacterial growth curve, which includes the lag phase where bacteria are metabolizing but not multiplying, the exponential growth phase where bacteria multiply exponentially, and the stationary phase where the bacterial cell number remains relatively constant. Figure 3 shows the pH growth curves along with the bacterial cell number during the 24 h growth of *Listeria* for three samples with initial cell numbers at (A) $2.39 \times 10^5$ and (B) $1.14 \times 10^6$ and $2.65 \times 10^6$ cfu/mL. As shown in Figure 3, the trends of the cell number curves for initial cell numbers in the same order ($10^6$ cfu/mL) or in different orders ($10^5$ and $10^6$ cfu/mL) are reproducible. For all three samples, when there were no changes in pH in the first period, bacterial cell number actually increased slowly with time. The possible reason is that the cell population was not high enough or their growth rates were not fast enough to produce detectable changes in pH. Therefore, strictly speaking, the first period on the pH growth curve is not identical to the lag phase on a traditional bacterial growth curve. This is the common limitation of other methods that are based on changes in physical properties (turbidity, conductivity, etc.) of the growth medium. These physical properties usually provide little information

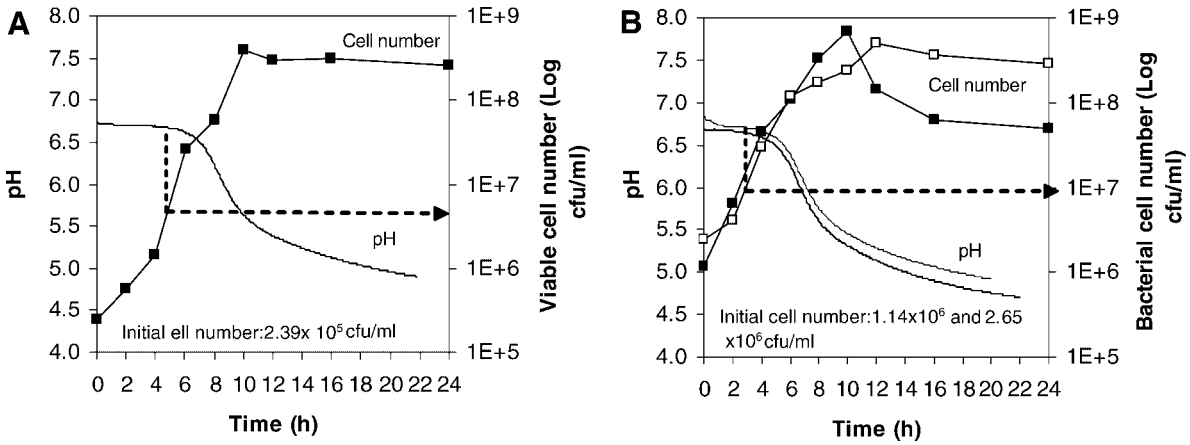


**Figure 3.** pH growth curvges of *Listeria monocytogenes* in LCGM with initial cell concentrations of (**A**) $2.39 \times 10^5$ cfu/mL and (**B**) $1.14 \times 10^6$ and $2.65 \times 10^6$ cfu/mL along with their cell numbers obtained from surface plate counting on BHI plates every 2 h during the first 16 h and the 24th h.

on the lag phase till the number of bacteria increases above the threshold of detection of the technique (Baranyi and Pin, 1999).

From the cell number curve, it can be seen that bacterial population enters into a fast increase period after the first slow growth period, and significant change in pH can be seen in this period. For the sample with $2.39 \times 10^5$ cfu/mL of *Listeria*, significant change in pH can be seen when bacterial cell number increases to approximately $8 \times 10^6$ cfu/mL. Change in pH can be seen when bacterial cell number reaches approximately $2 \times 10^7$ cfu/mL for the sample with an initial cell number of $2.65 \times 10^6$ cfu/mL. Such large cell population and fast growth release sufficient amounts of $H^+$ that produce significant change in pH. This observation is consistent with the observation in classical impedance measurement that the cell number of $10^6$–$10^7$ cfu/mL is required to produce significant changes in conductivity measurement (Adams and Moss, 1995). In other words, approximately $10^7$ cfu/mL of *Listeria* is required to produce a change in pH that exceeds the threshold (0.01 pH units per 5 min) observed in this study.

The time corresponding to the point where significant change in pH is observed on the pH growth curve is usually referred as *detection time*, and can be a very important indication of a growth characteristic of a certain initial bacterial concentration. For example, it takes 3.9 and 5.8 h for the bacterial concentrations of $2.65 \times 10^6$ and $2.39 \times 10^5$ cfu/mL to reach the detection time, respectively. The result of this study indicates that viable bacterial cell growth has experienced a lag phase, a slow growth phase, and a part of fast growth phase, when the pH curves reach the detection time.

The cell number remains relatively constant or starts to decrease very slowly when bacterial cell number reaches about $10^8$ cfu/mL or more, due to the depletion of nutrients, decreasing pH or increasing acidity of the medium (pH $4.9 \pm 0.2$), corresponding to the stationary phase of the bacterial growth. And pH of the medium becomes relatively stable again.

## Enumeration of *L. monocytogenes*

Typically, there are two ways to use the growth curves such as conductivity, optical density or turbidity curves in studies of bacterial growth. Some researchers linked the shape of the conductivity curve to specific growth characteristics for estimating bacterial growth parameters, usually in model studies (Baranyi and Pin, 1999; McKellar et al., 2002; Metris et al., 2003). Others used the observation of detection time instead of the whole growth curve for enumerating bacteria, which are based on a calibration curve by plotting the detection time against initial cell concentration (Yang et al., 2003, 2004).

In this study, the detection times on both conductivity and pH growth curves were used to evaluate the bacterial growth. The detection time was defined as the time point where the rate of the changes in conductivity or pH value starts to change significantly, as represented by arrows in Figure 2. It also can be seen that the detection time obtained on the pH growth curve is about 1 h less than that on the conductivity growth curve. This may be due to that $H^+$ concentration reached a detectable level earlier than the total ionic strength reached a detectable level. This result suggests that the pH growth curve may provide a more rapid way for estimating bacterial growth.

Figure 4 shows a group of representative (A) pH and (B) conductivity growth curves obtained from samples containing different concentrations of *L. monocytogenes* from $10^2$ to $10^7$ cfu/mL. As seen in the Figure, the group of pH growth curves is more uniform than the group of conductivity growth curves. This is possibly because the conductivity measurement is more dependent on environmental interferences such

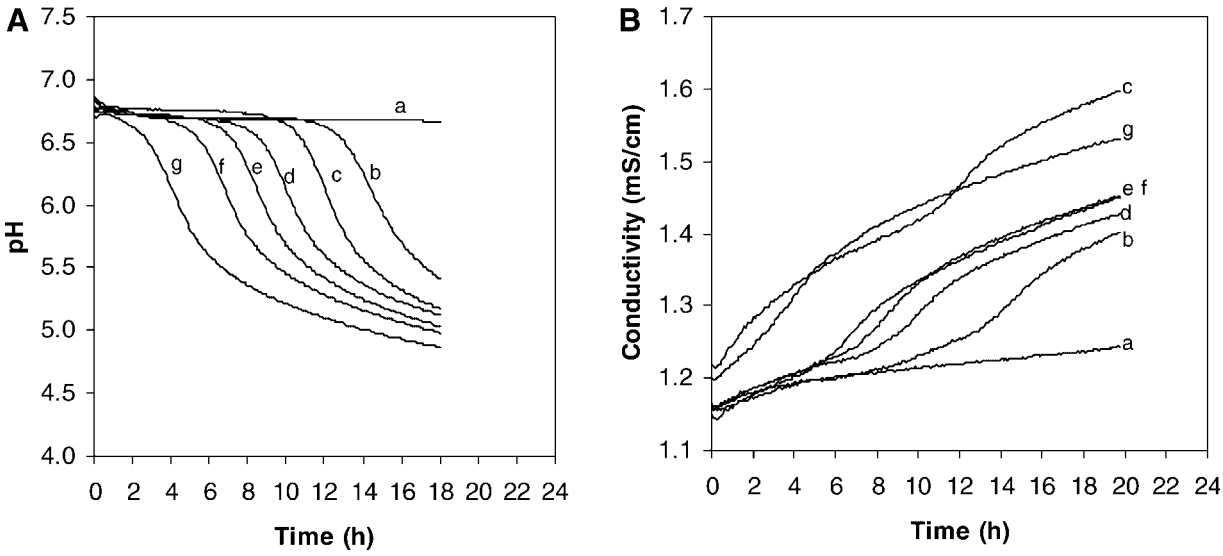


**Figure 4.** A group of representative pH-growth curves (**A**) and conductivity growth curves (**B**) obtained from samples containing different initial cell number of *Listeria monocytogenes*. Curve (a) no cell, (b) $2.5 \times 10^2$, (c) $2.39 \times 10^3$, (d) $2.64 \times 10^4$, (e) $2.66 \times 10^5$, (f) $2.65 \times 10^6$, (g) $2.7 \times 10^7$ cfu/mL.

as temperature variations or variations in ionic strength of the medium. For example, it is reported that a temperature increase of 1°C results in 1.8% change in conductance for a typical ionic medium (Eden and Eden, 1984). Variations in the initial values of conductivity among these conductivity growth curves could be attributed to small fluctuations in medium temperature of each experiment. However, these small variations in conductivity curves do not have influence on the observation of detection times to evaluate the growth of *L. monocytogenes* in this study. The control samples, which contain no cell or dead cells, showed no change in the rate of pH and conductivity changes, indicating that there was no ionic release resulted from bacterial metabolism in the medium over the test time. All the samples containing *L. monocytogenes* within concentration range from $10^2$ to $10^7$ cfu/mL reach a point where the rate of change in pH or conductivity starts to change, that is the detection times. It can also be seen that the detection time is related to the initial bacterial concentration in the medium. The higher the initial concentration is, the shorter the detection time.

Figure 5 shows the plots of logarithmic values of initial *Listeria* cell number as a function of detection times obtained from the pH growth curves and conductivity growth curves. The detection time was inversely proportional to the initial cell number. Linear relations between the detection times ($t_D$, h) and the logarithmic values of the initial cell number ($N$, cfu/mL) in the medium were found to be $t_D = -1.97 \text{ Log } N + 16.98$ with $R^2 = 0.98$ and $t_D = -1.82 \text{ Log } N + 14.96$ with $R^2 = 0.98$, for conductivity and pH measurements, respectively. The detection times for $10^2$ to $10^7$ cfu/mL initial cell numbers were between 10–12 and 1–2 h, respectively, which was close to the result of electrochemical cyclic voltammetry method based on the monitoring of consumption of oxygen in the medium (Ruan et al., 2003).

## Monitoring the Growth of *L. monocytogenes* Stressed by pH, Temperature, and Sodium Chloride

In food, bacteria are often subjected to various stresses induced by physical and chemical treatments during processing and storage. Stressed bacteria may recover in foods and regain their pathogenicity. Detection of the growth of *L. monocytogenes* stressed under conditions associated with food processing, preservation, and distribution is very important for ensuring food safety. Acidification and salting are widely used in the food industry as natural preservation processes. Exposure to acid, salt and high temperature is known to induce stress in *L. monocytogenes*. For example, Besse et al. (2000) reported a study on the recovery of salt and acid damaged *L. monocytogenes* on a solid repair medium. Acid-, salt-, or temperature-induced stresses also affected the antigen expression and antibody reaction in *L. monocytogenes* (Geng et al., 2003). However, conductivity and pH measurements for monitoring the growth of stressed *L. monocytogenes* in liquid media have not been reported. In this study, the growth of stressed *L. monocytogenes* in LCGM medium were monitored using pH and conductivity measurements. The bacteria were exposed to stress environments of acid (pH 5, 3, and 2), high temperature (45°), and NaCl (10%, w/v).

Figure 6 shows the results of a set of experiments on the growth of (A, B) acid-, (C, D) temperature-, and (E, F) salt-treated *L. monocytogenes*, monitored by pH and conductivity

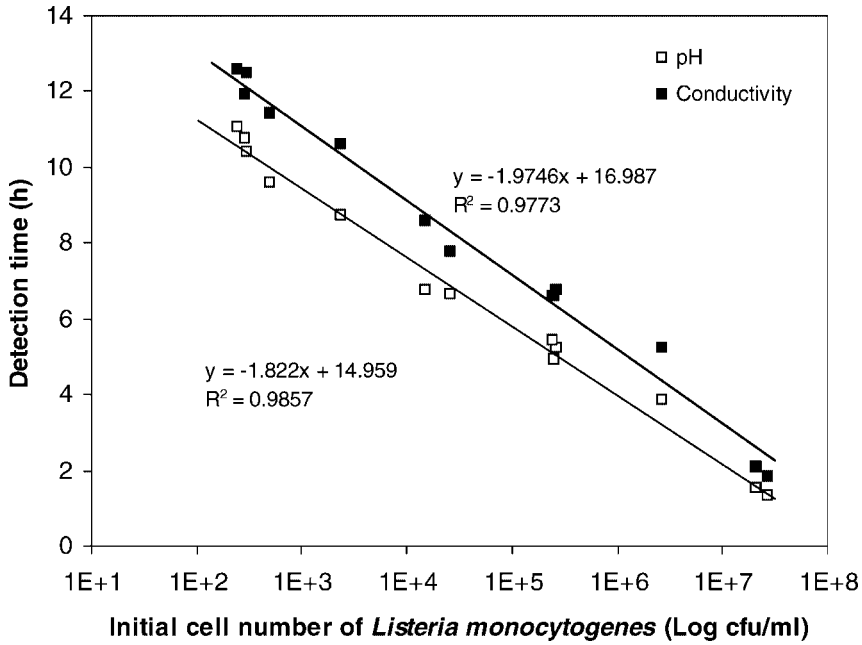


**Figure 5.** The linear relationship between the logarithmic values of initial cell number of *Listeria monocytogenes* in the medium and the detection times obtained from pH growth curves and conductivity growth curves in the cell number ranging from $10^2$ to $10^7$ cfu/mL.

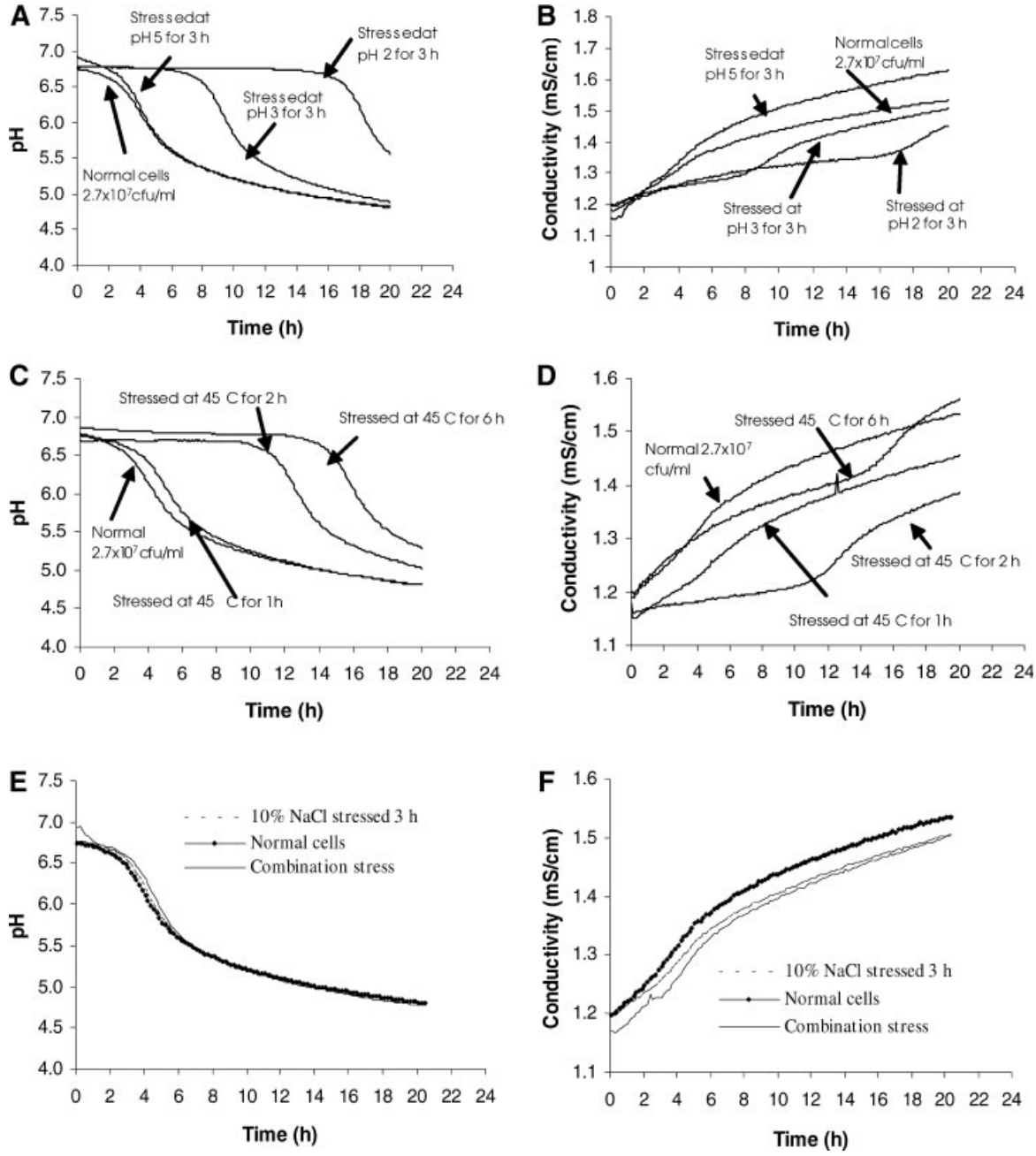


**Figure 6.** pH and conductivity growth curves obtained from samples containing pH stressed (**A**, **B**), temperature (45°C) stressed (**C**, **D**) and salt-stressed and combination stressed (**E**, **F**) *Listeria monocytogenes*. Initial cell numbers for normal and stressed cell samples were $2.7 \times 10^7$ cfu/mL.

measurements. In acid stress experiments, *Listeria* cells ($10^9$ cfu/mL) were stressed at pH 5, 3, and 2 for a short duration (3 h), respectively, and then 150 μL of stressed cells were grown in 15 mL LCGM medium for 20 h. As seen in Figure 6A and B, the shapes of the pH growth curves and conductivity growth curves are basically the same as those obtained from samples containing normal *Listeria* cells. However, by looking at the detection times on these curves, it is clear that different pH stress conditions result in differences in the growth responses monitored by conductivity and pH measurements. The results show that *Listeria* cells stressed at pH 5 in BHI medium for 3 h did not change their growth characteristics in the subsequent growth in LCGM medium. Lower pH stresses resulted in a significant delay in the detection times observed on pH and conductivity growth curves. For example, based on the pH growth curves in Figure 6A, while the growth of normal $10^7$ cfu/mL cells was detected within 2 h, the growth of $10^7$ cfu/mL cells that were stressed at pH 3 for 3 h could be detected in 6 h. It took 15 h for the growth of $10^7$ cfu/mL cells that were stressed at pH 2 for 3 h to reach the detection time. The long detection times suggests that exposure to pH 3 and 2 cause death of a large portion of cell population, resulting in a decrease in viable cell number in the inoculum that was initially introduced to

**Table I.** Results of acid-, temperature-, salt- stressed *Listeria monocytogenes* cells, including detection times obtained from pH growth curves, calculated initial cell number using detection time, and cell numbers obtained from surface plate counting on BHI plates.

| Stress condition | Detection time from pH curve (h) | Calculated cell number (cfu/mL) | Cell number[b] before stress (cfu/mL) | Cell number[b] after stress (cfu/mL) |
|---|---|---|---|---|
| pH 2, 3 h | 14.5 | 1.73 | $\sim 10^7$ | $<10^2$ |
| pH 3, 3 h | 6.0 | $8.38 \times 10^4$ | $1.69 \times 10^7$ | $2.25 \times 10^5$ |
| pH 5, 3 h | 1.91 | $1.50 \times 10^7$ | $2.9 \times 10^7$ | $2.15 \times 10^7$ |
| 45°C, 6 h | 11.83 | $5.13 \times 10^1$ | $2.4 \times 10^7$ | $<10^4$ |
| 45°C, 2 h | 9.33 | $1.22 \times 10^3$ | $2.32 \times 10^7$ | $1.03 \times 10^4$ |
| 45°C, 1 h | 1.83 | $1.66 \times 10^7$ | $2.6 \times 10^7$ | $1.96 \times 10^7$ |
| 10% NaCl, 3 h | 2.08 | $1.21 \times 10^7$ | $2.38 \times 10^7$ | $1.37 \times 10^7$ |
| Combination[a], 1 h | 2.33 | $8.83 \times 10^6$ | $2.45 \times 10^7$ | $>10^4$ |

[a]*L. monocytogenes* cells were stressed under pH5, 45°C, and 10% NaCl for 1 h.
[b]Cell numbers were obtained by averaging colony count on four BHI plates.

the LCGM growth medium. In fact, the decreases in viable cell numbers were validated by surface plate counting on BHI agar plates before and after stress treatments.

Table I listed the results of detection times obtained from pH growth curves, the calculated cell numbers using the detection times, and viable cell numbers before and after stress conditions. For pH stress experiments, the viable population of *L. monocytogenes*, as determined by colonies formed on BHI plates, had a 2 log reduction, decreasing from$1.69 \times 10^7$ to $2.25 \times 10^5$ cfu/mL after exposure to pH 3 for 3 h. Exposure to pH 2 for 3 h resulted in more than 5 log reduction in viable cell population. However, exposure to pH 5 did not cause a significant reduction in viable cell number. These results indicate that exposure to pH 5 does not cause a large portion of deaths in the cell population of *L. monocytogenes* cells, while exposure to low pH 3 and 2 results in significant death to the bacterial cell population. This observation is consistent with the results reported by George et al. (1988) that *L. monocytogenes* can generally grow in the pH range of 4.4–9.2. Although it is reported that acid (pH 2–3) stress environments had very little effect on antigen expression or antibody reaction in *L. monocytogenes* (Geng et al., 2003), such low pHs are beyond the regime of bacterial growth and cause severe damages to cells.

The decreases in viable cell numbers are common observations in acid treatment experiments on *L. monocytogenes.* Despite the decrease in viable cell number, there is a certain percentage in the remaining viable cell population that are injured due to acid exposure. Similar observation was reported by Besse et al. (2000) where viable cell number of *L. monocytogenes* decreased more than 2 log, and about $80 \pm 5\%$ of cells in the remaining viable population were injured after a low pH treatment (pH 4.7) for 14 days (Besse et al., 2000). However, the percentages of injured cells were not determined in the present study, because it was beyond the objective of the study.

Due to modification of cell permeability caused by the damage of the cell wall or cell membrane, acid treated cells became sensitive to many chemicals and lost their ability to grow on the selective media used in microbiological analysis. The viable cell numbers determined by the detection times on pH growth curves are 1.73, $8.34 \times 10^4$, and $1.50 \times 10^7$ cfu/mL for the samples exposed to pH 2, 3 and 5, while the results obtained from BHI plate counts are $<10^2$, $2.25 \times 10^5$, and $2.15 \times 10^7$ cfu/mL, respectively (Table I). The small difference in the viable cell numbers determined by these two methods might be due to the difference between the LCGM growth medium and BHI medium for the recovery of stressed *L. monocytogenes*. On the other hand, our results also demonstrated that pH and conductivity growth curves could be an effective method for enumerating viable cell number in liquid media and could provide valuable information of the growth of bacteria cells in liquid media for studying the recovery of stressed bacteria.

Figure 6C and D shows the pH and conductivity growth curves of temperature stressed *L. monocytogenens* in LCGM. High temperature (45°C) stress experiments show that exposure to 45°C for 2 and 6 h prolonged the detection time to 9.3 and 11.8 h, which are much longer than that of normal cells, whereas 1 h exposure to 45°C did not cause any visible delay in detection time. Increasing the duration of exposure to 45°C caused longer detection time, which suggest that there would be severe damages to the cells, leaving fewer viable cells in the medium. Based on the detection time on pH growth curves, it is estimated that exposure to 45°C for 2 and 6 h caused viable cell number to decrease from $10^7$ to $10^3$ and $10^1$ cfu/mL, respectively, compared to short exposure of 1 h to 45°C (Table I). These calculated cell numbers are in consistent trend with the results obtained using surface plate counting on BHI plates. Although it is reported that *L. monocytogenes* can grow in temperature range of 3–45°C (Walker et al., 1990), our results showed that prolonged exposure to high temperature (45°C) can cause a large portion of cell population to lose their capability to grow. Geng et al. (2003) have demonstrated that temperature stress at 45°C caused *L. monocytogenes* cells to be elongated and the cells formed chains; and reactions of whole cells to antibodies were severely affected as determined by ELISA.

Figure 6E and F shows the pH and conductivity growth curves of *L. monocytogenes* that were treated by 10% NaCl for 3 h and by a combined condition as 10% NaCl, pH 5 and 45°C for 1 h, indicating that there no visible difference

between normal cells and stressed cells on growth curves. Table I also shows the results of salt-stressed *L. monocytogenes*, indicating exposure to 10% NaCl for 3 h results in a decrease in viable cell number from $2.38 \times 10^7$ to $1.21 \times 10^7$ cfu/mL. Approximately 50% of *L. monocytogenes* cell in $10^7$ cfu/mL can survive after exposure to 10% NaCl for 3 h. This result agreed with the observation reported by McClure et al. (1989) that *L. monocytogenes* cells can survive at NaCl concentrations as high as 10%. The sight decrease in viable cell number did not show significant delay of the detection time on the pH and conductivity growth curve. However, even a portion of cell population can survive from salt-stress, salt-stressed cells are different from healthy cells in morphology and protein expression. It is reported that salt stress (5.5% NaCl) caused filamentous appearance with multiple septa along the length of the *L. monocytogenes* cells, and progressively decreased their ability to react with a specific antibody (Geng et al., 2003).

## CONCLUSIONS

We have clearly demonstrated a conductivity and pH dual detection system for evaluating the growth of *L. monocytogenes* in a liquid medium. Detection times obtained from the conductivity and pH growth curves were inversely related to the initial concentration of *L. monocytogenes* in the medium. Linear responses were found by plotting detection times obtained from both conductivity and pH growth curves as a function of initial cell concentration in the range of $10^2$ to $10^7$ cfu/mL, which could be used for enumeration of bacterial cells. The detection time was approximately 12 and 2 h for $10^2$ and $10^7$ cfu/mL of viable *L. monocytogenes*, respectively, using the conductivity growth curves, and most importantly, it was approximately 1 h less using the pH growth curves. The dual detection system could provide a more reliable analytical tool for bacterial detection, and our results have also suggested that pH measurement could possibly act as an individual tool, which would be more rapid as compared to other methods based on turbidity, impedance or conductance for monitoring bacterial growth.

This dual detection system was used for evaluating the growth of acid-, temperature-, and salt-treated *L. monocytogenes* in the medium. Acid stress at pH 2 and 3 for 3 h caused approximately 12 and 4 h delay of the detection time on pH growth curves, while stress at pH 5 for 3 h did not cause a significant delay in detection time. Delay in detection times was also observed for *L. monocytogenes* cells exposed to 45°C for more than 1 h (2 and 6 h). Exposure to 10% NaCl for 3 h did not cause visible delay in detection time. These observations on detection times for stressed *L. monocytogenes* had a consistent trend with the cell number decrease determined by surface plating on BHI plates, suggesting the growth-based pH and conductivity measurements could possibly provide a method for estimating viable bacteria cell number in the samples that are stressed in food processing treatments.

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