# Assessment of *Listeria* species in Fresh Produce Grown and Sold in Canterbury, New Zealand

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Qi Zhu

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science.

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*Listeria monocytogenes* is a member of the genus, *Listeria*, which is widely distributed in agricultural enviroments such as soil, manure and water. It is a pathogenic bacterium that causes disease including gastroenteritis, infections of the blood stream and/or the central nervous system and causes miscarriage in pregnant women. *Listeria* outbreaks have been regularly linked to fresh produce since the first outbreak in Boston, USA in 1979. Fresh produce, including lettuces, cabbages and carrots, are commonly used by most families as a part of their daily meals. Organic and conventional farms are the main source of fresh produce in most countries. Several investigations have focused on *L. monocytogenes* in fresh produce. However, research reports to compare the *L. monocytogenes* prevalence in fresh produce grown at organic farms vs inorganic farms are very few and there have been none from New Zealand. This masters research project aimed to investigate the prevalence of *Listeria* spp. (*L. monocytogenes*, in particular) in fresh produce grown and sold in the Canterbury region of New Zealand.

In order to generate new information and to improve the understanding about the current situation of *Listeria* spp. and *L. monocytogenes* in fresh produce grown in farms, 98 samples, including 78 fresh vegetable samples, twelve soil samples and eight water samples, were collected from three different types of farms (conventional farm [A], crop-livestock rotation farm [B], and a compost farm [C]), for microbiological analysis. A range of fresh produce, particularly cabbage, spinach, pakchoi and parsley, were collected from farms, depending on the seasonal availability. Fresh produce (cabbage, carrot, cucumber and lettuce) sold in four different retail shops were also assessed over a period of six weeks. A combination of detection methods such as aerobic plate counts (APC), *Listeria* spp. counts using a selective agar, rapid detection kits, and a 3M™ molecular detection system were used in this study. Microbiological (Gram staining) and biochemical (carbohydrate utilisation) methods were also used to further characterise positive *Listeria* spp. samples.

All soil samples from farms tested positive for the presence of *Listeria* whereas all water samples were negative. In terms of microbiological quality, all three farm types had similar APC levels. Significant differences were recorded for *Listeria* spp. contamination of soil and water with levels in the conventional farm > crop-livestock integration farm > compost farm. *Listeria* contamination of cabbage samples from Farm B (2.53±0.76 log cfu/g) was lower than in samples from Farm C (4.44±0.14 log cfu/g). Additionally, the number of colonies observed in spinach samples (5.13±0.32 log cfu/g, Farm C and 3.77±0.61 log cfu/g, Farm B) is comparatively more than parsley (4.51±0.09 log cfu/g, Farm C and 3.98±0.72 log cfu/g, Farm B) from these two farm types. The levels of *Listeria* spp. in cabbage and parsley were not significantly different when analysed by the Tukey test. The lowest population of *Listeria* spp. was recorded in the carrot samples, which had a mean of less than 1 log cfu/g. *Listeria* in pakchoi from Farm A (3.27±0.79 log cfu/g) > from Farm C (2.60±0.49 log cfu/g). Overall, samples from the compost farm had the highest *Listeria* counts and samples from the conventional farm had the least.

Microbiological analysis of the fresh produce samples showed that lettuce from the retail shops had the highest level (4.2 log cfu/g) > cucumber (3.2 log cfu/g) > cabbage (2.5 log cfu/g) > carrot (nil). In terms of *Listeria* prevalence, carrot samples had the lowest *Listeria* spp. load (< 1.05 log cfu/g) and lettuce samples had the highest (> 4 log cfu/g).

Carbohydrate utilitsation and 3M$^{TM}$ molecular detection system (MDS) analysis revealed that all *Listeria* positive samples were negative for *L. monocytogenes*.

In conclusion, this is the first research undertaken for the isolation and confirmation of *Listeria* spp. from soil and fresh produce in Canterbury region, New Zealand. The results showed that *Listeria* spp. are ubiquitous in the agricultural environment. *Listeria* spp. contamination of fresh produce was much less than those reported in the literature for meat and dairy products. Although the *L. monocytogenes* was not detected in this research, the potential hazards for contaminating fresh produce by this organism still exist. *L. monocytogenes* is a common pathogenic bacteria that can be present and grow on fresh produce. Therefore, a systematic monitoring plan is needed to avoid an outbreak.

**Keywords:** *Listeria monocytogenes*, foodborne pathogens, fresh produce

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# Symbols and Abbreviations

| | |
|---|---|
| °C | Degree Celsius |
| APC | Aerobic Plate Count |
| $a_w$ | Water Activity |
| CAMP | Christie, Atkins, Munch-Petersen |
| cfu | Colony Forming Unit |
| COP | Cold Oxygen Plasma |
| $CO_2$ | Carbon Dioxide |
| DSHS | Texas Department of State Health Services |
| g | Gram |
| g/L | Gram Per Litre |
| h | Hours |
| IAC | International Amplification Control |
| ISO | International Standard Organization |
| $K_2HPO_4$ | Dipotassium phosphate |
| *L. grayi* | *Listeria grayi* |
| *L. innocua* | *Listeria innocua* |
| *L. ivanovii* | *Listeria inanovii* |
| *L. monocytogenes* | *Listeria monocytogenes* |
| *L. seeligeri* | *Listeria seeligeri* |
| *L. welshimeri* | *Listeria welshimeri* |
| *Listeria* spp. | *Listeria* species |
| log | Logarithm |
| ml | Millilitre |
| MR-VP | Methyl Red and Voges-Proskauer agar |

| | |
|---|---|
| MPN-PCR | Most Probable Number - Polymerase chain reaction |
| $N_2$ | Nitrogen |
| NaCl | Sodium chloride |
| $O_2$ | Oxygen |
| P | Probability |
| PALCAM agar | Polymyxin, Acriflavine, Lithium chloride, Ceftazidime, Aesculin, Mannitol agar |
| PCR | Polymerase chain reaction |
| *R. equi* | *Rhodoccocus equi* |
| RH | Relative humidity |
| RLU | Relative Light Units |
| RTE | Ready-to-eat |
| RTi-PCR | Real Time Polymerase Chain Reaction |
| *S. aureus* | *Staphylococcus aureus* |
| US | ultrasonication |
| USA | United States of America |
| UV-C | ultraviolet-C |
| YSYEA | Tryptone Soya Yeast Extract agar |
| $\delta$ | Growth potential |
| µ | Micron |
| µl | Microlitre |

# Chapter 1

# Introduction

## 1.1 Background

*Listeria monocytgenes*, a member of genus *Listeria*, is found widely in agricultural enviroments, such as soil, manure and water (Jeyaletchumi et al., 2012). It is a pathogenic bacterium, which can cause a disease called listeriosis. Depending on the seriousness of the disease, it may result in mild gastroenteritis, severe infections of the blood stream and/or the central nervous system, and even abortion in pregnant women (Carpentier et al., 2011; Jeyaletchumi et al., 2012). About 17 % of listeriosis cases occur in pregnant women (Jeyaletchumi et al., 2012). People who have an immune deficiency (Okutani et al., 2004), such as the elderly (CDC, 2015; Gillespie et al., 2010; Levidiotou et al., 2004; Okutani et al., 2004), children (Buzby, 2001; Dilber et al., 2009; Okutani et al., 2004; Smith et al., 2009), pregnant women (Okutani et al., 2004; Smith et al., 2009) and unborn children (FDA, 2011; Smith et al., 2009) are more easily infected by *L. monocytogenes*. According to data from the FDA, there were about 2500 people in the USA that had listeriosis and with a high death rate (FDA, 2011). Approximately 20 to 30% of listeriosis patients end up losing their lives (Carpentier et al., 2011).

*L. monocytogenes* had previously been isolated from market or restaurant produce such as cabbages (Palumbo et al., 2005; Ponniah et al., 2010), corn (Aureli et al., 2000), carrots (Kamat et al., 2005; Ruiz-Cruz et al., 2007; Sy et al., 2005), lettuces (Abadias et al., 2008; Althaus et al., 2012; Ding et al., 2013; Francis & O'Beirne, 2006; Johannessen et al, 2002; Soriano et al., 2001; Thunberg et al., 2002), cucumbers (Jamali et al., 2013b; Jeyaletchumi et al., 2012; Meldrum et al., 2009), parsley (Gómez-Govea et al., 2012; Öktem et al, 2006; Ponniah et al., 2010) and salad vegetables (Easa, 2010; Jamali et al., 2013b; Ponniah et al., 2010; Skalina et al., 2010). Several outbreaks of *L. monocytogenes* infections associated with fresh produce have been reported from many parts of the world (Meldrum et al., 2009). In 2010, *L. monocytogenes* was responsible for the deaths of 10 people a in food poisoning outbreak from chopped celery in Texas, USA (Gaul et al., 2013) and, in the following year 30 people were infected from contaminated melons in Colorado, USA (CDC, 2011a). Furthermore, in December 2014, there was a *Listeria* outbreak from apples that occured in Califonia, USA (CDC, 2015). Therefore, foodborne outbreaks due to contaminated fresh fruit and vegetables have been recognised as a problem which could be regarded as a continuing food safety challenge in coming years.

## 1.2 Importance and aim of this study

New Zealand is an agricultural country; farming and pastures strongly support the food chain supply and the national economy. As a pathogen, *L. monocytogenes* could pose food safety risks in a range of foods, especially fresh produce which can lead to listeriosis. Therefore, information about the presence and survival of *L. monocytogenes* in fresh produce has been used by scientists and researchers to find ways to protect people from listeriosis. In addition, several outbreaks related to contaminated fruits and vegetables had serious consequences, such as the two outbreaks associated with melons and lettuces in the USA (CDC, 2011b; Shrivastava, 2011). Although there have been no serious *Listeria* outbreaks linked to fresh produce reported in New Zealand so far, protecting from a *Listeria* outbreak is more important than handling the problems after outbreaks. This study was designed to investigate the influence of the growing environment (organic farms and conventional farms), on the prevalence of *L. monocytogenes* in fresh produce. It also investigated the prevalence of *Listeria* spp. (especially *L. monocytogenes*) in fresh produce sold in the Canterbury region of New Zealand.

## 1.3 Specific objectives

The aim of this research is to investigate the presence of *Listeria* spp. presence in fresh produce from organic farms and inorganic farms. The specific objectives are:

1. To clarify whether organic farming can result in a higher risk of *Listeria* contamination compared to conventional farming (Chapter 4);

2. To investigate Listeria contamination levels of the fresh produce in retail shops (Chapter 5); and

3. To identify the extent of *Listeria monocytogenes* contamination of fresh produce in the Canterbury region (Chapter 6).

# Chapter 2

# Literature review

## 2.1 Outbreak

In the 1920s, listeriosis was first recognised as a disease caused by a bacterial infection. Later, the bacterium was named as *Listeria monocytogenes*. In 1981, *L. monocytogenes* was considered to have a connection with listeriosis in a foodborne outbreak; the scientists proved this viewpoint subsequently and researched the transmission method of *L. monocytogenes* (Liu, 2008). The death rate of listeriosis did not show a decreasing trend for many years compared with salmonellosis and campylobacteriosis; therefore, *L. monocytogenes* has become a major foodborne pathogen (Todd & Notermans, 2011).

Listeriosis is due to infection of organs such as the liver (Wing & Gregory, 2002), spleen (Aoshi et al., 2009), cerebral spinal fluid (Cone et al., 2003) or blood (Bhat et al., 2012) by *L. monocytogenes*. If a healthy adult acquires listeriosis, he or she will suffer diarrhoea and fever (Salazar et al., 2013). For pregnant women, the symptoms are fever, diarrhoea, abortion or stillbirth (Pérez-Trallero et al., 2014). *L. monocytogenes* even infects new-born babies and leads to sepsis, pneumonia or meningitis (Brouwer et al., 2006; Camacho-Gonzalez et al., 2013; Gaschignard et al., 2011; Khoury et al., 2012; Okike et al., 2013). The symptoms of listeriosis patients are mostly similar, with non-invasive gastroenteritis in many outbreaks associated with eating deli meat (Gottlieb et al., 2006; Lin et al., 2006), chocolate milk (Proctor et al., 1995), cheese (CDC, 2012a, 2013, 2014), smoked fish (Rørvik, 2000; Tham et al., 2000) and corn (Aureli et al., 2000). Most outbreaks have occurred in Europe, the USA, Canada and, to a lesser extent, Australia and New Zealand (Todd et al., 2011). Foodborne listeriosis is a serious disease with high death rates (30%) compared with other foodborne microbial pathogens (Ponniah et al., 2010). The incidence of foodborne pathogens outbreaks linked to fresh produce has shown an increasing trend in recent years (Mukherjee et al., 2006).

A serious *Listeria* outbreak associated with canned corn contaminated by *L. monocytogenes* occurred in two primary schools and a university of Italy in 1997. The main symptoms in this outbreak were febrile illness and gastroenteric disease. Large numbers of people (2930 in total) got febrile gastroenteritis in these two schools, including primary school students aged six to ten years, adult staff in the primary school, and university students. Investigation into this incident showed that the symptoms occurred after eating food supplied by the same caterer. There were no other cases that happened outside schools during the same period (Aureli et al., 2000).

Another listeriosis outbreak linked to chopped celery occurred in, as 2010 announced by the Texas Department of State Health Services (DSHS). Theten infected patients were 56 to 93 years-old. As a result of this outbreak five patients died in the three months since the samples were collected by hospitals (Gaul et al., 2013).

In 2011, an outbreak was caused from contaminated melons in which a total of 147 persons were affected, with 33 deaths in the USA. The subtype of *L. monocytogenes* in this outbreak was analysed using pulsed-field gel electrophoresis (PFGE), and *L. monocytogenes* DNA fingerprint patterns were matched by comparing colony isolations from samples of cut cantaloupe and from the patients. The people who developed listeriosis were from 28 different states of the USA. In addition, a pregnant woman also developed listeriosis and miscarried during that period (CDC, 2012b).

In the same year, another outbreak associated with romaine lettuces was recorded in North America. In this outbreak, 15 people died and 84 were sick, across 19 States. The FDA collected and tested samples randomly from True Leaf Farms of California, USA. The results of microbiological analyses results were positive for *L. monocytogenes*. Approximately 30,000 pounds of chopped and bagged romaine lettuces in 90 cartons were recalled (Shrivastava, 2011).

More recently, a listeriosis outbreak associated with apples occurred in December, 2014. The vehicle of this outbreak was pre-packaged caramel apples. The survey results from environmental testing confirmed that the problem came from the firm's apple-packing facility. In total, 35 people, including 11 pregnant women, were infected by *L. monocytogenes* in 12 states of USA. As a result, one of these infected pregnant woman miscarried. In addition, 7 out of 35 people lost their lives during that listeriosis outbreak (CDC, 2015). Table 2.1 below gives the summary of several outbreaks caused by fresh fruit and vegetables.

**Table 2.1 *Listeria* outbreaks associated with fresh produce**

| Listeriosis outbreak | Deaths/cases (% mortality) | Food vehicle | Reference(s) |
|---|---|---|---|
| Boston, USA, 1979 | 3/20 (15) | Raw vegetables | Ho et al., 1986 |
| Nova Scotia, Canada, 1981 | 17/41 (41) | Vegetable mix for coleslaw | Schlech et al., 1983 |
| Moncalieri and Giaveno, Italy, 1997 | 0/2930 (0) | Corn | Aureli et al., 2000 |
| Texas, USA, 2010 | 5/10 (50) | Chopped celery | Gaul et al., 2013 |
| Colorado, USA, 2011 | 33/147 (22) | Whole cantaloupes | CDC, 2012b |
| Colorado, USA, 2011 | 15/99 (15) | Lettuce | Shrivastava, 2011 |
| California,USA,2014 | 1/35(3) | Apple | CDC, 2015 |

## 2.2 Prevalence of *L. monocytogenes* in the growing environment

*L. monocytogenes* is present in many animals and humans (Nightingale et al., 2005; Srinivasan et al., 2005; Vela et al., 2001), so it is possible to isolate the bacteria from faeces of these sources and their environment (Esteban et al., 2009; Nightingale et al., 2004; Olier et al., 2002). Moreover, fresh produce can be contaminated by sewage applied to water plants and the soil in which the plants were grown (Food Safety Authority of Ireland, 2005). Therefore, *L. monocytogenes* transfers as a cycle among vegetable, human and soil contaminated with faeces (Figure 2.1).

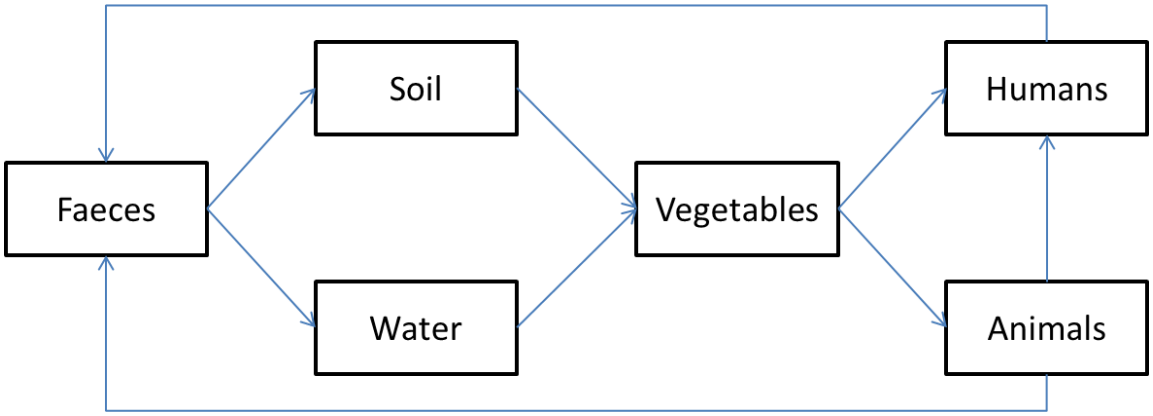



**Figure 2.1 Potential pathways for transmission of *L. monocytogenes* to humans via vegetables**

*L. monocytogenes* have been tested for from ready-to-eat (RTE) food, such as freshly-cut fruit (Conway et al., 2000; Oliveira et al., 2014), and fresh-cut vegetables (Vandamm et al., 2013). Additionally, *L. monocytogenes* has been isolated from the vegetable growing environment as shown in Figure 2.1; for example, from ruminant animals (Esteban et al., 2009). *L. monocytogenes* can multiply rapidly in decaying plants, dirty environments, and in nooks where it is cool and wet (Food Safety Authority of Ireland, 2005).

Temperature, water activity ($a_w$) and the pH of food are the main factors which can influence the multiplication and survival of *L. monocytogenes.* Compared with other bacteria, *L. monocytogenes* has the widest optimal conditions for growth during food processing and storage as shown in Table 2.2. Nonetheless, it has been proved that those microbial cells which have been damaged before, such as under acidic conditions, can set up a stress response and bear stronger additional stress (Food Safety Authority of Ireland, 2005).

**Table 2.2 Growth and survival limits of *L. monocytogenes* (adapted from the Food Safety Authority of Ireland, 2005)**

| Parameter | Minimum | Maximum | Optimal[d] | Can survive (but no growth)[e] |
|---|---|---|---|---|
| Temperature (°C) | -1.5 to +3 | 45 | 30 to 37 | -18°C [f] |
| pH [a] | 4.2 to 4.3 | 9.4 to 9.5 | 7.0 | 3.3 to 4.2 |
| Water activity ($a_w$)[b] | 0.90 to 0.93 | > 0.99 | 0.97 | < 0.90 |
| Salt (%)[c] | < 0.5 | 12 to 16 | N/A | ≥ 20 |

a. Hydrochloric acid as acidulant (inhibition is dependent on type of acid present)
b. Sodium chloride as the humectant
c. Percent sodium chloride, water phase
d. When growth rate is highest
e. Survival period will vary depending on the nature of the food and other factors
f. A temperature of 70°C/2min is required for a $10^6$ reduction in numbers of *L. monocytogenes* cells
N/A Not applicable

The most optimal environment for *L. monocytogenes* to survive in is in a slightly alkaline pH with a temperature range from 30 to 37°C. However, *L. monocytogenes* can also survive in refrigerator temperatures (less than 5°C). As influenced by different temprature and other factors, *L. monocytogenes* can multiply in 1.1 to 131 hours (Food Safety Authority of Ireland, 2005). The growth limits of *L. monocytogens* are shown in Table 2.2. The results of the *L. monocytogenes* survival-limiting conditions were from laboratory experiments. However, the conditions in food are more complex than in cultural media in laboratory. To conclude, conditions which can stop *L. monocytogenes* growing would included:

1. pH 5 to 5.5 and $a_w < 0.95$

2. pH < 5 at any $a_w$

3. $a_w \leq 0.92$ at any pH.

In the case of the contaminated melons that happened in Jensen Farms (2011), the temperature created an ideal environment for *Listeria* growth. In addition, the machines were impossible to clean and had dirt on them and the potato washing machine was used for washing cantaloups. This created an opportunity to pollute the cantaloups. Furthermore, the truck used to haul the reject cantaloupes to cattle feedlots was parked next to the packing plant. It was, therefore, easy to get *Listeria* from cattle farms (CDC, 2011a).

Many infectors could influence the prevalence of *L. monocytogenes* in fresh produce, including direct or indirect infection from the environment, such as from soil, water, compost and faeces (Table 2.3). In a research project, 174 samples were tested for *L. monocytogenes* and 48 gave a positive reaction. However, all *L. monocytogenes*-positive water samples were from natural water sources like creek and pond water, and none of the 28 samples from piped water and well water was positive for *L. monocytogenes* (Strawn et al., 2013a). A similar phenomenon was found in research associated with compost and irrigation water (Strawn et al., 2013b). Linke et al. (2014) emphasised that soil and water may lead to the transmission of *L. monocytogenes* to plant material, animals, and the food chain. To support this suggestion, these authors analysed a total 467 soil samples and 68 water samples from national parks or mountain summits in 12 sampling areas in Austria between 2007 and 2009. Of the soil samples, 140 of the 467 samples showed a positive result for in *Listeria* spp. and 28 of these positive samples were identified as *L. monocytogenes*. *Listeria* was isolated from 26.5% of the water samples. Szymczak et al.(2014) completed research on the prevalence of *L. monocytogenes* in fresh produce in relation to the type of soil, including natural fertilizers, artificial fertilisers, wastelands and, garden plots, etc. As a result, the artificial environment was more suitable for *L. monocytogenes* to survive. Exciting research on the factors (including temperature and, moisture) which could influence the survival of *L. monocytogenes* in soil was carried out by McLaughlin et al. (2011). They used three marked colonies to monitor the situation of *L. monocytogenes* survival in different kinds of soil. They found that *L. monocytogenes* can survive in normal soil and it preferred higher moisture containing soil. In other research Locatelli et al. (2013) worked on *L. monocytogenes* survival and showed that the physical and chemical properties of soil may influence the survival of *L. monocytogenes*. Biotic and abiotic reasons could, together, influence survival ratio of *L. monocytogenes*. To conclude, the external environment (contaminated soil, water and nutriment, soil properties) could affect the survival of *L. monocytogenes* individually, however, there could be other factors acting concurrently on the *L. monocytogenes* survival ratio in moist conditions.

**Table 2.3 Prevalence of *L. monocytogenes* in growing environment**

| Location | Environment | Frequency [a] | Reference (s) |
|---|---|---|---|
| USA | Soil (178)<br>Drag swab (175)<br>Fecal (61)<br>Water (174)<br>Engineered (28)<br>Surface (146) | 16 (9)<br>15 (9)<br>9 (15)<br>48 (28)<br>0 (0)<br>48 (33) | Strawn et al., 2013a |
| USA | Field<br>Water | 263(17.5)<br>74(30) | Strawn et al., 2013b |
| Austria | Soil (140) | 28 (6) | Linke et al., 2014 |
| Poland | Soil (1000) | 55(5.5) | Szymczak et al., 2014 |
| Ireland | Soil | | McLaughlin et al., 2011 |
| Malaysia | Soil<br>Animal manure | 21(38.1)<br>23(61.1) | Jeyaletchumi et al., 2012 |
| France | Soil | | Locatelli et al., 2013 |

a: Frequency data represent the number of samples (percent).

## 2.3 Prevalence and survival of *L. monocytogenes* in fresh produce

Many methods, such as UV radiation and, pesticides, have been used to reduce microbe survival in fresh produce; however, it is difficult to determine that all *L. monocytogenes* in fresh produce have been destroyed completely (Table 2.4). In research by Szymczak et al., (2014), 5% of parsley grown in naturally fertilised soil was positive for *L. monocytogenes*. In addition, an assessment on lettuce for *L. monocytogenes* was undertaken from the farm to the table (Ding et al., 2013). Numbers of 1.05 log cfu/g *L. monocytogenes* were found in samples from restaurants, while 0.146 log cfu/g were found in samples from homes. Although both these two samples had been treated before cooking or eating, samples from the home treatment were cleaner than those from restaurants (Ding et al., 2013). Other similar studies worked on the effect of washing treatments for decreasing levels of *L. monocytogenes* (Nastou et al., 2012). Lettuce, cucumber and parsley were the objects in this research. They studied the influence of storage temperature, water temperature, acetic acid concentration and immersion time which would affect the survival of *L. monocytogenes*. As a result, a higher storage temperature increased the number of *L. monocytogenes* colonies. Although acetic acid has some effect on decreasing *L. monocytogenes*, the structure of the vegetable itself decided

the numbers of pathogens left after washing. It could be speculated that washing treatment is more effective to fruit in decreasing *L. monocytogenes* than leafy vegetables. A quantitative assessment relating to *L. monocytogenes* was published on minimally treated leafy vegetables, including collard greens, cabbages, lettuces, Chinese cabbages, arugula, etc. (Oliveira et al., 2010a). In total, the researchers explored 162 minimally processed leafy samples in this assessment; however, only six of the tested samples were confirmed as *Listeria* spp. Moreover, six samples were confirmed as *Listeria* spp. and two samples were confirmed as *L. monocytogenes* from one collard greens and one bunched parsley and spring onions. In research on market vegetables (Sant'Ana et al, 2012b), *L. monocytogenes* was detected in 3.1% of the samples. Five salad samples were countable with numbers between $1.0\times10^1$ and $2.6\times10^2$ cfu/g. Isolates belonging to serotypes 1/2b or 4b were most which were positive for genes *inlC* and *inlJ*. Among the minimally-processed vegetable samples tested, 0.3% of them were positive in sprout; this was from a microbial evaluation in Korea (Seo et al., 2010). Uzeh et al. tested many salad vegetables (lettuces, cabbages, carrots and cucumbers), and only cabbages and lettuces gave a positive reaction (Uzeh et al., 2013). To conclude, although *L. monocytogenes* levels may decrease after treatment, some colonies with huge vitality still could survive.

**Table 2.4 The prevalence of *L. monocytogenes* in fresh produce**

| Produce | Country | Prevalence [a] | References |
|---|---|---|---|
| Parsley | Poland | 10(0.5) | Szymczak et al., 2014 |
| | Malaysia | 16(25) | Ponniah et al., 2010 |
| | Greece | | Nastou et al., 2012 |
| Collard greens | Brazil | 30(0.03) | Oliveira et al., 2010a |
| | Brazil | 24(4.2) | Sant'Ana et al., 2012b |
| Lettuces | Korea | | Ding et al., 2013 |
| | Brazil | 152(3) | Sant'Ana et al., 2012b |
| | Nigeria | | Uzeh & Adepoju, 2013 |
| | Greece | | Nastou et al., 2012 |
| Cabbages | Malaysia | 32(21.9) | Ponniah et al., 2010 |
| | Brazil | 11(18.2) | Sant'Ana et al., 2012b |
| | Nigeria | | Uzeh & Adepoju, 2013 |
| Spinach | Brazil | 11(1) | Sant'Ana et al., 2012b |
| Carrots | Malaysia | 33(24.2) | Ponniah et al., 2010 |
| Tomatoes | Malaysia | 32(21.9) | Ponniah et al., 2010 |
| Cucumbers | Malaysia | 32(21.9) | Ponniah et al., 2010 |
| | Greece | | Nastou et al., 2012 |
| Sprouts | Korean | 112(0.3) | Seo et al., 2010 |

a Number of analysed samples/percent of positive tests

## 2.4 Biofilm production and methods of control

Besides the growing environment, transportation is another important pathway to contaminate fresh produce and cause the production of biofilms by bacteria. Oliveira et al. (2010b) stated that the term biofilm was created to describe the sessile form of microbial life, characterised by adhesion of microorganisms to biotic or abiotic surfaces, with consequent production of extracellular polymeric substances.

Fresh produce may contact many different kinds of surface during processing or transport. This means that, to a certain extent, temperature and surfaces touched are other factors that can influence the production of a *L. monocytogenes* biofilm. According to a recent study (Bonsaglia et al., 2014), a *L. monocytogenes* biofilm could be affected by different temperatures and surfaces touched. They compared *L. monocytogenes* biofilms growing on three kinds of touched surfaces, including polystyrene, glass and stainless steel, at three different temperatures (4, 20 and 35$^{o}$C). The results from this research showed that *L. monocytogenes* was easier to attach to hydrophilic surface (glass and stainless steel) than hydrophobic surfaces (polystyrene). Higher temperatures and longer incubation times may decrease the microbes adhering surfaces but not significantly.

For bacteria, including *L. monocytogenes*, the biofilm is produced from the bacteria itself to enhance its living environment. Therefore, damaging the biofilm of *L. monocytogenes* is a practicable method to decrease the survival levels of *L. monocytogenes*. The most common methods to reduce biofilm formation included physical (such as UV-C) and chemical (such as chlorine, acid) pathways. In the study of Banire and Jia (2014), chlorine was used to treat onions. They compared the effectiveness of chlorine on suspending *L. monocytogenes* and *L. monocytogenes* biofilms. Chlorine was more effective on suspending *L. monocytogenes* with a more significant reduction..

According to a recent study (Srey et al., 2014), physical methods are more likely to be recommended because of their minimal influence on produce quality and their relatively stable effect. These authors used three physical methods to treat *L. monocytogenes* biofilms: 32 Hz ultrasonication (US), 390 mJ/cm$^2$ ultraviolet-C (UV-C), and 750 mJ/cm$^2$ cold oxygen plasma (COP). UV-C and COP showed a higher reduction in the *L. monocytogenes* biofilm. Another effective method to reduce *L. monocytogenes* biofilm production is using organic acid combined with modified atmosphere packaging (Bae et al., 2011). In a study by Bae et al., they treated cabbages with 2% lactic acid for 10 minutes combined with modified atmosphere packaging, and the nearly half numbers of *L. monocytogenes* where reduced by half (from 6.2 cfu/g to 3.1 cfu/g). In addition, the packing method (air, $N_2$ gas, $CO_2$ gas) has been proved to be effective in delaying the growth of *L. monocytogenes*.

## *2.5* Studies reporting presence of *L. monocytogenes* in fresh produce

Several groups have undertaken research using different methods of analysis for *L. monocytogenes* in fresh produce, and the microbiological status on farms or in retail shops, as shown below (Table 2.5).

In research associated with the microbiological quality of fresh lettuce from organic and conventional production systems, a total of 72 lettuce samples from 18 farms with four repetitions each were collected to examine the prevalence of selected pathogens. The lettuce samples were analysed for the presence of many kinds of microbes including *L. monocytogenes*. The mean aerobic mesophilic counts were 6.35 ± 0.69 $\log_{10}$ cfu/g and 5.67 ± 0.80 $\log_{10}$ cfu/g from the organic and conventional lettuces, respectively. However, none of the lettuce samples was positive for *L. monocytogenes* (Oliveira et al., 2010c).

In a Korean risk assessment for *L. monocytogenes*, lettuces were tested though the whole food chain from farm to table and included the initial contamination on the farm, growth and cross contamination during transportation, storage and consumption. The results showed that the final mean *L. monocytogenes* contamination levels were -1.50 log cfu/g and -0.146 log cfu/g at restaurant s and homes, respectively. However, the quantitative risk assessment model developed in this study calculated that 7.5 log cfu/g was the worst situation, assuming a maximum-dose level (Ding et al., 2013).

A bacteriological assessment of RTE lettuce, freshly-cut fruit and sprouts was completed in Switzerland. The prevalence of *L. monocytogenes* was tested as a reference for the microbial quality of produce. In total, 223 samples were collected from a large local production plant which supplied RTE fresh produce to retail shops all over Switerland. They used two different types of agar, including PALCAM agar and Chromogenic *Listeria* Agar (CM1084, OxoidAG) with additional of *Listeria* selective supplement (SR0226,Oxoid AG) and *Listeria* differential supplement (SR0244, OxoidAG), to detect the present of *L. monocytogenes*. Haemolysis testing with sheep blood agar was used for further confirmation (Althaus et al., 2012).

Other research with *L. monocytogenes* in RTE food compared the differences when samples were grown in a range of different selective culture agar. In total, 396 RTE food samples, including 170 salads and vegetable samples, were purchased from hypermarkets and street stalls for *Listeria* spp. and *L. monocytogenes* testing. The suspicious colonies were confirmed by biochemical tests and the polymerase chain reaction (PCR). The group of salad and vegetables had the highest levels of *L. monocytogenes* present (14.7%). Moreover, the prevalence of *L. monocytogenes* in fresh lettuces, cucumbers and cabbages was 5.6%, 10.5%, and 10.5%, respectively. In a culture agar comparison,

CHROM agar ™ was more sensitive than *Listeria* selective agar and PALCAM agar with 96.9% of sensitivity and 99.1% of specificity in *L. monocytogenes* detection (Jamali et al., 2013a).

As mentioned in Section 2.2, the growing environment of vegetables, especially the soil, can influence the prevalence of *L. monocytogenes*. A group of scientists has worked on determining the connection between the type of soil and the presence of *L. monocytogenes* in fresh fruit and vegetables. In total, 1,000 soil samples were collected from 15 different locations and also 210 vegetable samples and 140 fruit samples were collected from these locations for *Listeria* spp. and *L. monocytogenes* testing. As a result, *Listeria* spp. and *L. monocytogenes* were detected in soil fertilised with manure and composted by faeces of animals. In contrast, in soil samples fertilised artificially, which did not contain animal faeces, *L. monocytogenes* was not detected. Furthermore, *L. monocytogenes* was detected in 10% of the strawberry samples, 15% of the potato samples and 5% of the parsley samples. All *L. monocytogenes*-positive samples were collected from manure-fertilised land (Szymczak et al., 2014).

In order to explore more effective method to detect *L. monocytogenes* in fresh produce, a group of technicians evaluated a method which can complete detection in fewer than three days. They combined the International Standard Organization (ISO) method and real–time PCR, together with internal amplification control but without DNA purification. *L. monocytogenes* was detected at 1cfu in 25g after ISO enrichment. This method had been proven to can be an alternative process to detect the presence of *L. monocytogene*, that is more effective, faster and reliable than other methods currently used (Badosa et al., 2009).

Another group of technicians in Brazil used a similar method, which combined enrichment and 16S rRNA real-time PCR (Rti-PCR), to detect *L. monocytogene* in leafy vegetables. The immunoassay *Listeria* Rapid Test (Oxoid) was used to detect the prevalence of *Listeria* spp. in vegetable samples. As well as traditional enumeration, the Most Probable Number (MPN) technique was used, combined with Rti-PCR for the dectection of the 16rRNA gene of *L. monocytogenes*. The ABSOLUTE™ QPCR SYBR® Green Mix (ABgene, UK) was used for positive MPN enrichment sample Rti-PCR analysis with special primers for *L. monocytogenes*. However, no significant differences were seen between the results of the MPN-PCR method and that of traditional culture methods ($p<0.05$). In addition, MPN-PCR, which requred just used two days for detection, was faster than the traditional method which took seven days (Oliveira et al., 2010a).

In other related research with *Listeria* spp. and *L. monocytogenes* dectection with a PCR method, 306 vegetable samples were purchased from four local markets (including two wet markets and two hypermarkets) in Malaysia. They used the most probable number-polymerase chain reaction (MPN-PCR) to detect the present of *Listeria* spp. and *L. monocytogenes*. As a result, in total, 33.3% samples

of vegetables tested positive for *Listeria* spp., and *L. monocytogene* was present in 22.5% samples of vegetables. Japanese parsley and yardlong beans were the most contaminated with prevalence percentages of 31.1% and 27.2%, respectively (Ponniah et al., 2010).

A group of researchers carried out a microbial evaluation of minimally-processed vegetables in Korea. *L. monocytogenes* was tested as one of the detection items in this research. A total of 345 samples were purchased from three local shops, including a department store, a local maket, and a restaurant. The samples tested included mixed vegetable salads, sprouts, RTE fruit, lettuces and green onions. They used two different selective agar to isolate *L. monocytogenes* (PALCAM and OXFORD agars). An ISO method was used for evaluation of aerobic mesophils. The range of aerobic mesophils counted in these products ranged from 2.0 to 9.7 log cfu/g; and 0.3% of sprouts samples were positive for *L. monocyotgenes* (Seo et al., 2010).

Further research on *L. monocytogenes* present in RTE vegetables was completed in Brazil. As well as analysing for the prevalence of *L. monocytogenes* with the ISO method, they also investigated the characteristics of the phenotypes as well. A total of 512 samples of RTE vegetables were purchased from local markets to determine their prevalence and level present. The isolated colonies were then described for their serotypes, ribotypes, which were positive for virulence genes *inlA*, *inlC* and *inlJ*. In addition, their optical densities (OD) were tested at 620 nm every 20 h to describe the growth rate and hydrion micro chlorine test papers were used for evaluation of chlorine resistance. As a result, *L. monocytogenes* was found in 3.1% of the RTE vegetable samples. Serotypes 4b and riborype DUP-1038 were the highest ones isolated from *L. monocytogenes* (Sant'Ana et al., 2012b).

Specific research on salad vegetables was developed to determine the incidence and survival of *L. monocytogenes*, in Nigeria. Cabbages, carrots, cucumbers and lettuces were purchased from local markets and packaged in sterile containers before being sent directely to the laboratory. In order to explore the influence of strorage temperatures on *L. monocytogenes* survival, they chose 5$^{o}$C (refrigerator temperature) and 28$^{o}$C (room temperature) for storage temperatures for comparison. Fresh produce samples with known (quantitative) populations of *L. monocytogenes* were incubated separarely at these two temperatures for 10 days and monitored by enumerating with PALCAM agar. The results showed that most cases in this experiment had increasing population trends in the first two or three days, followed by a decreasing trend until the end. No *L. monocytogenes* survived to the tenth day at 28$^{o}$C (Uzeh et al., 2013).

Other researchers also worked on the effect of temperature on *L. monocytogenes* survival in fresh produce (Sant'Ana et al., 2012a). According to this reseach, the definition of growth potential ($\delta$) is the difference between the population of a microorganism at the end of shelf-life of specific food and its initial population. They considered 0.5 $\log_{10}$ as a critical value to decide if the situation

(including vegetable type and storage temperature) supported the growth of *L. monocytogenes*. In this research, the growth potential of aerobic microorganisms and *L. monocytogenes* were tested at refrigeration (7°C) and abuse temperatures (15°C) over six days of storage time. As a result, when vegetables were storaged at 15°C for six days, the $\delta$ value was the highest. In another words, *L. monocyotgenes* can reach huge populations in RTE vegetables when the storage conditions are optimal for growth.

In addition, in other research that explored the effect of storage conditions on *L. monocytogenes* growth, approximately 3 log cfu/g *L. monocytogenes* were cultured from fresh celery in polyethylene bages or closed containers at different temperatures (4°C, 12°C and 22°C). The behaviour of *L. monocytogenes* was monitored by enumeration at several time points. When stored at 4°C and 12°C, contaminated celery were enumerated at 0, 1, 3, 5, 7 days, however, for 22°C, the samples were enumerated at 0, 8, and 17 h, and one, and two days. The population of *L. monocytogenes* decreased by 0.5 to 1.0 log cfu/g after seven days of storage at 4°C. Meanwhile, the population increased by approximately 0.5 log cfu/g over seven days at 12°C. At 22°C, the population increased most during the first 17 h, and was increased by approximately 0.3 log cfu/g. In addition, populations of *L. monocytogenes* in cut celery were higher than those in uncut celery. This research proved that *L. monocytogenes* can survive better in higher temperatures (Vandamm et al., 2013).

Many researches focus on *L. monocytogenes* in fresh produce, however, there are small numbers of studies comparing levels of *L. monocytogenes* between organic and nonorganic environments. Therefore, this study was planned to investigate the *L. monocytogenes* contamination status of selected vegetables. This paper reports on prevalence and characteristics of *L. monocytogenes* on fresh produce grown in organic farms and conventional farms in the Canterbury region of New Zealand.

**Table 2.5 Thirteen recent studies reporting *Listeria* contamination in fresh fruits and vegetables**

| Reference | Location | Samples | Method |
|---|---|---|---|
| Badosa et al., 2009 | — | Tomato, apple, grape, soybean sprouts, watercress | Enumeration RTi-PCR |
| Oliveira et al., 2010a | Brazil | Minimally processed vegetables | Enumeration 16S rRNA RTi-PCR |
| Oliveira et al., 2010c | Spain | Lettuce | Enumeration |
| Ponniah et al., 2010 | Malaysia | Raw salad vegetables | MPN–PCR |
| Seo et al., 2010 | Korea | Minimally processed vegetables | Enumeration |
| Althaus et al., 2012 | Switzerland | Ready-to-eat lettuce, fresh-cut fruit, and sprouts | Enumeration |
| Sant'Ana et al., 2012a | Brazil | Packages of RTE vegetables | Enumeration |
| Sant'Ana et al., 2012b | Brazil | Ready-to-eat vegetables | Enumeration |
| Vandamm et al., 2013 | — | Freshly-cut celery | Enumeration |
| Uzeh & Adepoju, 2013 | Lagos, Nigeria | Salad vegetables; carrots, cucumbers, lettuces and cabbages | Plate counts |
| Ding et al., 2013 | Korea | Lettuces | Enumeration |
| Jamali et al 2013a | Malaysia | Lettuces, cucumbers, tomatoes, cabbages | Enumeration PCR |
| Szymczak et al., 2014 | Poland | Soil, fruit, and vegetables | Enumeration Multiplex PCR Agarose gel electrophoresis |

# Chapter 3

# Material and methods

## 3.1 Sample collection

This study had two aspects: investigating *Listeria* spp. contamination in fresh produce grown at different farms, and fresh produce sold at different retail shops.

From Week 1 to Week 6, fresh samples were collected from three different types of farms located in Canterbury, New Zealand. The first farm (Farm A) was a conventional farm, which used fertiliser and chemicals to enhance the plants growth. The second farm (Farm B) was a special organic system (Crop-Livestock Integration) that, allowed plants to absorb nutrients left by sheep fed on lucerne. The third farm (Farm C) was also organic and used compost made in the farm for growing vegetables. Vegetables were collected from the three farms depending on availability. Ten different vegetables were investigated for prevalence of *Listeria*, as indicated in Table 4.1. The microbial differences between organic and traditional farming methods were investigated with collection of three vegetables common in Farm A and Farm C, and crops in common in Farm A and Farm B. All fresh samples were bagged separately when collected. Samples of soil and water were also collected and analysed from three farms.

Similar vegetables from different farms were difficult to be collected due to seasonal variation and each farms growing preferences. From Farm A leek (6), pakchoi (6), radish (6), silver beet (2), spring onion (6) were collected; from Farm B cabbage (6), lettuce (4), pakchoi (5), silver beet (2), spinach (6), parsley (6) were collected; from Farm C cabbage (4), carrot (4), coriander (5), leek (1), parsley (4), spinach (5) were collected.

The retail samples were collected from four local markets: Markets A, B, C and D. Lettuce, cabbage, cucumber and carrot samples were collected from these markets over a period of six weeks (Figure 3.1).

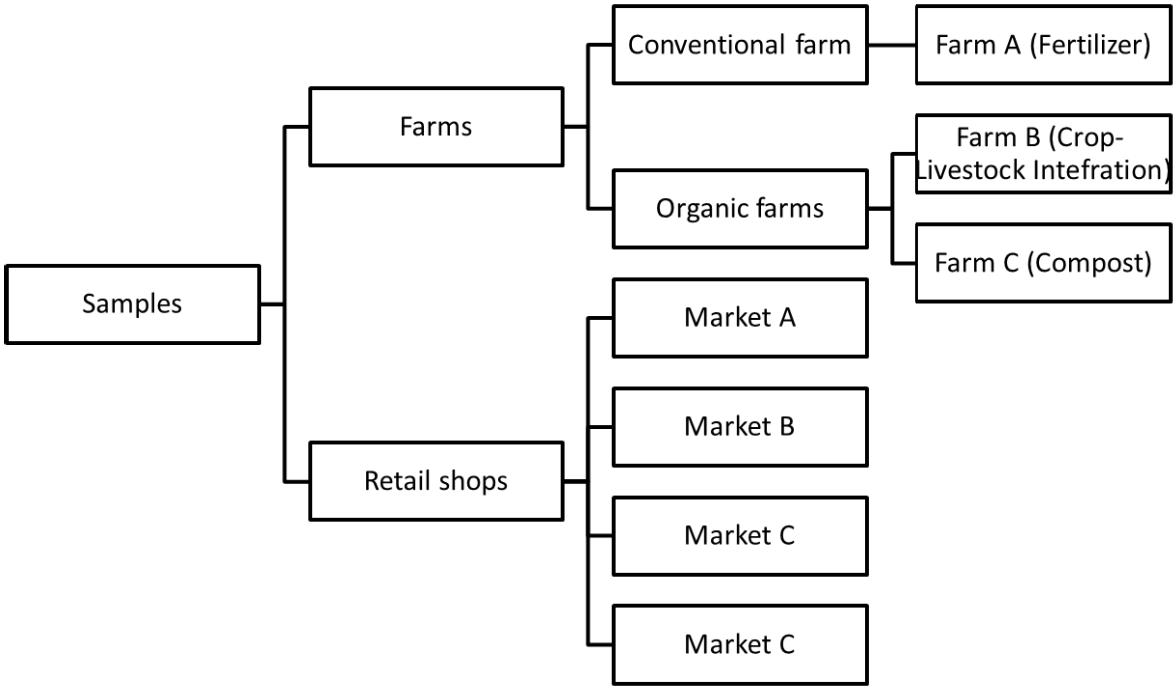



**Figure 3.1 Sample collection plan for the study**

## 3.2 Growth media and broth

### 3.2.1 *Listeria* selective agar

*Listeria* selective agar was used to count the numbers of *Listeria* spp. in the samples collected. This agar was prepared by adding 27.75 g of *Listeria* Selective Agar Base (CM0856, Oxoid Ltd.) to 500 ml of distilled water and sterilising by autoclaving at 121 $^{o}$C for 15 min. Plates were poured after adding a vial of supplement (SR140, Oxoid Ltd.) which dissolved by 5ml 70% alcohol.

### 3.2.2 APC agar

Aerobic plate count agar (APC agar) was used to enumerate the aerobic bacteria. The agar was prepared by adding 23.5 APC agar powder (Difco $^{TM}$) to 1 L $dH_2O$, sterilised by autoclaving at 121 $^{o}$C for 15 min, then poured into disposable sterilised plates.

### 3.2.3 TSYEA agar

Tryptone Soy Yeast Extract Agar (TSYEA agar) was used for *Listeria* spp. purification. TSYEA agar plates were prepared by combing 17 g/L typtone, 6 g/L yeast extract, 5 g/L NaCl, 3 g/L pepton, 2.5 g/L $K_2HPO_4$, 2.5 g/L glucose monohydrate and 15 g/L bacteriological agar; sterilised by autoclaving at the 121 $^{o}$C for 15 min and then poured into disposable sterilised plates.

### 3.2.4 Phenol red carbohydrate broth base

Phenol red carbohydrate broth base was prepared for carbohydrate utilisation to identify the species of *Listeria* present in the samples. The base was prepared by combining 10 g/L trypticase, 5 g/L NaCl and 0.018 g/L phenol red. The liquid was sterilized by autoclaving at 121$^{o}$C for 15 min before use. Ten percent of a sterilised sugar solution (xylose, rhamnose, mannitol, glucose and maltose) was prepared at the same time. 1ml of 10% sterilised sugar solution was combined with 9ml of phenol red carbohydrate broth base on a clean bench. This was called phenol red carbohydrate when used below.

### 3.2.5 Half Fraser broth

Half Fraser broth was used for the normal *Listeria* spp. first enrichment and was prepared by adding 12.9 g Fraser Broth (CM0895, Oxoid Ltd.) powder to 225 ml of $dH_2O$, and mixed well to dissolve completely. The broth was sterilised by autoclaving at 121°C for 15 minutes. After the broth was cooled to below 50°C, one vial of Half Fraser Selective Supplement (SR0166E, Oxoid Ltd.) was aseptically added into the broth as directed in the product insert.

### 3.2.6 Fraser broth

Half Fraser broth was used for the normal *Listeria* spp. second enrichment and was prepared by adding 28.7 g Fraser Broth (CM0895, Oxoid Ltd.) powder to 500ml $dH_2O$, and mixed well to dissolve completely. The broth was sterilised by autoclaving at 121°C for 15 minutes. After the broth was cooled to below 50°C, one vial of Half Fraser Selective Supplement (SR0156, Oxoid Ltd.) was aseptically added into the broth as directed in the product insert.

### 3.2.7 3M™ Demi Fraser broth

3M™ Demi Fraser broth was used for the primary enrichment of *Listeria* spp. specifically for 3M molecular detection. The broth was prepared by adding 55 g 3M™ Demi Fraser broth powder to 1 L $dH_2O$ and sterilised by autoclaving at 121°C for 15 min before use.

### 3.2.8 3M™ Fraser broth

3M™ Fraser broth was used for secondary enrichment of *Listeria* spp., specifically used for 3M molecular detection. The broth was prepared by adding 55 g 3M™ Demi Fraser broth powder into 1 L $dH_2O$ and sterilised by autoclaving at 121°C for 15 min before use.

## 3.3 Sample preparation

The methodological approach to analyse the samples for the prevalence, enumeration and confirmation of *Listeria species* in fresh produce is shown in Figure 3.2.

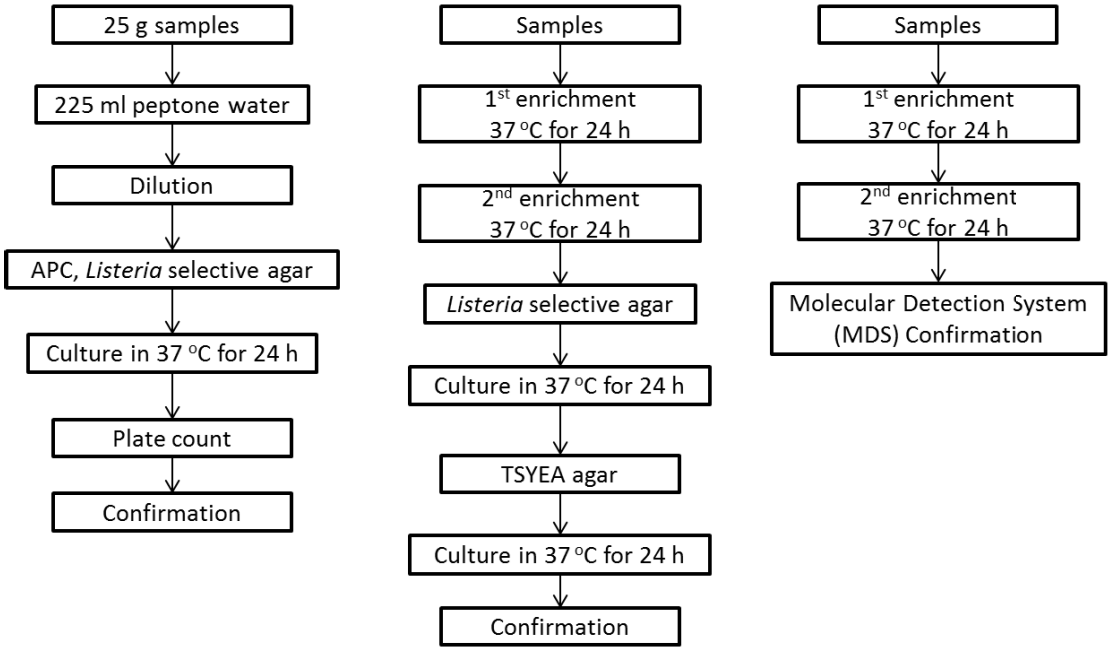



**Figure 3.2 Methodological approach used to study *Listeria* presence in fresh produce**

Fresh produce samples were packaged separately and stored at 5°C before the analysis. To reduce the influence of objective factors, samples were washed in the same tap water and cut with sterile tools (including knives and cutting boards). All samples were tested for the presence of *L. monocytogenes* following the procedure recommended by the International Organisation for Standardisation procedure. Twenty-five g of a representative portion from each sample was introduced aseptically into a sterile stomacher bag containing 225 ml of peptone water. *Listeria* selective agar (CM0856, OXOID) and Difco™ plate count agar (for aerobic plate counts, as APC for short) were plated the after making suitable dilutions. Following incubation at 35°C for24 – 48 h, the colonies were counted and results were reported in cfu/g.

The following formula was used to calculate cfu/g:

$$^{\text{cfu}}/_{g}\ \text{of sample} = \frac{\text{A} \times 10^{n}}{\text{V1}} \times \frac{V2}{m}$$

where A — number of colonies (average of the plates);

$10^n$ — level of dilution at which the counting was carried out;

V1 — volume of inoculum;

V2 — total volume of peptone water;

m — total sample weight

For reconfirmation of the results, the 3M™ molecular detection system was used and samples were prepared and incubated separately in Half Fraser Broth (Oxoid, Ltd., Basingstoke, UK, CM0895) and 3M™ *Listeria* Primary Fraser broth as per the manufacturer's instructions. The samples were incubated for 24 h at 30°C. After the incubation period, a 0.1 ml sub-sample from each Half Fraser Broth culture was added to 10 ml of Fraser Broth (Oxoid, CM0895) and 3M *Listeria* Secondary Fraser broth, then incubated for 24 h at 37°C for secondary enrichment. A loopful of the Fraser Broth enrichment culture will be streaked on the surface of *Listeria* Selective Agar (Oxford Formulation) (Oxoid, CM0856). These selective agars were then incubated for up to 48 h at 37°C. Selective agars were observed for suspected colonies after 24 h and 48 h of incubation. Suspected colonies were those that appeared as greyish colonies surrounded by black halos and sunken centres with a possible greenish sheen on the Oxford agar. Whenever possible, up to five suspected colonies showing typical morphology of *Listeria* on these isolation media were streaked onto Tryptone Soya Yeast Extract Agar (TSYEA) and incubated at 37°C for 24 h. The following tests were used for confirmation: Gram staining, Carbohydrate utilisation and 3M ™ Molecular Detection.

## 3.4 Sample examination

### 3.4.1 Gram staining

Smears were prepared from a suspect colony (from TSYEA agar after culturing) and heated gently to fix. The slide was flooded with 0.5% crystal violet and left for 30s. The slide was tilted, and then rinsed gently with water; the slide was flooded with sufficient (1%) Lugol's iodine to rinse off excess water, covered with fresh iodine, and allowed to remain for 30s. The slide was tilted and washed off with water; decolourised with 95 - 100% ethanol or acetone until the colour ceased to run off the smear; rinsed with water then flooded with 0.1% counterstain safranin and left to act for about 30 s to 1 min. The slide was then washed briefly with water and blotted dry. This slide was examined under oil immersion to observe cell morphology and the Gram reaction. *L. monocytogenes* is a gram-

positive bacterium; therefore, the reaction should be deep blue/purple if the colony is *L. monocytogenes*.

### 3.4.2 Carbohydrate utilisation

For the carbohydrate utilisation test, isolated colonies from TSYEA were transferred into NUNCLON ™ Surface unit containing phenol red carbohydrate broth (glucose, maltose, mannitol rhamnose and xylose) and incubated at 37°C for 24 h (as shown in Figure 3.3). Positive reactions were indicated by a yellow colour (acid formation) and occurred mostly within 24 to 48 h.

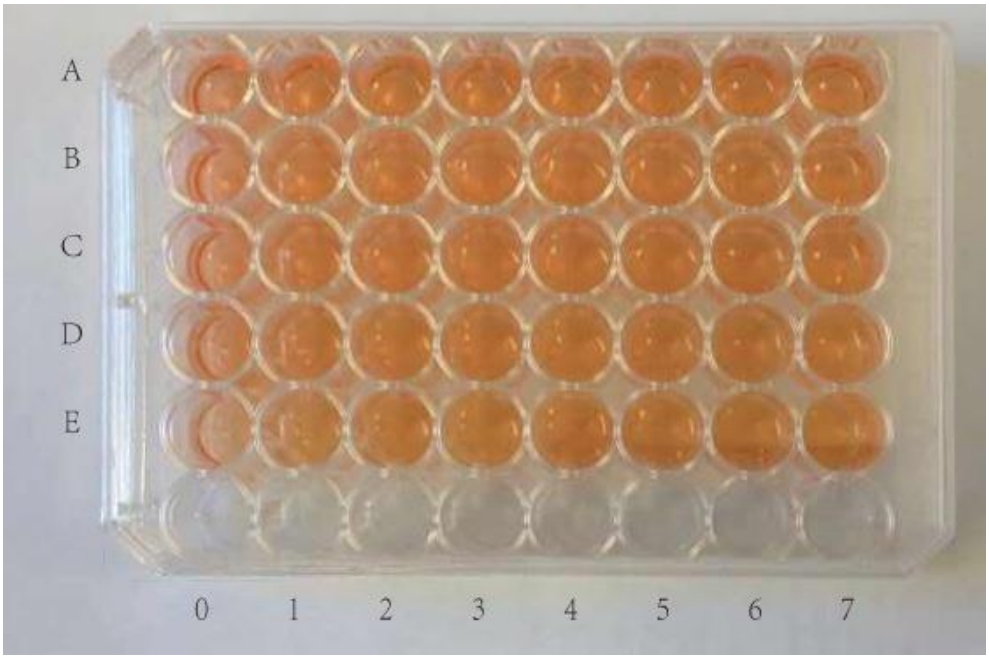



**Figure 3.3 NUNCLON ™ Surface units containing phenol red carbohydrate broth after colonies transferred but before culturing. (A=glucose; B=maltose; C=mannitol; D=rhamnose; E=xylose; for numbers, 0=negative control, 1 to 7 = marked colonies from different plates)**

### 3.4.3 3M™ Molecular Detection

The theory of 3M™ molecular detection is based on PCR. The superiority of this technique is the combination of isothermal DNA amplification and bioluminescence (from firefly) detection that are used together to detect the prevalence of target genes without sacrificing productivity. Samples were enriched in 225 mL 3M™ Modified *Listeria* Recovery Broth directly in the 3M™ sample collection bag, followed by incubation at 30°C for 24 h and tested with the 3M™ Molecular Detection System at 24 hours. The procedure followed the manufacturer's protocol (Figure 3.4).

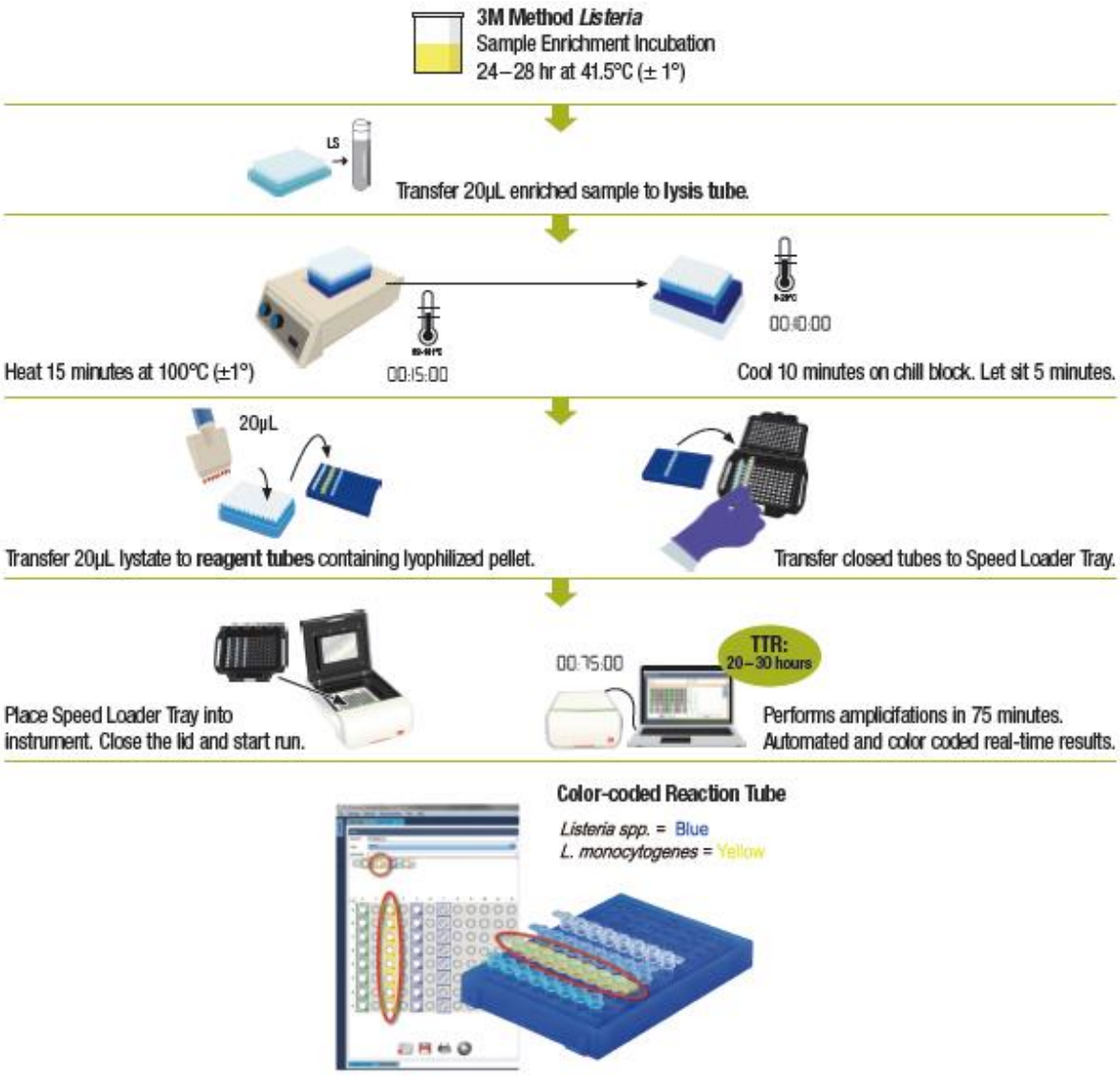



**Figure 3.4 Manufacturer's protocol of 3M ™ Molecular Detection.**

### 3.4.4 Statistical analysis

Microsoft Excel (Microsoft Office 2010) was used for basic data calculations (cfu/g). All data calculated from the formula above were log transformed for normal distribution data before further analysis using Minitab Statistical Software version 17 (Minitab Pty Ltd). The software functions were used to analyse the experimental data. The Tukey test was used to compare the means. When $P<0.05$, the results were considered to be as a significantly different.

# Chapter 4

# Prevalence of *Listeria* spp. in Fresh Produce Grown Using Different Farming Systems

## 4.1 Introduction

Farming environment has the potential to spread *Listeria* spp to food commodities. A farming environment is a complex system governed by many factors. These factors can be categorised into concrete and abstract. Soil, water and vegetables are concrete factors whereas the abstract factors consist of moisture, temperature and sunshine time. Both types of factors influence the survival of *Listeria* spp. in and on fresh produce. In the outbreak of listeriosis associated with cantaloupes in Colorado (CDC, 2011a), both factors in the farming environment collectively caused the contamination of *L. monocytogenes*. There were many potential reasons for the contamination of the cantaloupes. The primary factor was the temperature, which was ideal for facilitating the growth of *Listeria* spp. As well as temperature, machinery and trucks contributed equally. This was because the machines used previously for potato washing were re-used for washing the cantaloupes. In this process, *L. monocytogenes* may have been transferred from the potato soil - via the washing machine - to the cantaloupes. Similarly, the trucks used to transfer the bad/reject cantaloupes to the cattle farms were parked adjacent to the packing plant for the cantaloupes. It was highly possible that the cantaloupes could have been contaminated by *L. monocytogenes* from the cattle farms.

New Zealand's economy is driven to a large extent by agriculture. The Canterbury Plains are in the east central coast of the South Island. This region is very important for crop growing. Although stock farming is popular in New Zealand, vegetable farming is equally important and gaining attention (Moot et al., 2010). Besides providing food to local citizens, a reasonable amount/volume of the vegetables (fresh and processed) produced locally are exported each year. An increased export rate has been noted over recent years. For example, from 1994 to 2000, the value of fresh carrots exported increased from less than one million NZ dollars per year to nearly five million NZ dollars per year (*Canterbury Process and Fresh Vegetables*, 2001).

The research work presented in this chapter investigated the prevalence of *Listeria* spp. in different farming environments (organic vs conventional). The primary objective was to clarify whether organic farming has a higher risk of *Listeria* contamination compared with the conventional farming system. Samples of fresh produce, water and soil were collected to detect the presence of *Listeria* spp. The characterisation of selected *Listeria* isolates from the positive samples is reported in Chapter 6.

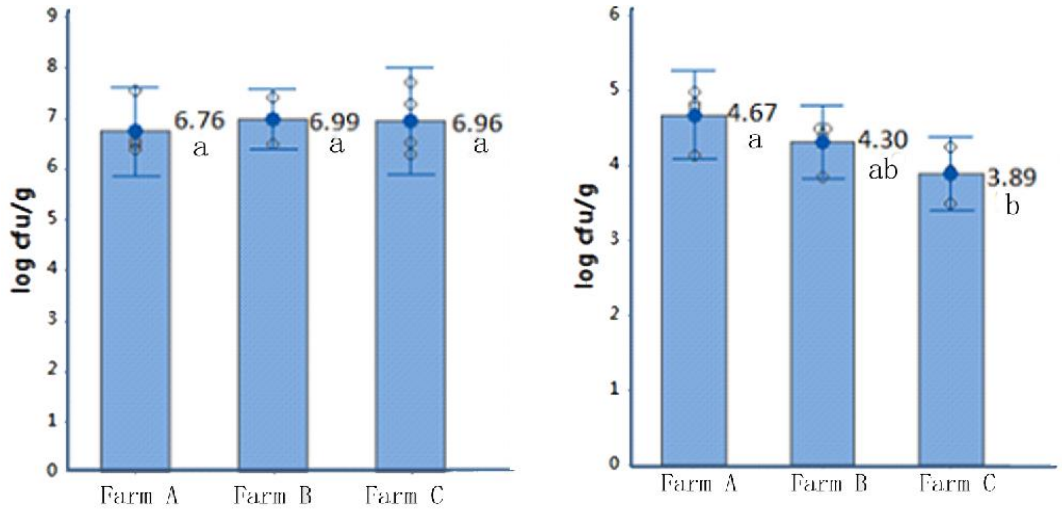
## 4.2 Material and Methods

Material and methods used in the experimental work reported in this chapter are described in Sections 3.2.1, 3.2.2, 3.3 and 3.4.

## 4.3 Results

### 4.3.1 The prevalence of *Listeria* spp. in soil samples

In order to investigate the contribution of soil as a source of *Listeria* spp. contamination in vegetables grown at different farms, soil samples were collected twice during weeks 5 and 6 of sampling to analyse for total microbial load and the presence of *Listeria* spp. Two soil samples were collected from each farm per week. Aerobic plate counts (log cfu/g) in the soil samples from three different farms were grouped and statistically and compared using the Tukey test ($p>0.05$). Statistical analysis showed no significant differences among the soil samples from the three farms (Figure 4.1A). The mean of APC was approximately 7 log cfu/g. However, significant differences were observed in the levels of *Listeria* spp. in the soil samples ($p<0.05$). The average counts of *Listeria* spp. in the soil samples of Farms A, B and C were 4.67, 4.30 and 3.89 log cfu/g, respectively (Figure 4.1B).




**(A)** **(B)**

**Figure 4.1 Comparative microbial analyses of soil samples from three farms: (A) Aerobic plate count, and (B) *Listeria* spp. Error bars represent standard deviations of the means. Different letters on each bar show that the results have significant differences using the Tukey test, P<0.05. Farm A, conventional farming; Farm B, crop-livestock rotation organic farming; and Farm C, compost organic farming**

### 4.3.2 The prevalence of *Listeria* spp. in fresh produce samples from the conventional farm

Due to seasonal changes in growing different vegetable types, not all types of vegetable samples were available during the six-week sampling regime. Therefore, sliver beet samples were only picked in the first two weeks. The remaining four vegetable samples, which included leek, pakchoi, radish and spring onion, were collected for six weeks. Thus, microbial levels and *Listeria* spp. levels were able to be compared for the leek, pakchoi, radish and spring onion samples, whereas microbial levels and *Listeria* spp. levels in sliver beet were not able to be evaluated using the relevant values, for the microbial quality and *Listeria* spp. levels in the fresh produce samples obtained from the conventional farm. The microbial quality (APC) of the leek samples was similar to the pakchoi samples, where the total count was around 6 log cfu/g and the *Listeria* spp. contamination level was about 3 log cfu/g. The prevalence of *Listeria* spp. in sliver beet was comparatively higher in comparison to the other samples. The average *Listeria* spp. count in silver beet was around 5 log cfu/g (mean APC level was about 6.5 ). Spring onions showed the second lowest numbers of *Listeria* spp., which were approximately 1.5 log cfu/g (the APC was around 2.5 log cfu/g). Radish had the lowest numbers of *Listeria* spp. (mean 1.11 log cfu/g) and the APC was around 3 log cfu/g (Figure 4.2).

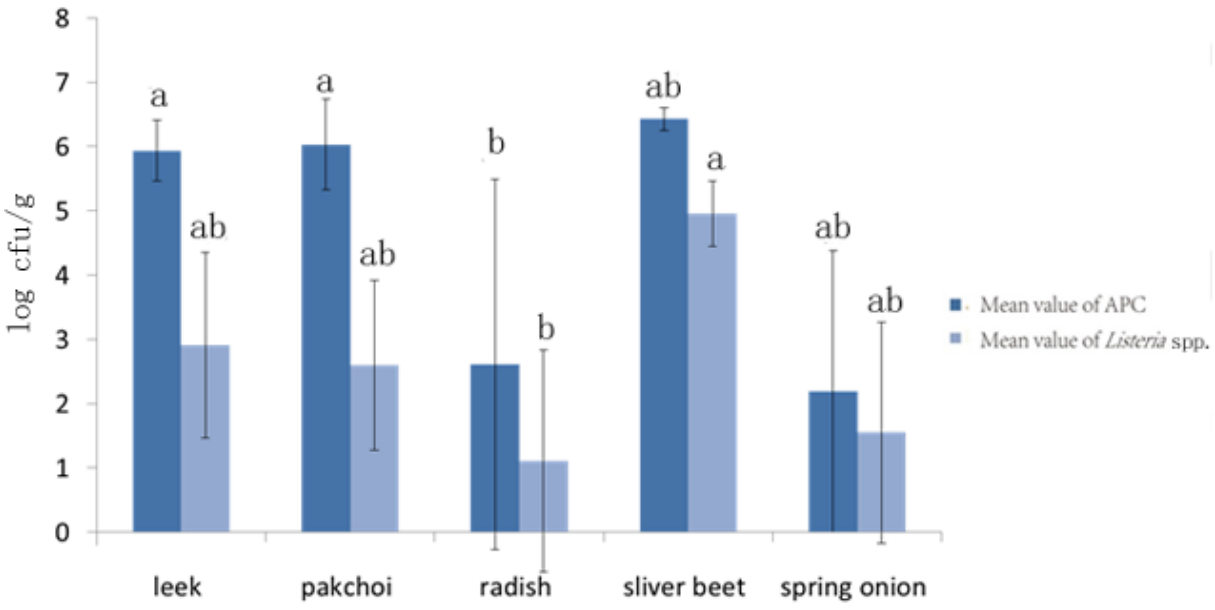



**Figure 4.2 Comparative microbial analyses of five different fresh produce samples obtained from conventional farms. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, $P < 0.05$**

### 4.3.3 The prevalence of *Listeria* spp. in fresh produce samples from crop-livestock integration farm

In the crop-livestock farm (Farm B), pakchoi was not available during week 3 of sampling and sliver beet was only available in the first two weeks. Lettuce was available in weeks 3, 4 and 5 because of the seasonal change. Therefore, microbial levels (APC) and *Listeria* spp. levels in cabbage, parsley and spinach samples which were collected for six weeks were not comparable. However, APC levels and *Listeria* spp. levels of samples of lettuce (three weeks' collection), packchoi (five weeks' collection) and sliver beet (two weeks' collection) were compared together using one-way ANOVA analysis. Although there were no significant differences in APC levels and *Listeria* spp. contamination levels among the six types of fresh produce samples according to Tukey's test ($p > 0.05$), the *Listeria* spp. levels in the cabbage samples (2.53 log cfu/g; mean) were lower than in parsley (3.98 log cfu/g ; mean) and spinach samples (3.77 log cfu/g; mean). The mean values of APCs in all six fresh produce samples were in the range of 6.5 to 7.5 log cfu/g (Figure 4.3).

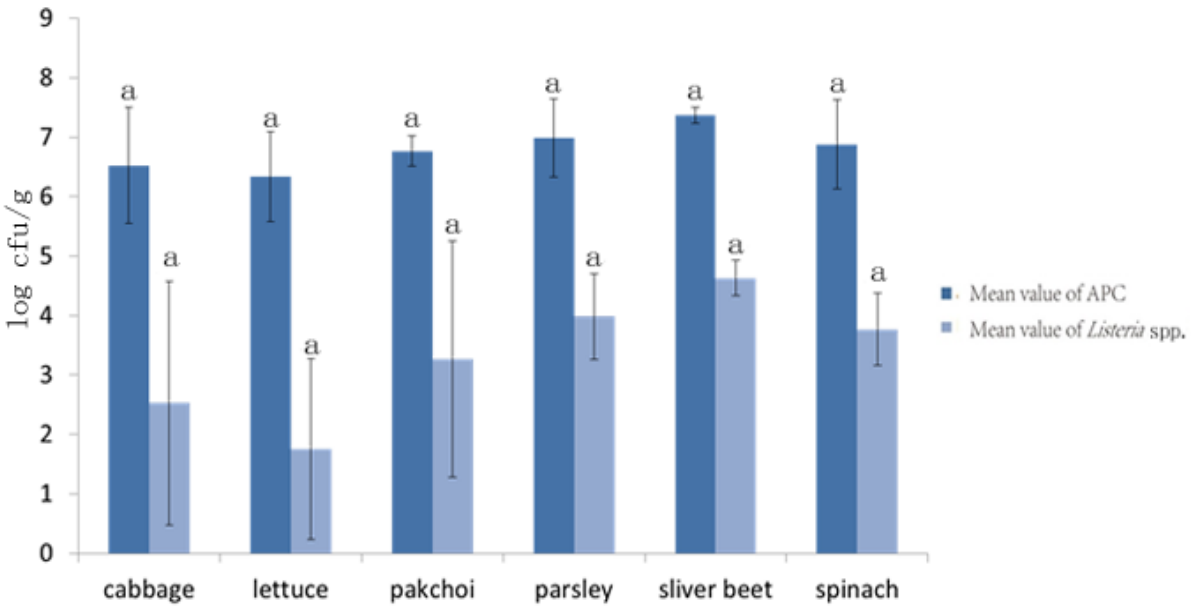



**Figure 4.3 Comparative microbial analysis of six different fresh produce samples obtained from crop-livestock farms. Error bars represent standard deviations from the mean. Different letters on each bar show that results have significant differences using the Tukey test, P < 0.05**

### 4.3.4 The prevalence of *Listeria* spp. in fresh produce samples from the compost farm

Sample collection from the compost farm was also influenced by seasonal changes. Cabbage, carrot and parsley were not available in week 5 and 6 whereas coriander and spinach were not available in week 6. Leek samples were collected only in the sixth week. Therefore, microbial levels and *Listeria* levels were not able to be compared between the coriander and spinach samples (week 5 collection), and between carrot samples and parsley samples (week 4 collection). Meanwhile, APC levels and *Listeria* spp. levels in cabbage (week 2 collection) and leek samples (week 1 collection) were compared using one-way ANOVA analysis to evaluate the microbial levels and *Listeria* spp. levels of the fresh produce samples from the compost farm. There were no significant differences in APC levels between these fresh produce samples ($p > 0.05$). However, there were significant differences in the *Listeria* spp. contamination levels ($p < 0.05$). The prevalence of *Listeria* spp. in fresh produce from Farm B was separated into five groups according to the Tukey test with the highest observed in spinach (5.13 log cfu/g; mean). The population of *Listeria* spp. in the cabbage and parsley were categorised as being in the same group by the Tukey test, which meant there were no significant differences between these two types of vegetables. The lowest population of *Listeria* spp. was present in the carrot samples with a mean of <1 log cfu/g (Figure 4.4).

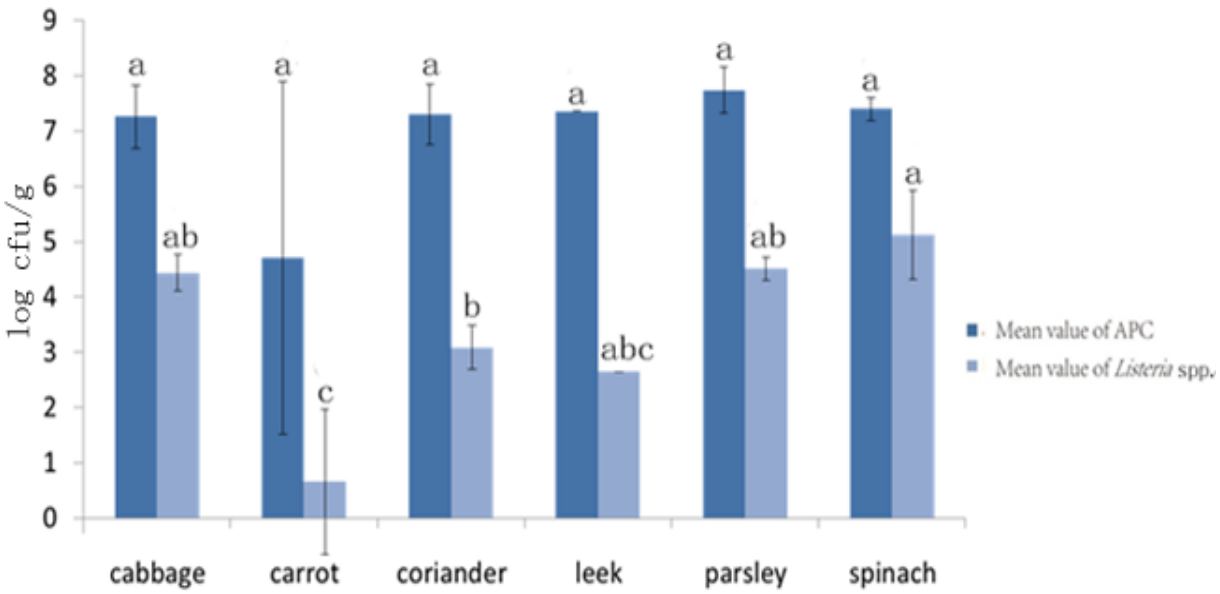



**Figure 4.4 Comparative microbial analysis of six different fresh produce samples obtained from compost farms. Error bars represent standard deviations from the mean. Different letters on each bar show that results have significant differences using the Tukey test, P < 0.05**

### 4.3.5 Overview of the presence of *Listeria* spp. in fresh produce samples from the different farms

In total, 98 samples including 78 fresh vegetable samples, 12 soil samples and 8 water samples from different farms were analysed (see Table 4.1). *Listeria* spp. were detected in all soil samples from all farms but not in water samples. Most vegetable samples were positive for the presence of *Listeria* spp.

**Table 4.1 Incidence of *Listeria* spp. in soil, water and vegetable samples from selected farms**

| Farm | Soil No. (%)[a] | Water No. (%)[a] | Vegetable | No. (%)[a] | *Listeria* [b] (log cfu/g) | | APC [b] (log cfu/g) | |
|---|---|---|---|---|---|---|---|---|
| A (conventional) | 4 (100) | 2 (0) | leek | 6 (83.3) | 2.91±1.44 | abcde | 5.93±0.48 | ab |
| | | | pakchoi | 6 (83.3) | 2.60±1.32 | abcde | 6.03±0.71 | ab |
| | | | radish | 6 (33.3) | 1.11±1.73 | ce | 2.61±2.88 | cd |
| | | | silver beet | 2 (100) | 4.97±0.51 | abc | 6.43±0.18 | abcd |
| | | | spring onion | 6 (50) | 1.55±1.72 | bcde | 4.27±2.91 | bd |
| B (Crop-livestock rotation) | 4 (100) | 4 (0) | cabbage | 6 (66.7) | 2.53±2.05 | abcde | 6.53±0.98 | ab |
| | | | lettuce | 4 (75) | 2.06±1.52 | abcde | 6.34±0.76 | ab |
| | | | pakchoi | 5 (80) | 3.27±1.99 | abcde | 6.77±0.26 | ab |
| | | | silver beet | 2 (100) | 4.63±0.30 | abcde | 7.37±0.14 | ab |
| | | | spinach | 6 (100) | 3.77±0.61 | abc | 6.88±0.75 | ab |
| | | | parsley | 6 (100) | 3.98±0.72 | ab | 6.99±0.65 | ab |
| C (Compost) | 4 (100) | 2 (0) | cabbage | 4 (100) | 4.44±0.14 | ab | 7.26±0.57 | ab |
| | | | carrot | 4 (25) | 0.66±0.57 | de | 4.71±3.19 | abc |
| | | | coriander | 5 (100) | 3.09±0.16 | abcde | 7.31±0.55 | a |
| | | | leek | 1 (100) | 2.65 | abcde | 7.36 | abcd |
| | | | parsley | 4 (100) | 4.51±0.09 | ab | 7.74±0.41 | a |
| | | | spinach | 5 (100) | 5.13±0.32 | a | 7.41±0.21 | a |

[a] Numbers of analysed samples/per cent of positive test is given via plate counts

[b] Values come from means ± standard deviations. Letters that differ from each other within a column indicate that the results are significantly different based on the Tukey test ($P<0.05$)

## 4.4 Discussion

The studies reported in this chapter described the influence of the environment on the growth of *Listeria* spp. in fresh produce obtained from different farm types. Samples of fresh produce from three different farms were collected and analysed for microbiological quality and *Listeria* contamination. In addition, some soil and water samples were also tested to determine any possible relationships between microbial loads.

The APC levels and *Listeria* spp. levels for soil in three farms are shown in Figure 4.1. The means of APC for soil in Farms A, B, and C were 6.76, 6.99, and 6.96 log cfu/g, respectively. There were no significant differences for APC among the soil sample in these three farms ($P>0.05$). However, a soil sample from Farm A showed the highest number of *Listeria* spp. (4.67 log cfu/g; mean) followed by a soil sample from Farm B (4.30 log cfu/g; mean). Soil samples from Farm C had the lowest counts for *Listeria* spp. (3.89 log cfu/g by mean). The total microbial loads in the soil had no significant differences. However, significant differences appeared in the counts of *Listeria* spp. (conventional farm > crop-livestock integration farm > compost farm). Similar work on the presence of *L. monocytogenes* in soil and its associated fresh produce has been reported from Poland. According to that study, just 1.2% of 173 samples of naturally fertilised soil tested positive for *L. monocytogenes*. No *L. monocytogenes* were found in the other types of soil (including artificially fertilised soil and wasteland soil) (Szymczak et al., 2014). Similar data was obtained from our study; *L. monocytogenes* was not found in any soil sample type. In another report from Korea (Thapa et al., 2008), which analysed fresh produce and soil from farms, the values of APC in soil were between 7 log cfu/g - 9 log cfu/g, which were higher than our results.

All vegetable samples from these three farms can be roughly divided into three types, strong leafy vegetables (leek, spring onion, pakchoi, silver beet and cabbage), weak leafy vegetables (lettuce, parsley, spinach and coriander) and root vegetables (radish, carrot). The average APCs of the fresh produce were approximately 6 to 7 log cfu/g. The values of APC in the vegetables were similar to the values of APCs in soil from the farms. Similar APC values in fresh produce from farms were shown in a Korean study (Thapa et al., 2008). A group of researchers (Maffei et al., 2013) took 130 samples of fresh produce from different organic farms and conventional farms in Brazil and most APC values were within the 6 to 7 log cfu/g range in both types of farms and results were similar to ours. Radishes in Farm A and carrots in Farm C, which are root vegetables, had lower APC levels, with values of 2.61 log cfu/g and 4.71 log cfu/g, respectively. However, parsley and spinach in Farm C, which are weak leafy vegetables, showed higher levels of APC (over 7 log cfu/g). For the *Listeria* spp. levels, root vegetables radish and carrot were the least contaminated with values of 1.11 log cfu/g and 0.66 log cfu/g, respectively. In contrast, in Farm A, spring onions also had a lower level of

contamination (1.55 log cfu/g). Other research by Yucel Sengun & Karapinar, (2005) has shown that the compounds in spring onions have effective antimicrobial functions. This explains why spring onions had fewer *Listeria* counts. In Farm B, parsley had a higher level of contamination of *Listeria* spp. than other samples based on the Tukey test. In Farm C, the lowest level of *Listeria* spp. was in carrot (0.66 log cfu/g). There were no significant differences between strong leafy vegetables and weak leafy vegetables based on *Listeria* spp. level.

A comparison between Farm A and Farm B, showed that pakchoi in both farms had similar microbial contamination levels based on the Tukey test. However, sliver beet in Farm A (4.97 log cfu/g by mean, APC at 6.43 log cfu/g by mean) showed slightly higher *Listeria* spp. levels than those in Farm B (4.63 log cfu/g by mean, APC at 6.77 log cfu/g by mean). For Farms B and C, parsley samples showed a similar level of *Listeria* spp. whereas the *Listeria* spp. contamination of spinach samples in Farm B (3.77 log cfu/g; mean) was lower than that of Farm C (5.13 log cfu/g mean).

As mentioned in Chapter 2, Section 2.2, soil is a potential source factor for *Listeria* contamination of cantaloupes. In our results, 100% of soil samples were positive for *Listeria* spp.. However, not all vegetable samples tested positive. Thus for farm vegetables, if *Listeria* spp. were detected in the soil, the crops grown in that field should also be positive for *Listeria* spp. Others have shown the prevalence of *Listeria* spp. associated with fresh produce and growth environment (Szymczak et al., 2014), *L. monocytogenes* was detected in the soil from natural fertilised land and garden plots, and only parsley and potato were positive for *L. monocytogenes*. However, no fresh vegetables grown in artificially fertilised land were positive for *L. monocytogenes*, and soil in this region also tested negative for *L. monocytogenes*.

## 4.5 Conclusions

Water samples from all farms were negative for the presence of *Listeria* spp. All soil samples tested positive for *Listeria*. The three farms had similar total microbial loads. However, significant differences were recorded for *Listeria* spp. contamination with levels in conventional farm > crop-livestock integration farm > compost farm). Generally, most of the fresh produce samples from these three farms had similar total microbial loads, but differences in *Listeria* spp. loads were observed in the different types of vegetables. When comparing the same vegetables from Farm A and Farm B, the contamination of *Listeria* spp. in vegetables from the conventional farm was similar to that of the crop-livestock rotation farm. Fresh produce from the compost farm had the highest levels of *Listeria* spp.

# Chapter 5

# Detection of *Listeria* spp. in Fresh Produce Samples from Different Retail Shops

## 5.1 Introducion

Fresh produce is transported from farm to distribution centres and then to retail shops. It is a general practice that fresh vegetables are washed in water before they are supplied to retailers. After washing, fresh produce commodities are packed and transferred to different shops by different food chains. At any of these steps careless omissions would give *L. monocytogenes* the chance to contaminate fresh vegetables, such as under storage conditions (including temperature, moisture, and oxygen content) and/or sanitation conditions during the packing processes and transport. At the end of 2014 (CDC, 2015), a listeriosis outbreak related to apples happened, which led to 35 people being infected by *L. monocytogenes*. After testing, *L. monocytogenes* was detected in the apple-packing factory.

Most fresh produce is pre-treated, i.e. by UV (Srey et al., 2014), washing (Nastou et al., 2012) or chlorine (Banire & Jia, 2014) before being sold. However, *Listeria* spp. and *L. monocytogenes* were found in fresh produce from retail shops; for example, in re-packaged mixed vegetable salads (Little et al., 2007), parsley (Gómez-Govea et al., 2012) and lettuces (Althaus et al., 2012).

Fresh vegetables are consumed by almost all families. According to a local report (*Fresh Fact - New Zealand Horticulture*, 2013), in 2013, local consumers spent 41.8 million NZ dollars on fresh lettuces and 30 million NZ dollars on fresh carrots. New Zealand produced nearly 1.4 million tonnes of vegetables, while 500,000 tonnes were exported and over 900,000 tonnes were consumed locally. Therefore, to understand the safety of fresh produce in retail shops is at least as significant as that in farms.

The research described in this chapter investigated the prevalence of *Listeria* spp. in selective salad vegetables from different retail shops. Four different retail shops were selected in this study: 1, an open market (Market A); 2, a supermarket (Market B); 3, an Asian grocery store (Market C); and 4, a fruit and vegetable market (Market D).

## 5.2 Material and Methods

Material and methods used in the experimental work reported in this chapter are described in Section 3.2.1, 3.2.2, 3.3 and 3.4.

## 5.3 Results

### 5.3.1 Comparsion after classification by the different sources

**Comparsion of microbial levels in vegetables from Market A**

Market A was an open market that operated each Sunday. Four types of vegetable samples were collected over a six-week period to compare the total microbial counts and the presence of *Listeria* spp. Data was statistically analysed using the Tukey test. The total microbial populations in the cabbages, carrots and lettuces did not have significant differences and the mean counts were around 7 log cfu/g. The cucumber samples had the lowest microbial counts (4.5 log cfu/g; mean). The *Listeria* spp. detected in four different types of fresh produce samples were significantly different ($p < 0.05$). The highest population of *Listeria* spp. was detected in lettuce (about 4.5 log cfu/g; mean), followed by cucumber (about 3 log cfu/g; mean). The level of *Listeria* spp. had the second lowest load in cabbages (about 1.5 log cfu/g; mean). The level of *Listeria* spp. contamination was the lowest in carrot with less than 1 log cfu/g (Figure 5.1).

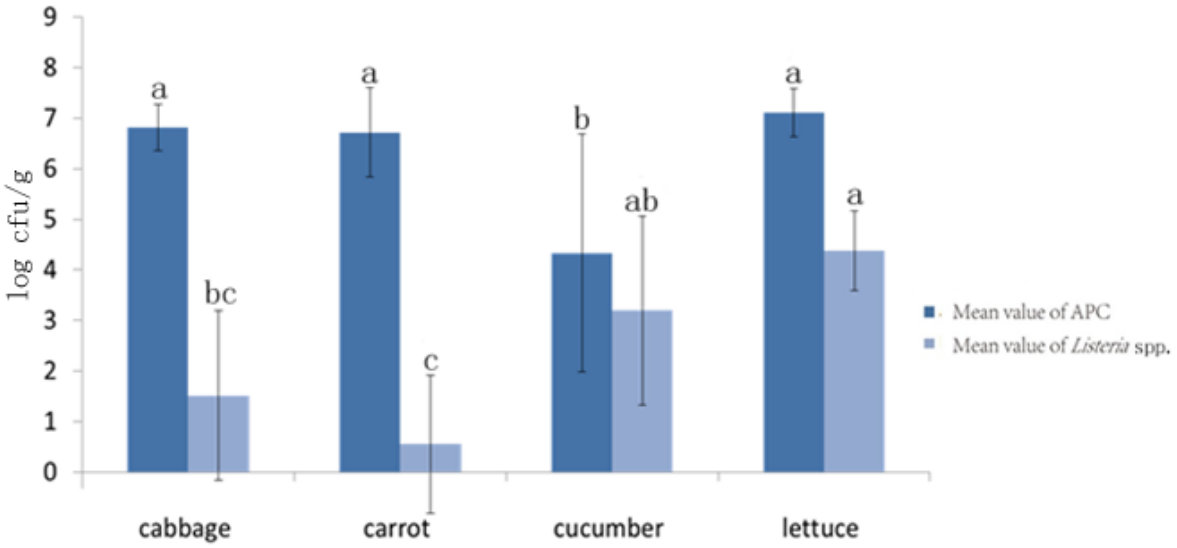



**Figure 5.1 Comparative microbial analysis of fresh produce samples from Market A. Error bars represent standard deviations of the means. Different letters on each bar show that the results have significant differences using the Tukey test, P < 0.05**

### Comparsion of microbial levels in vegetables from Market B

Market B was an outlet from a supermarket chain, which had constant suppliers providing fresh vegetables. Four types of vegetable samples were completed over the six-week collection period; therefore, it was not suitable to compare the microbial levels and *Listeria* spp. levels for these four types of vegetable samples from Market B. This was a similar situation as in Market A, where cabbage, carrot and lettuce belonging to the same group were divided by the Tukey test, with contamination in a range between 6 to 7 log cfu/g. In contrast, the APC value of cucumbers was lower (about 3 log cfu/g by mean). The level of *Listeria* spp. in lettuce was the highest (about 4 log cfu/g by mean) among the four types of vegetables from Market B. The second highest population of *Listeria* spp. was in cabbage (about 2.5 log cfu/g by mean) followed by cucumbers (about 2 log cfu/g; mean). The population of *Listeria* spp. in carrot was the lowest (about 1 log cfu/g; mean) (Figure 5.2).

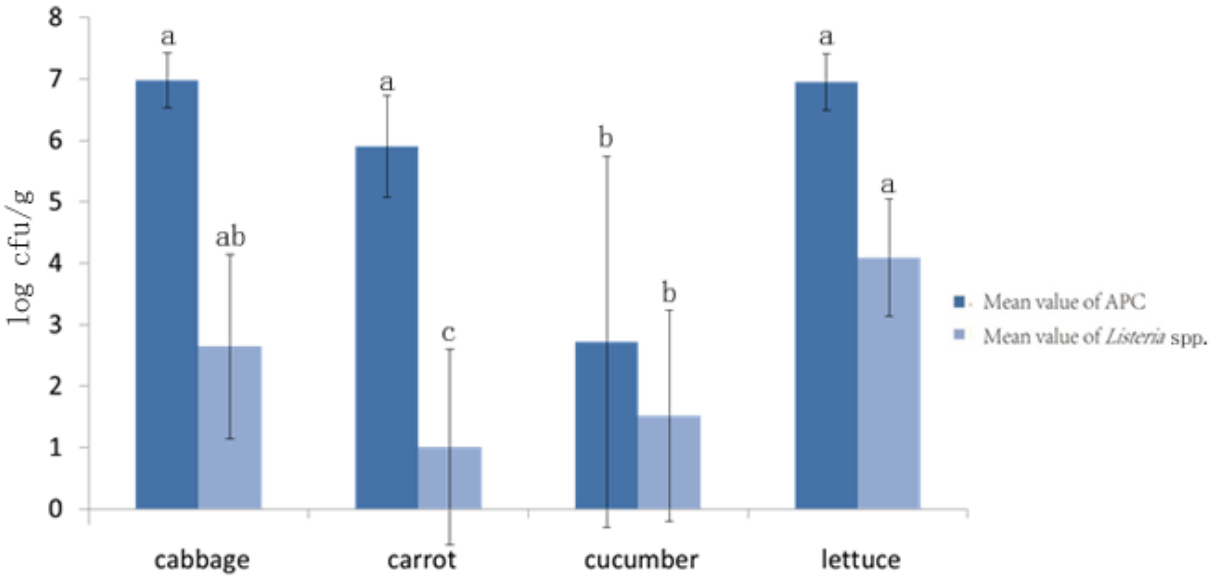



**Figure 5.2 Comparative microbial analysis of fresh produce samples from Market B. Error bars represent standard deviations of the means. Different letters on each bar show that the results have significant differences using the Tukey test, P <0.05**

### Comparsion of microbial level in vegetables from Market C

Market C was an ethnic retail shop that sold a variety of commodities, including fresh produce. Four types of vegetable samples were collected over six weeks to test for total microbial counts and the presence of *Listeria* spp. There were no significant differences ($p > 0.05$) in the total plate counts of the fresh produce samples sold in Market C (Figure 5.3). The mean APC values of the fresh produce samples in Market C were in the range of 5.5 to 7 log cfu/g. Leafy vegetables (cabbages and lettuces) in Market C had similar *Listeria* spp. contamination levels when analysed by the Tukey test. However, the *Listeria* spp. levels in lettuce (about 4 log cfu/g by mean) were higher compared to those in cabbages (about 3 log cfu/g by mean), followed by those in cucumbers (about 3 log cfu/g; mean). Carrot contained the lowest population of *Listeria* spp. (less than 1 log cfu/g; mean) (Figure 5.3).

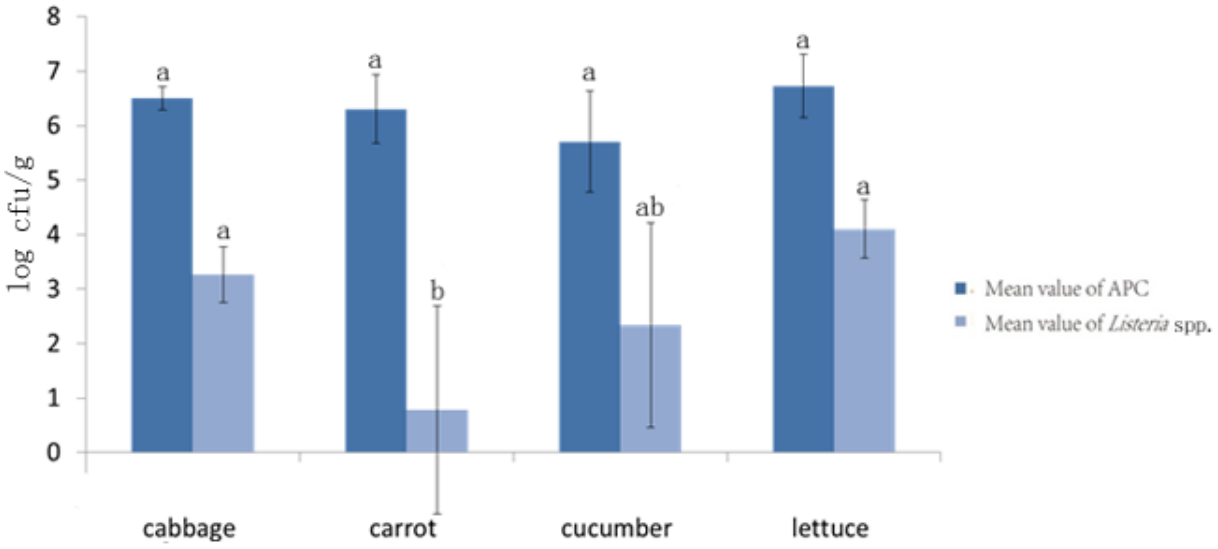



**Figure 5.3 Comparative microbial analysis of fresh produce samples from Market C. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, $P < 0.05$**

### Comparsion of microbial level in vegetables from Market D

Market D was a fruit and vegetable retail shop outlet where fresh produce was the major commodity sold. Four different types of vegetable samples were collected to evaluate the microbiological quality and the presence of *Listeria* spp. contamination levels during six weeks of sampling. Statistically significant differences were recorded for total plate counts using the Tukey test. The microbial populations were highest in the cabbage and lettuce samples (7 log cfu/g; mean). The population of total microbes in carrots (6.5 log cfu/g; mean) was slightly lower and had significant differences with the group of cabbages and lettuces (7 log cfu/g; mean) ($p < 0.05$). The APC in cucumber were lowest in the samples from Market D (fewer than 5 log cfu/g; mean). In contrast, the levels of *Listeria* spp. existing in the cabbages and lettuces were higher than in other types of vegetable. No *Listeria* spp. were present in carrot samples from Market D (Figure 5.4).

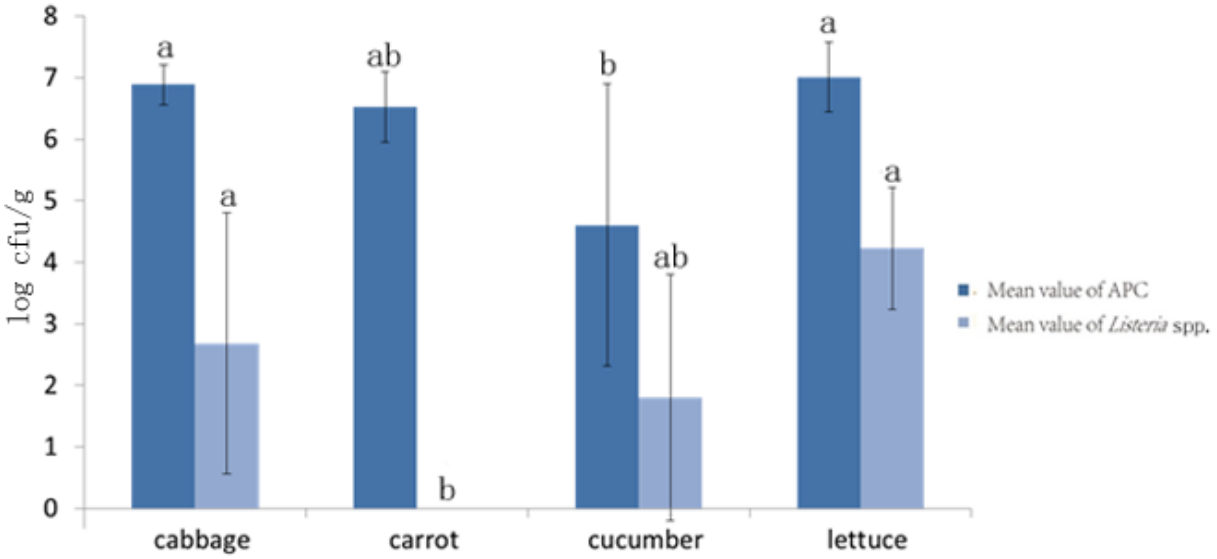



**Figure 5.4 Comparative microbial analysis of fresh produce samples from Market D. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, P < 0.05**

### 5.3.2 Comparsion after classification by vegetable type

The microbial quality (*Listeria* spp. and APC) of fresh produce was compared between four retail shops. Statistical analysis (Tukey test) showed that, microbiologically, the fresh produce samples did not have significant differences ($p > 0.05$) among the retail shops selected (Figure 5.5). *Listeria* spp. levels in the cabbage samples ranged from 1.5 to 3 log cfu/g by mean (APC values were about 7 log cfu/g by mean). *Listeria* spp. levels in the carrot samples ranged from 0 to 1 log cfu/g (APC values from 6 to 7 log cfu/g). *Listeria* spp. levels in the cucumber samples ranged from 1.5 to 3.5 log cfu/g (APC levels ranged from 2.5 to 6 log cfu/g). *Listeria* spp. levels in lettuce samples were about 4 log cfu/g by mean (APC levels were about 7 log cfu/g; mean).

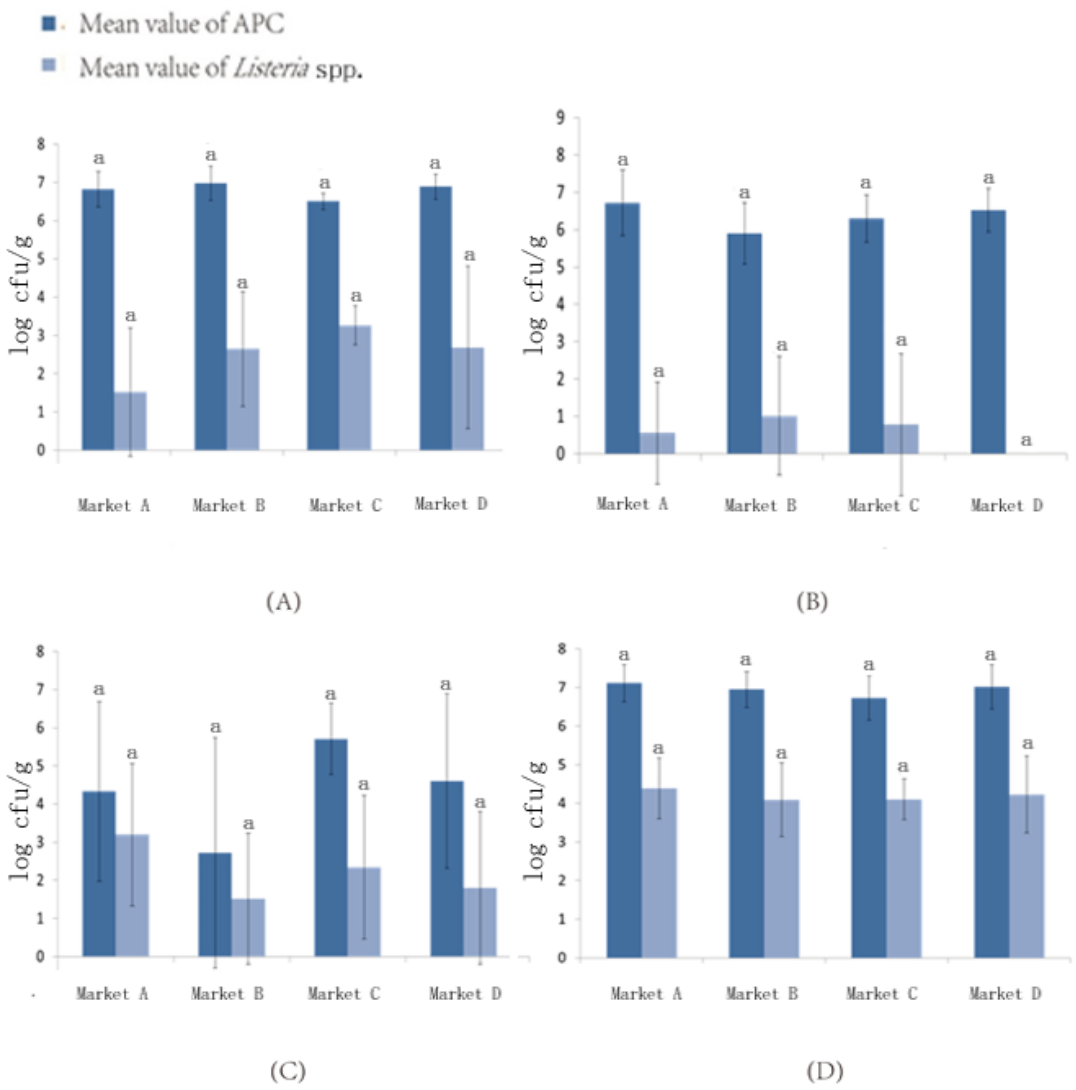
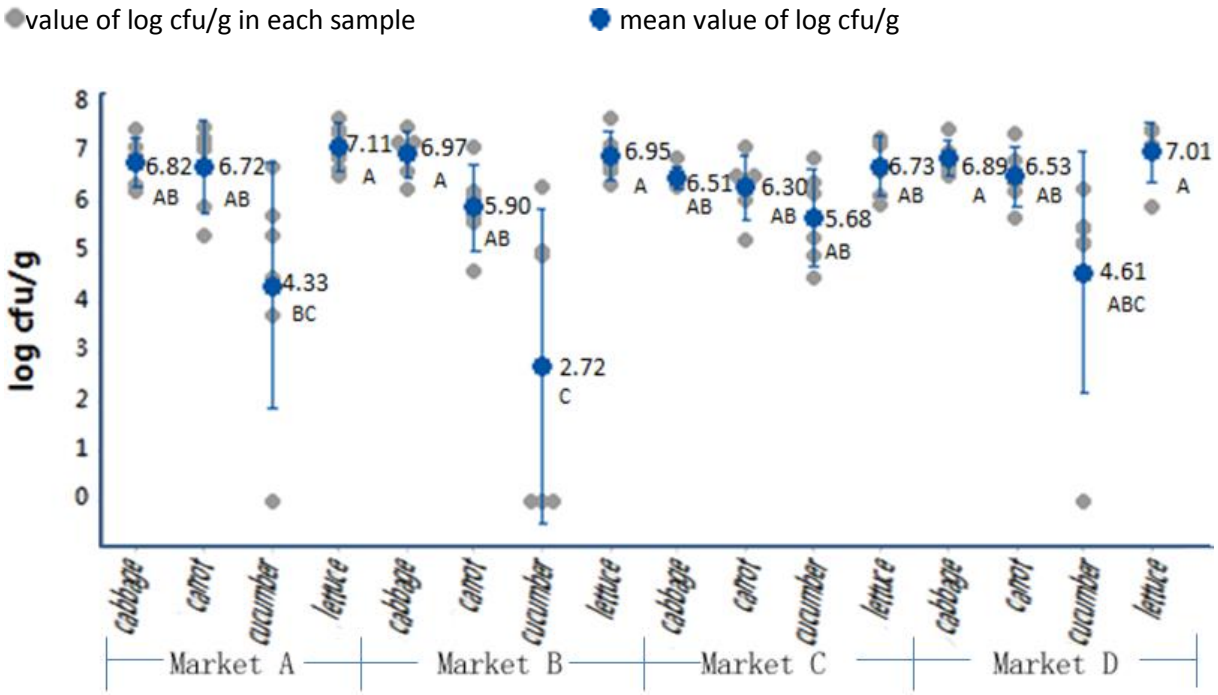


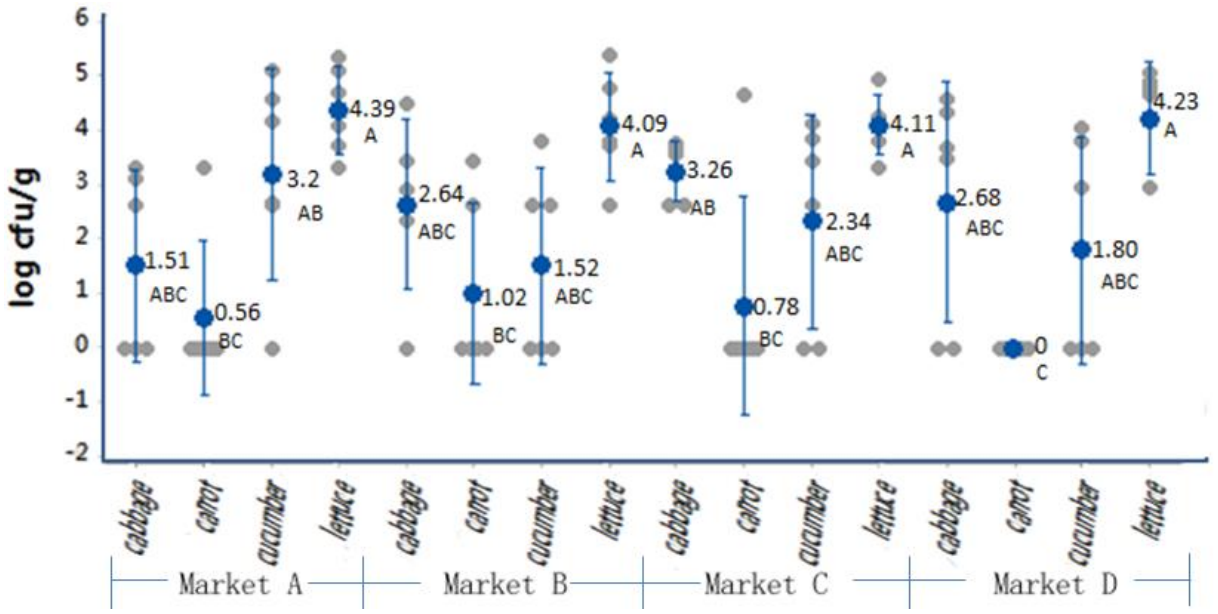

**Figure 5.5 Comparative microbial analysis of fresh produce samples from Market A to D. (A) cabbage (B) carrot (C) cucumber (D) lettuce. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, P > 0.05**

### 5.3.3 Overview of *Listeria* spp. in fresh produce samples from retail shops

In general, there were similar values for average APCs in cabbages, carrots and lettuces in the four retail shops, according to Figure 5.6 (A). Similar to the samples from the farms, most values for APC were in the range from 6 to 7 log cfu/g. The mean APC of the carrot samples from Market B had the lowest value, which was fewer than 6 log cfu/g. On average, lettuce samples from Market A and Market D had comparatively higher APC (>7 log cfu/g). The cucumber samples had the lowest microbial load, ranging from 2.72 to 5.68 log cfu/g. However, a research report on the microbiological analysis of salad vegetables in markets in India (Viswanathan et al., 2001) found the average APCs were 9 log cfu/g. The *Listeria* spp. counts for fresh produce from retail shops are presented in Figure 5.6B. The majority of APCs in cabbages and cucumbers were distinguished in the same group using the Tukey test. Carrot samples had the lowest *Listeria* spp. load (< 1.05 log cfu/g); however, lettuce samples had the highest *Listeria* spp. load recorded (> 4 log cfu/g). As a whole, there were no significant differences in the same type of fresh produce among the four retail shops. Other research from Dhaka, which evaluated the prevalence of bacteria in salad vegetables (Rahman et al., 2013), found that the *Listeria* spp. loads were much higher (6 to 8 log cfu/g) than the results of this study.




**(A)**




**(B)**

**Figure 5.6 Comparative microbial analyses of samples from four retail shops. A, APC and B, *Listeria* spp. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, P < 0.05**

## 5.4 Discussion

The results presented in this chapter mainly compared the microbial populations and presence of *Listeria* spp. in selected vegetables from four retail shops in the Canterbury region. According to Section 5.3.2, each type of vegetable purchased from the selected shops did not show significant differences in microbial quality (APC). However, slight differences can be seen Figure 5.5 and Figure 5.6. For the cabbage samples, the highest *Listeria* spp. loads were in samples from the Market C, with 3.26 log cfu/g by mean, while the lowest population of *Listeria* spp. found in the samples was from Market A, with only 1.51 log cfu/g (mean). Carrots had the lowest *Listeria* spp. load. Among four types of fresh produce in four retail shops the highest loads were in Market B (1.02 log cfu/g; mean) and lowest loads were in Market D (nil). The population of *Listeria* spp. in cucumber was closer to the data for cabbages, with the highest value of *Listeria* spp. load in Market A (3.2 log cfu/g ; mean) and the lowest value of *Listeria* spp. load in Market B (1.52 log cfu/g; mean). The lettuce samples had the highest bacterial load among the four types of vegetables with the highest APC values and *Listeria* spp. levels. Lettuce samples from Market A (4.39 log cfu/g; mean) had the highest population of *Listeria* spp. compared to the lowest *Listeria* spp. in the samples, from Market B (4.09 log cfu/g ; mean).

In the assessment of *L. monocytogenes* associated with lettuce, *L .monocytogenes* was positive in five RTE lettuce samples (Althaus et al., 2012). All colonies isolated belonged to serotype 1/2a. In another research on a risk assessment for *L. monocytogenes* from the farm to the table in Korea (Ding et al., 2013), the mean final *L. monocytogenes* contamination levels were -1.50 log cfu/g and -0.146 log cfu/g at restaurants and in homes, respectively. In the bateriological assessment of fresh produce in Norway (Johannessen et al., 2002), just one in 200 lettuce samples was positive for *L. monocytogenes*. In the study on the detection of *Listeria* spp. in RTE products in Malysia (Jamali et al., 2013a), *Listera* spp. were found in 20% of salad and vegetable samples, including two lettuce samples, two cucumber samples, one tomato sample and three cabbage samples. *L. monocytogenes* was positive in one lettuce sample, two cucumber samples and two cabbage samples. In the microbial evaluation for minimally-processed vegetables, just one in 112 sprouts samples was positive for *L. monocytogenes*, and lettuce and other fruit and vegetable samples were negative for this bacterium (Seo et al., 2010). In another microbial assessments of fresh produce (Abadias et al., 2008), *L. monocytgenes* was found in 3.4% of lettuce samples (fewer than 2 log cfu/g). In a research paper on the prevalence of pathogens in fresh produce (Rahman et al., 2013), the population of *Listeria* spp. on fresh produce (cucumber, carrot, lettuce) was in a range from 6 log cfu/g to 8 log cfu/g, which was much higher than our results.

Sample collection from retail shops did not seem to be influenced by changing seasons. Therefore, all fresh produce from retail shops were collected over six weeks. Microbial quality may be influenced by storage situations, including temperature, relative humidity (RH), $O_2$ and $CO_2$ contents, storage time and surfaces touched.

A group of scientists completed research on the effect of storage temperature and RH on the growth of *L. monocytogenes* (Likotrafiti et al., 2013). They used lettuce leaves, parsley leaves and cucumber epidermis as samples, three temperatures, and two RH values as variables, to analyse by group. However, *L. monocytogenes* was just grown on the cucumber's epidermis. The results showed that *L. monocytogenes* preferred higher temperature and RH. In addition, *L. monocytogenes* had difficultly surviving on intact vegetable surfaces (Likotrafiti et al., 2013).

In other research which studied the effect of atmosphere and acid adaptation on *L. monocytogenes* growth (Francis et al., 2001) used intact lettuce leaves to prepare lettuce agar to monitor the growth of *L. monocytogenes*. Lettuce agar plates with *L. monocytogenes* were stored separately under three conditions (a. air; b. 10% $CO_2$, 5% $O_2$, 85% $N_2$; and c. 25% $CO_2$, 75% $N_2$) in 8$^{o}$C. Microbial levels were tested over the storage period. The results showed that the vitality of the *Listeria* spp. was raised when samples were stored under 25% $CO_2$ conditions, and the most optimal condition for vegetable packing was in a 5 to 10% $CO_2$ content atmosphere (Francis et al., 2001).

In addition, in research on storage temperatures (Sant'Ana et al., 2012a), *L. monocytogenes* growth in fresh produce at 7°C and 15°C for six days was compared. The result showed that the population of *L. monocyogenes* increased more in 15°C over the storage period. Similar research on the effect of storage conditions on *L. monocytogenes* used three different temperatures (4°C, 12°C and 22°C) for comparison (Vandamm et al., 2013). As a result, *L. monocytogenes* was more fit to survive in higher temperatures with increased growth by about 0.3 log cfu/g in the first 17 h.

## 5.5 Conclusions

The results of this study showed that cabbages and lettuces had similar levels of total microbial loads; however, the contamination of *Listeria* spp. in lettuce was higher than in cabbage. In addition, the total microbial load of the carrot samples was higher than the cucumber samples while *Listeria* spp. were higher in the cucumber samples. Overall, all four different types of vegetable samples had similar microbiological quality regardless of the source of the market. However, some variations were observed in the samples when analysed on a weekly basis.

# Chapter 6

# Characterisation of selected *Listeria* spp. isolated from fresh produce samples collected from 3 farm types

## 6.1 Introduction

The *Listeria* genus has six species: *L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri* and *L. welshimeri* (Allerberger, 2003). Of these, *L. monocytogenes* is a type of human pathogen that can cause many disease conditions including gastroenteritis, severe blood / central nervous system infections, and miscarriages in pregnant women (Carpentier et al., 2011; Jeyaletchumi et al., 2012). As the primary pathogen, *L. monocytogenes* has not been described as much as the other members in the *Listeria* genus. However, if any species of *Listeria* is present in high levels in the food, that food will be described as having a low microbial quality (McLauchlin, 1997). *L. monocytogenes* is pathogenic. Therefore, it is important to distinguish the type of *Listeria* spp. using effective methods.

This chapter describes the characterisation and identification of selected *Listeria* isolates from the samples collected from the 3 farms. Carbohydrate utilisation, Gram staining and 3M$^{TM}$ molecular detection methods were employed.

## 6.2 Material and Methods

Material and methods used in the experimental work reported in this chapter are described in Sections 3.1, 3.2.3 to 3.2.8, 3.3 and 3.4.1 to 3.4.3.

## 6.3 Results

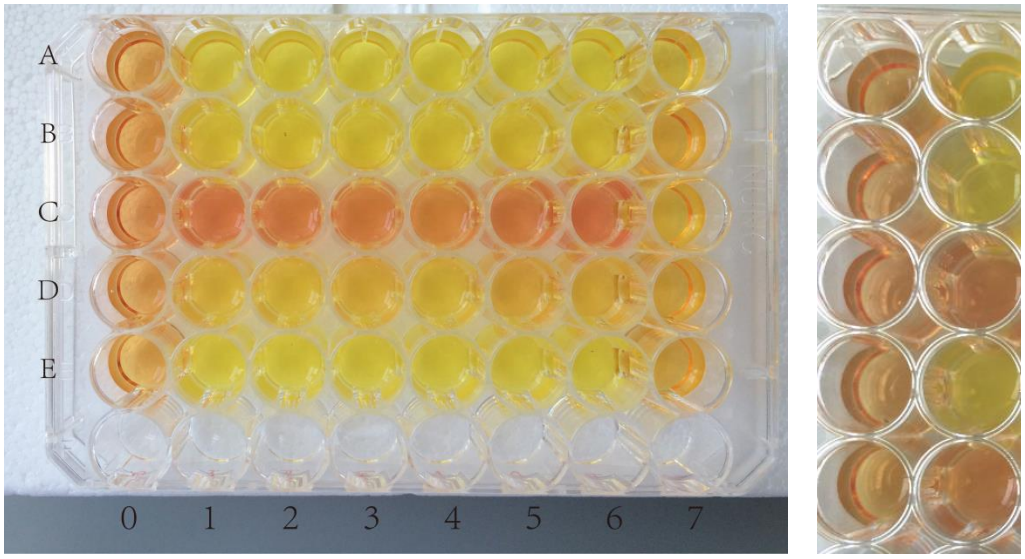
### 6.3.1 Carbohydrate utilisation

Different *Listeria* species showed differences in their ability to ferment sugars (see Table 6.1). A total of 61 different potential *Listeria* colonies were selected from the positive samples (fresh produce, soil and water) and streaked on individual agar plates. Subsequently, colonies were selected and used for carbohydrate utilisation profiling. Figure 6.1 shows a typical results action in a 48 well microplate; row 0 was a negative control. One row had no colour change and in all positive reaction wells, the

colour changed to yellow. Samples 1 to 6 in Figure 6.1 (A) were identified as *L. welshimeri*. The right row in Figure 6.1 (B) was identified as *L. monocytogenes which* was the positive control used in the assay. Thus *L. monocytogenes* was not present in any of the selected samples.

**Table 6.1 Typical sugars fermentation of profiles of various *Listeria* spp**

| Species | Glucose | Maltose | Mannitol | Rhamnose | Xylose |
|---|---|---|---|---|---|
| ***L. monocytogenes*** | + | + | - | + | - |
| ***L. grayi*** | + | + | + | - | - |
| ***L. seeligeri*** | + | + | - | - | + |
| ***L. welshimeri*** | + | + | - | V | + |
| ***L. ivanovii*** | + | + | - | - | + |
| ***L. innocua*** | + | + | - | V | - |
| **Note：+: positive -:negative V: variable** | | | | | |




**(A)** **(B)**

**Figure 6.1 An example of carbohydrate utilisation methods used in this study: (A) Colonies identified as *L. welshimeri*; and (B) typical profile of *L. monocytogenes* (positive control)**

Figure 6.2 shows the identity of sixty-one colonies of *Listeria* spp. (one from each positive sample) isolated and profiled for carbohydrate utilisation. The data suggested the presumptive distribution and identity of *Listeria* isolates: *L. welshimeri* (41%), *L. grayi* (14.8%), *L. innocua* (18%), unknown (26.2%) and *L. monocytogenes* (Nil) (Figure 6.2).

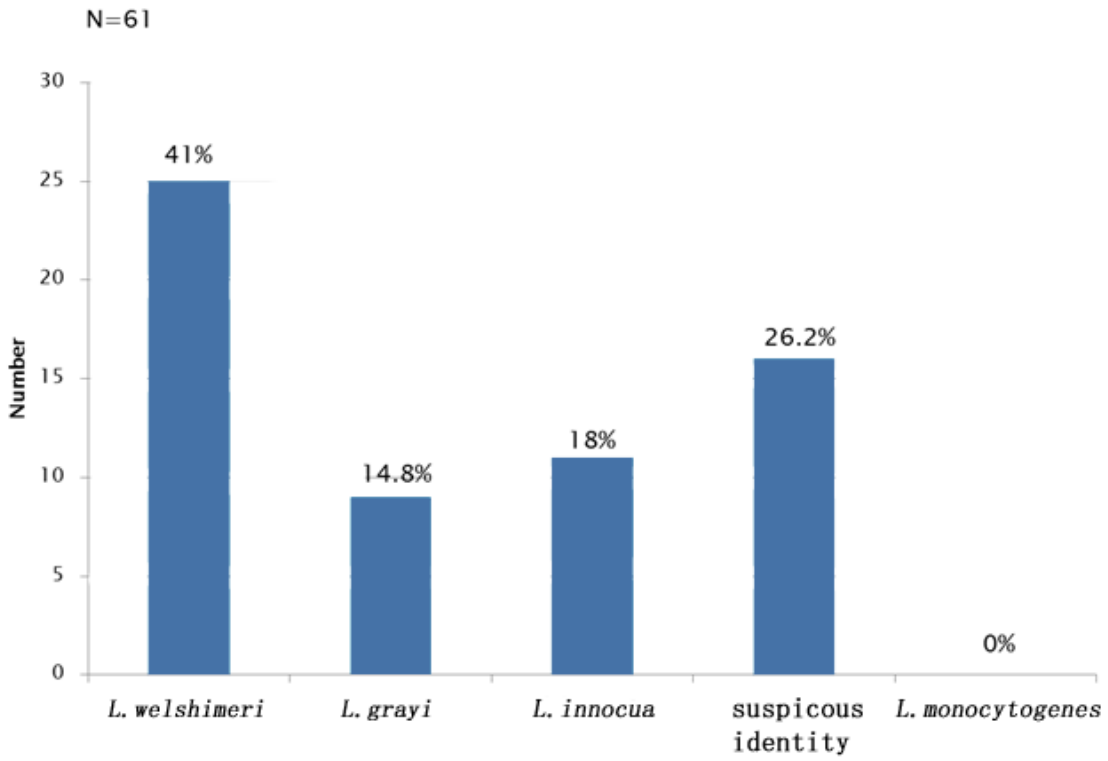
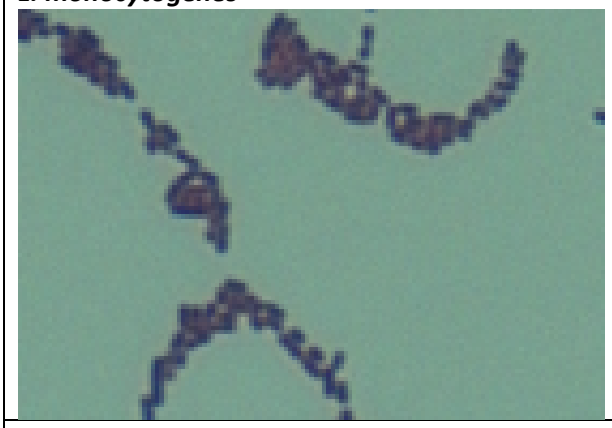
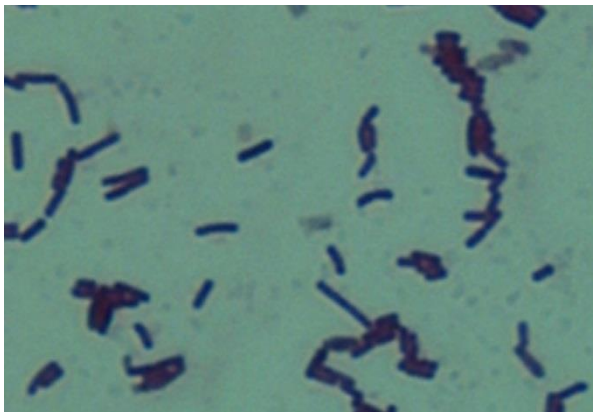



**Figure 6.2 Distribution of different *Listeria* spp. stochastically isolated positive samples using carbohydrate utilisation profiles**

### 6.3.2 Gram stain and the shape of colony

All 61 colonies used for carbohydrate utilisation profiles were also characterised for their colony and cell morphologies. Cells were Gram stained and observed under a microscope. All selected colonies appeared as purple rods, indicating Gram positive. Figure 6.3, shows rod-shaped Gram positive images of selected *Listeria* spp. The first image is for *L. monocytogenes* cells that were used as the positive control in this study. *L. monocytogenes* cells appeared as short rod-shaped bacterium. The colonies on YSTEA plates showed as semi-transparent and close to a white colour. On *Listeria* selective agar (Figure 6.4), *L. monocytogenes* showed as grey metallic lustre ball-shaped colonies with a black point in the centre, surrounded by a black halo. Compared to other species of *Listeria* (Figure 6.4A ) which were random-shaped, *L. monocytogenes* was much easier to distinguish on *Listeria*-selective agar.

| Gram strain (100x) | Colonies on TSYEA agar plate |
|---|---|
| ***L. monocytogenes***  |  |
| ***L. grayi***  |  |
| ***L. innocua***  |  |
| ***L. welshimeri***  |  |
| **unknown identity** | |

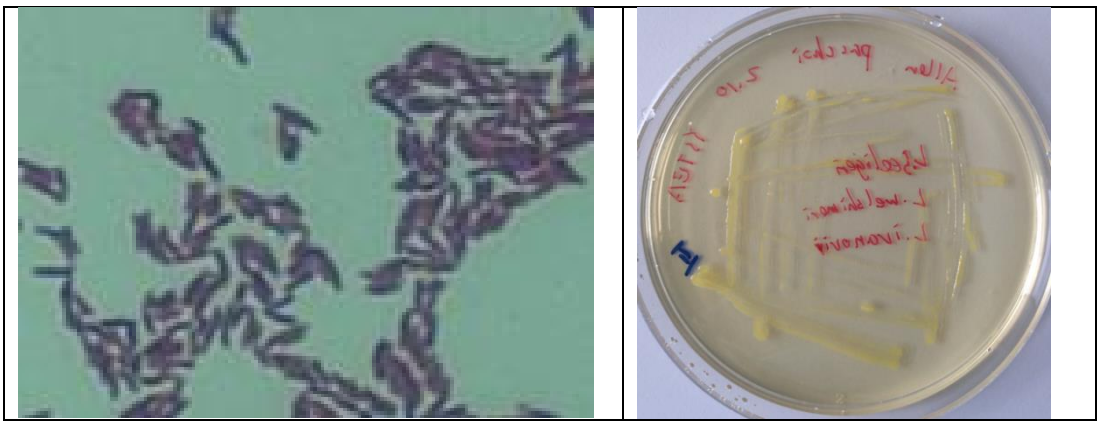
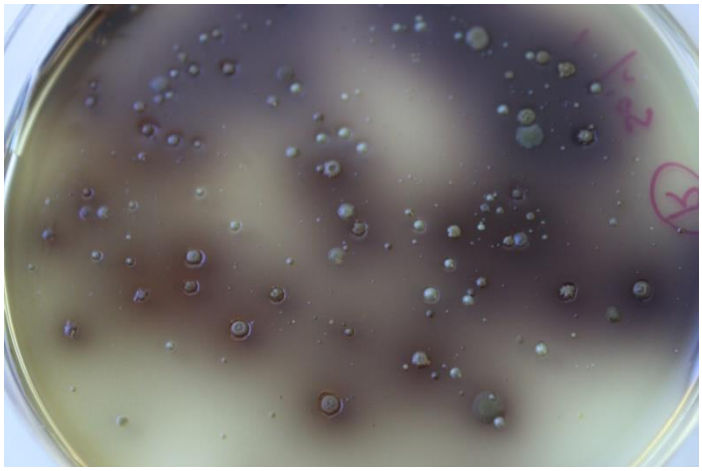
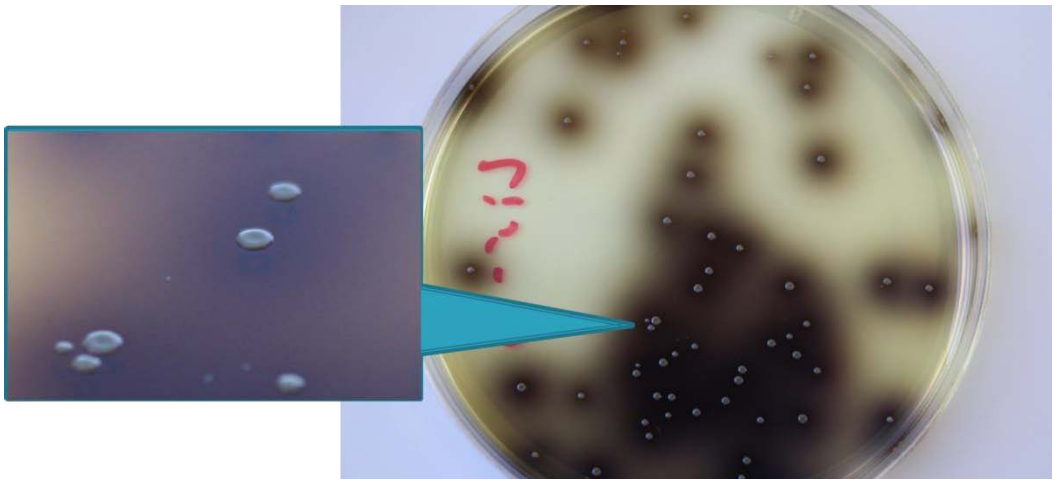


**Figure 6.3 Comparison of different *Listeria* spp. refer to cells growth on TSYEA plates**



**(A)**



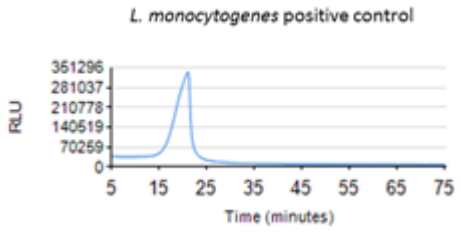
**(B)**

**Figure 6.4 Typical colonies on *Listeria* selective agar (CM0856, Oxoid Ltd); (A) *Listeria* spp. from a soil sample; and (B) a pure *L. monocytogenes* culture**

### 6.3.3 3M™ Molecular Detection

Carbohydrate utilisation profiling (Section 6.3.1) suggested that *L. monocytogenes* was not present in any of the positive samples (Chapter 4 and Chapter 5). It was important to verify these results and the 3M Molecular Detection System (MDS) was used for this purpose. Two different detection kits; one for *Listeria* spp. and one for *L. monocytogenes* were used for to specific identification. A total of forty-three samples were tested for *Listeria* spp. and forty- nine samples were tested for *L. monocytogenes* using MDS. Examples of the sample analysis outputs (positive) are shown in Figure 6.5, samples spiked with *L. monocytogenes* and tested using the *L. monocytogenes* kit (Figure 6.5A) and the *Listeria* spp. Kit (Figure 6.5B). *Listeria* spp. were detected in spring onions from Farm C (without washing), leeks from Farm B, cucumbers from Farm C and soil from Farm A and Farm B. Again, none of the samples were positive for *L. monocytogenes*. This re-confirms the identity data obtained in Section 6.3.1.

In order to check the efficiency of 3M ™ molecular detection system, known *L. monocytogenes* colonies were enriched with Demi Fraser broth and analysed. The analysis results were positive for the presence of *Listeria* spp. and *L. monocytogenes* using both kits, demonstrating the detection accuracy of MDS.

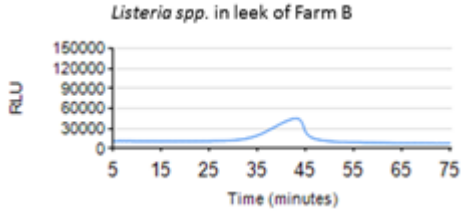



(A)


(B)

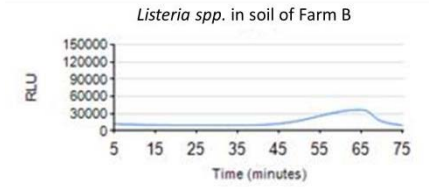

(C)


(D)

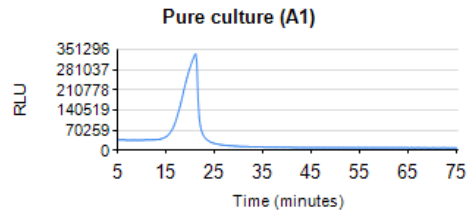



(E)

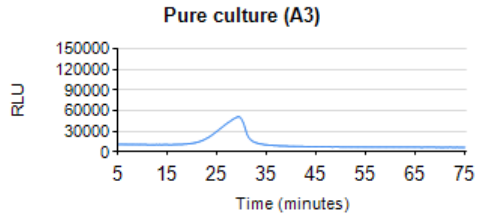

(F)




(G)




(H)




(I)

**Figure 6.5 Examples of 3M ™ Molecular Detection system output using *Listeria* spp. and *L. monocytogenes.***

## 6.4 Discussion

Gram staining and carbohydrate utilisation are the most commonly used methods, along with many others (i.e. oxidase, catalase, motility, MR-VP) to identify and characterise *Listeria* spp. (Alsheikh, 2014; Jamali et al., 2013b). However, many researchers have used the haemolysis test (Althaus et al., 2012; Olier et al., 2002)and CAMP test (Thapa et al., 2008) for *Listeria* spp. identification and used the API kit (Seo et al., 2010; Szymczak et al., 2014) for characterisation.

The haemolysis test was used to classify isolated colonies via their haemolytic properties. Based on the method described by Olier et al. (2002) , the agar plates were prepared using sheep blood agar base (Oxoid, CM 0854) with 7% sterile sheep blood added as a supplement. Isolated colonies were taken from TSYEA agar with an inoculating needle and stabbed into the sheep blood agar. Positive samples showed ß-haemolysis, which is narrow, clear and light zones after incubation at 37$^{o}$C for 24 h (Althaus et al., 2012; Olier et al., 2002). The CAMP test first was first used by a group of scientists and was named after them (i.e. CAMP = Christie, Atkins, Munch-Petersen). The CAMP test was used to analyse pathogens due to the synergistic reaction between *Staphylococcus aureus* and group B *Streptococcus* to lyse red blood cells (McKellar, 1994). As described in this paper, they used *S. aureus* and *Rhodoccocus equi* for a CAMP test of *Listeria* spp. *S. aureus* and *R. equi* were separately streaked in parallel lines across a sheep blood agar plate. The test strains were vertically streaked to the *S. aureus* and *R. equi* lines with a 1 to 2 mm gap at both sides. Positive reactions were shown as ß-haemolysis at the ends of the lines. The results showed that *L. monocytogenes* was positive to *S. aureus*, *L. ivanovii* was positive to *R. equi*, and *L. innocua* was negative to both strains after culturing at 37$^{o}$C for 18 to 24 h (Molla et al., 2004). These tests were not used in this study as *L. monocytogenes* was absent in all identified samples and so it was irrelevant to check the virulence

The API test has been used for *Listeria* spp. characterisation (Seo et al., 2010; Szymczak et al., 2014). This method combined many traditional biochemical test methods (e.g. carbohydrate utilisation and a haemolysis test) together to identify the *Listeria* species of (Bille et al., 1992). In an evaluation of the API system, there were 85% of test *Listeria* spp. strains identified at the species level. In addition, the API test can test for the presence of arylamidase to distinguish *L. monocytogenes* and *L. innocua*, which should be negative to *L. monocytogenes*. *L. monocytogenes* strains (97.7%) and 99.4% of *L. innocua* strains were tested positive correctly. Significant differences in biochemical reactions between *Listeria* spp. and the other Gram-positive bacteria tested were observed. The whole procedure of API testing can be completed within 18 to 24 h (Bille et al., 1992). In this study carbohydrate utilisation was used to identify selectively-picked *Listeria* colonies from positive samples.

PCR has been used for *Listeria* spp. detection and confirmation in many research reports (Badosa et al., 2009; Jamali et al., 2013a; Jeyaletchumi et al., 2011; Oliveira et al., 2010a; Ponniah et al., 2010). Ponniah et al. (2010) worked on *L. monocytogenes* in fresh produce used MPN-PCR to confirm *Listeria* spp. and *L. monocytogenes*. The protocol of this experiment followed the FDA-BAM Standard for enrichment, which included culturing at 30°C for 4 h for pre-enrichment before a selective agent was added (e.g. acriflavin, sodium nalidixate, and cycloheximide). Before the PCR reaction, the tubes used for MPN analysis were cultured for 44 h at 30°C. With this method, *Listeria* spp. and *L. monocytogenes* tested positive in 33.3% and 22.5% of fresh produce samples respectively in a retail shop study in Malaysia (Ponniah et al., 2010). In our study, the 3M™ molecular detection system was used because it is efficient and a cheap option to confirm the results. However in our study, *L. monocytogenes* was not detected in the *Listeria* positive samples. This method was useful and is an effective method to verify the data obtained from the carbohydrate utilisation method. However, no *L. monocytogenes* was not found in our study.

# Chapter 7

# General Discussion and Conclusion

## 7.1 General Discussion

According to microbiological limits for *Listeria* described in guidelines for the control of *L. monocytogenes* in RTE products (fresh produce such as carrots, cucumbers and lettuces ), the maximum safety level limit is 100 cfu/g (2 log cfu/g). If the level of *L. monocytogenes* contamination is over 10,000 cfu/g (5 log cfu/g), the product will be extremely dangerous as a food (MPI, 2012). So, although *L. monocytogenes* is a dangerous pathogen, fresh produce does not necessarily have to be free of it but should appear in as low levels as possible, preferably < 100 cfu/g.

In our study enumeration of total viable counts and *Listeria* spp. counts were used to evaluate microbial quality of fresh produce samples from several sources. In total, 174 vegetable samples (including 78 from farms and 94 from retail shops) were collected in this study. The analysis of samples collected showed that *Listeria* spp. was present in 79.5% of fresh produce from farms compared to 62.8% fresh produce samples from retail shops. Among the *Listeria* spp.-positive samples from the farms, 78.9% were in strong-leafy vegetables (leek, spring onion, pakchoi, silver beet, cabbage) and 96.7% in weak-leafy vegetables (lettuce, parsley, spinach, coriander). Root vegetable samples had the lowest positive rates (50%) of *Listeria* spp. detection. In retail shops, 100% of lettuce samples (weak-leafy vegetables) were positive for the presence of *Listeria* spp. Therefore, lettuce had the highest contamination of *Listeria* spp. observed in this study. This was followed by cabbage (strong leafy vegetable) and cucumber (a fruit vegetable) with *Listeria* spp. contamination percentages of 75% and 62.5%, respectively. Carrot (a root vegetable) had the lowest percentage of *Listeria* spp. present, e.g. only 16.7% of samples were positive. In general, it was noted that *Listeria* spp. prevalence has a relationship with the vegetable's structure. This study showed that the prevalence of *Listeria* spp. in weak-leafy vegetables was highest > strong-leafy vegetables > root vegetables.

Results of this study can be supported by the observations reported in recent research (Szymczak et al., 2014). These authors analysed for the prevalence of *L. monocytogenes* in three types of farmland, including natural fertilisers, artificial fertiliser and garden plots, and the crops grown in these soils. Soil in garden plots had the highest *L. monocytogenes* present with 10.6% in 47 samples. Soil in naturally fertilised land had 1.2% in 173 samples polluted by *L. monocytogenes*. No *L. monocytogenes* were present in the artificially fertilised soil. In the two paddocks contaminated by *L. monocytogenes*, 5% of parsley (weak-leafy vegetable) samples were positive. Beetroot, carrots and cabbages were not

tested for the presence of *L. monocytogenes*. These results provide evidence that *Listeria* spp. survive more easily in weak-leafy vegetables. In other research (Ponniah et al., 2010) a group of scientists compared some types of vegetables in selected local retail shops in Malaysia. As in our research, they also collected cabbages, carrots and parsley for analysis. MPN-PCR was used to detect the prevalence of *Listeria* spp. They showed the prevalence of *Listeria* spp. in carrots, cabbages, Japanese parsley and wild parsley were 24.2%, 37.2%, 39.4% and 50%, respectively. Furthermore, in other research (Jamali et al., 2013b) on the prevalence of *Listeria* spp. in salad vegetables (including cabbage, carrot, cucumber and lettuce – the same vegetable types as in our research), they also used PCR to detect the prevalence of *Listeria* spp. Each type of vegetable had 50 samples tested. The results showed that the prevalence of *Listeria* spp. in cabbage, lettuce and carrots were 6%, 12% and 0%, respectively. The prevalence of *Listeria* spp. in cucumber was 18%. These results indicate that *Listeria* spp. survived more easily in weak-leafy vegetables which is in agreement with our findings. Other evidence to support the results obtained in this study was an investigation that explored *L. monocytogenes* growth in different situations (Likotrafiti et al., 2013). These authors used lettuce leaves, parsley leaves and cucumber epidermis to research *L. monocytogenes* growth at different temperatures and relative humidities. The bacterium grew only in the cucumber epidermis. The reason for this phenomenon was that the surfaces of the leaves were intact without damage. In addition, other research found that the level of *L. monocytogenes* survival in cut celery was higher than in uncut celery samples (Vandamm et al., 2013). Therefore, it appears that it is easier for *Listeria* spp. to survive in weak-leafy vegetables due to their fragile leaf surfaces. One of the possible reason of different *Listeria* spp. contamination levels detection between leafy vegetables and root vegetables was different treatment before retailing. Root vegetables are normally washed, cleaned and treated before sale but not the leafy vegetables.

In our research, some types of vegetables were common for both farms and retail shops. Cabbages were available in Farm B, Farm C and retail shops; carrots were available in Farm C and retail shops; and lettuce available in Farm B and retail shops. These samples were compared to obtain further analysis for the prevalence of *Listeria* spp. in fresh produce from the farm to the shop (Appendix C). For the cabbage and carrot samples, there were no significant differences in the populations of *Listeria* spp. between samples from farms and shops ($p > 0.05$). However, for lettuce samples, there were significant differences between the populations of *Listeria* spp. in Farm B and the shops ($p < 0.05$). The *Listeria* spp. load in lettuce from Farm B was 1.75 log cfu/g (mean) compared to 4 log cfu/g (mean) from shops. Vegetables from farms were picked directly from the paddocks, while vegetables from the markets had gone through a long transport and storage before being purchased. *Listeria* spp. can multiply quickly during the first few days of storage when the conditions are optimal for growth (Chapter 5). In addition, lettuce belongs to weak- leafy vegetables, which provide better

conditions for *Listeria* spp. to survive (Chapters 4 and 5). From farm to shop, the transport and storage conditions are extremely complex and variable and is very difficult to monitor and control. Colonies of *Listeria* spp. were characterised and identified using Gram staining, carbohydrate utilisation and 3M™ MDS for confirmation in this study (Chapter 6). This study used NUNCLON™ 48-well microplates instead of traditional fermentation tubes. The benefits of this method were: less reagent usage; small inoculum size; and less space used. However, gas production was not possible to record in this modified method. Moreover, for *Listeria* identification, gas production is not a necessary test. This study also used another method to detect *Listeria* spp.; this was the 3M™ molecular detection system, which is sensitive and reliable (Chapter 6). 3M™ MDS is a relatively new amplification technology which combines PCR and continuous luminometry. Primers labelled with inorganic pyrophosphate were released due to positive amplification, and joined to form ATP. The ATP was then detected by the luciferin–luciferase system. All these reactions were carried out in small tubes and within 75 min. Most positive sample had a luminometric peak after approximately 20 min (Kuchta et al., 2014). This technique has been used for food pathogen detection (Crowley, 2013; Fortes et al., 2013, 2013; Vongkamjan, 2013). This technique was successfully applied in this study to verify the *Listeria* spp. identification results.

No listeriosis outbreaks associated with fresh produce have been reported in New Zealand so far. Although *Listeria* spp. were detected in fresh produce using *Listeria* selective agar, no bacterial colony was identified as *L. monocytogenes* in this research. The results of 3M™ MDS also re-confirmed these observations that L. monocytogenes was absent in the samples used in this research.

## 7.2 Concluding remarks

*L. monocytogenes* is a member of *Listeria* spp. and a human pathogen. In this study enumeration of *Listeria* spp. present in selected fresh produce samples from different farms and retail shops was carried out and reported as log cfu/g. Cabbages, carrots, cucumbers and lettuces were routinely sampled from retail shops to check the prevalence of *Listeria* spp. Several kinds of vegetables were collected from farms. Due to changing seasons, it was difficult to have the same range of vegetables samples available from each farm at each sampling day. This research can be used a reference study, which is a preliminary evaluation of the level of *Listeria* spp. present in fresh vegetable samples collected from three different farms in the Canterbury region. However, in this research it was difficult to pinpoint the source of the *Listeria* spp. in the fresh produce. Many potential factors can influence the contamination of fresh vegetables by *Listeria* spp. in the food supply chain, i.e. from farm to fork. Therefore, samples common to all types of farms and markets may be collected from markets and farms that are suppliers for these markets. Soil samples from vegetable growing areas

should also be analysed.  This will provide stronger evidence to prove the influence of the growing environment and transport lines on the prevalence of *Listeria* spp. .

To conclude, this is the first research undertaken for the isolation and confirmation of *Listeria* spp. from soil and fresh produce in New Zealand. These results provided valuable data on the quality of fresh vegetables with respect to *Listeria* spp.. *Listeria* spp. are present ubiquitous in the agricultural environment. However, fresh produce contaminated by *Listeria* spp. were at much lower levels than in meat and dairy products so far. Although there were no *L. monocytogenes* detected in this research, the potential hazards for contaminating fresh produce by this bacterium still existed. Therefore, it is necessary to keep monitoring the occurrence of *Listeria* spp. in fresh produce from the farms to the markets.

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# Appendix A

# One-way ANOVA analysis (Retail shop) by Minitab

## A.1 Comparison of APC and *Listeria* spp. for cabbage in Retail shops

**APC**

### One-way ANOVA: log cfu/g versus Market

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
Market 4 A, B, C, D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Market 3 0.7617 0.2539 1.80 0.179
Error 20 2.8148 0.1407
Total 23 3.5766

Model Summary
S R-sq R-sq(adj) R-sq(pred)
0.375157 21.30% 9.49% 0.00%

Means
Market N Mean StDev 95% CI
A 6 6.818 0.459 ( 6.499, 7.138)
B 6 6.977 0.448 ( 6.657, 7.296)
C 6 6.5050 0.2153 (6.1855, 6.8245)
D 6 6.892 0.325 ( 6.572, 7.211)
Pooled StDev = 0.375157

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Market N Mean Grouping
B 6 6.977 A
D 6 6.892 A
A 6 6.818 A
C 6 6.5050 A
Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus Market

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
Market 4 A, B, C, D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Market 3 9.611 3.204 1.31 0.300
Error 20 49.023 2.451
Total 23 58.634

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.56562 16.39% 3.85% 0.00%

Means
Market N Mean StDev 95% CI
A 6 1.515 1.675 (0.182, 2.848)
B 6 2.643 1.501 (1.310, 3.977)
C 6 3.262 0.507 (1.928, 4.595)
D 6 2.683 2.119 (1.350, 4.017)
Pooled StDev = 1.56562

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Market N Mean Grouping
C 6 3.262 A
D 6 2.683 A
B 6 2.643 A
A 6 1.515 A
Means that do not share a letter are significantly different.

## A.2 Comparison of APC and *Listeria* spp. for carrot in Retail shops

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus Market** | **One-way ANOVA: log cfu/g versus Market** |
| Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Market 4 A, B, C, D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Market 3 2.231 0.7436 1.37 0.281<br>Error 20 10.853 0.5427<br>Total 23 13.084<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.736663 17.05% 4.61% 0.00%<br><br>Means<br>Market N Mean StDev 95% CI<br>A 6 6.722 0.875 (6.094, 7.349)<br>B 6 5.902 0.822 (5.274, 6.529)<br>C 6 6.303 0.629 (5.676, 6.931)<br>D 6 6.527 0.578 (5.899, 7.154)<br>Pooled StDev = 0.736663 | Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Market 4 A, B, C, D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Market 3 3.392 1.131 0.56 0.646<br>Error 20 40.145 2.007<br>Total 23 43.537<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.41678 7.79% 0.00% 0.00%<br><br>Means<br>Market N Mean StDev 95% CI<br>A 6 0.557 1.364 (-0.650, 1.763)<br>B 6 1.015 1.592 (-0.192, 2.222)<br>C 6 0.778 1.907 (-0.428, 1.985)<br>D 6 0 0 (-1.207, 1.207)<br>Pooled StDev = 1.41678 |
| **Tukey Pairwise Comparisons** | **Tukey Pairwise Comparisons** |
| Grouping Information Using the Tukey Method and 95% Confidence<br>Market N Mean Grouping<br>A 6 6.722 A<br>D 6 6.527 A<br>C 6 6.303 A<br>B 6 5.902 A<br><br>Means that do not share a letter are significantly different. | Grouping Information Using the Tukey Method and 95% Confidence<br>Market N Mean Grouping<br>B 6 1.015 A<br>C 6 0.778 A<br>A 6 0.557 A<br>D 6 0.000000 A<br><br>Means that do not share a letter are significantly different. |

## A.3 Comparison of APC and *Listeria* spp. for cucumber in Retail shops

**APC**

### One-way ANOVA: log cfu/g versus Market

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.
Factor Information
Factor Levels Values
Market 4 A, B, C, D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Market 3 27.43 9.145 1.76 0.187
Error 20 103.88 5.194
Total 23 131.32

Model Summary
S R-sq R-sq(adj) R-sq(pred)
2.27907 20.89% 9.03% 0.00%

Means
Market N Mean StDev 95% CI
A 6 4.333 2.355 (2.392, 6.274)
B 6 2.72 3.02 ( 0.78, 4.66)
C 6 5.708 0.931 (3.767, 7.649)
D 6 4.605 2.292 (2.664, 6.546)

Pooled StDev = 2.27907

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Market N Mean Grouping
C 6 5.708 A
D 6 4.605 A
A 6 4.333 A
B 6 2.72 A

Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus Market

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
Market 4 A, B, C, D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Market 3 9.877 3.292 0.94 0.439
Error 20 69.920 3.496
Total 23 79.797

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.86976 12.38% 0.00% 0.00%

Means
Market N Mean StDev 95% CI
A 6 3.200 1.867 ( 1.608, 4.792)
B 6 1.517 1.714 (-0.076, 3.109)
C 6 2.342 1.882 ( 0.749, 3.934)
D 6 1.800 2.005 ( 0.208, 3.392)

Pooled StDev = 1.86976

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Market N Mean Grouping
A 6 3.200 A
C 6 2.342 A
D 6 1.800 A
B 6 1.517 A

Means that do not share a letter are significantly different.

## A.4 Comparison of APC and *Listeria* spp. for lettuce in Retail shops

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus Market**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br>Factor Information<br>Factor Levels Values<br>Market 4 A, B, C, D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Market 3 0.4789 0.1596 0.58 0.635<br>Error 20 5.5089 0.2754<br>Total 23 5.9879<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.524831 8.00% 0.00% 0.00%<br><br>Means<br>Market N Mean StDev 95% CI<br>A 6 7.113 0.476 (6.666, 7.560)<br>B 6 6.950 0.465 (6.503, 7.397)<br>C 6 6.728 0.577 (6.281, 7.175)<br>D 6 7.013 0.571 (6.566, 7.460)<br>Pooled StDev = 0.524831<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>Market N Mean Grouping<br>A 6 7.113 A<br>D 6 7.013 A<br>B 6 6.950 A<br>C 6 6.728 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus Market**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Market 4 A, B, C, D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Market 3 0.3397 0.1132 0.16 0.921<br>Error 20 13.9830 0.6992<br>Total 23 14.3228<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.836153 2.37% 0.00% 0.00%<br><br>Means<br>Market N Mean StDev 95% CI<br>A 6 4.388 0.786 (3.676, 5.100)<br>B 6 4.093 0.953 (3.381, 4.805)<br>C 6 4.105 0.530 (3.393, 4.817)<br>D 6 4.228 0.995 (3.516, 4.940)<br>Pooled StDev = 0.836153<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>Market N Mean Grouping<br>A 6 4.388 A<br>D 6 4.228 A<br>C 6 4.105 A<br>B 6 4.093 A<br>Means that do not share a letter are significantly different. |

## A.5 Comparison of APC and *Listeria* spp. for fresh vegetables in Market A

**APC**

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 4 cabbage, carrot, cucumber, lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 3 29.79 9.929 5.88 0.005
Error 20 33.75 1.688
Total 23 63.54

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.29905 46.88% 38.91% 23.51%

Means
sample N Mean StDev 95% CI
cabbage 6 6.818 0.459 (5.712, 7.925)
carrot 6 6.722 0.875 (5.615, 7.828)
cucumber 6 4.333 2.355 (3.227, 5.440)
lettuce 6 7.113 0.476 (6.007, 8.220)

Pooled StDev = 1.29905

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
lettuce 6 7.113 A
cabbage 6 6.818 A
carrot 6 6.722 A
cucumber 6 4.333 B

Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 4 cabbage, carrot, cucumber, lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 3 52.62 17.540 8.01 0.001
Error 20 43.81 2.190
Total 23 96.43

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.47999 54.57% 47.75% 34.58%

Means
sample N Mean StDev 95% CI
cabbage 6 1.516 1.676 ( 0.256, 2.777)
carrot 6 0.557 1.363 (-0.704, 1.817)
cucumber 6 3.200 1.865 ( 1.940, 4.460)
lettuce 6 4.388 0.785 ( 3.128, 5.648)

Pooled StDev = 1.47999

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
lettuce 6 4.388 A
cucumber 6 3.200 A B
cabbage 6 1.516 B C
carrot 6 0.557 C

Means that do not share a letter are significantly different.

## A.6 Comparison of APC and *Listeria* spp. for fresh vegetables in Market B

| **APC** | ***Listeria*** spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus sample** | **One-way ANOVA: log cfu/g versus sample** |
| Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>sample 4 cabbage, carrot, cucumber, lettuce<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>sample 3 72.64 24.215 9.50 0.000<br>Error 20 51.00 2.550<br>Total 23 123.65<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.59693 58.75% 52.56% 40.60%<br><br>Means<br>sample N Mean StDev 95% CI<br>cabbage 6 6.977 0.448 (5.617, 8.337)<br>carrot 6 5.902 0.822 (4.542, 7.262)<br>cucumber 6 2.72 3.02 ( 1.36, 4.08)<br>lettuce 6 6.950 0.465 (5.590, 8.310)<br><br>Pooled StDev = 1.59693 | Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>sample 4 cabbage, carrot, cucumber, lettuce<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>sample 3 33.59 11.197 5.19 0.008<br>Error 20 43.16 2.158<br>Total 23 76.76<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.46909 43.76% 35.33% 19.02%<br><br>Means<br>sample N Mean StDev 95% CI<br>cabbage 6 2.646 1.501 ( 1.395, 3.897)<br>carrot 6 1.015 1.591 (-0.236, 2.266)<br>cucumber 6 1.518 1.715 ( 0.266, 2.769)<br>lettuce 6 4.093 0.952 ( 2.842, 5.344)<br><br>Pooled StDev = 1.46909 |
| **Tukey Pairwise Comparisons** | **Tukey Pairwise Comparisons** |
| Grouping Information Using the Tukey Method and 95% Confidence<br><br>sample N Mean Grouping<br>cabbage 6 6.977 A<br>lettuce 6 6.950 A<br>carrot 6 5.902 A<br>cucumber 6 2.72 B<br><br>Means that do not share a letter are significantly different. | Grouping Information Using the Tukey Method and 95% Confidence<br><br>sample N Mean Grouping<br>lettuce 6 4.093 A<br>cabbage 6 2.646 A B<br>cucumber 6 1.518 B<br>carrot 6 1.015 B<br><br>Means that do not share a letter are significantly different. |

## A.7 Comparison of APC and *Listeria* spp. for fresh vegetables in Market C

| **APC** | ***Listeria*** spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus sample** | **One-way ANOVA: log cfu/g versus sample** |
| Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>sample 4 cabbage, carrot, cucumber, lettuce<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>sample 3 3.450 1.1501 2.80 0.066<br>Error 20 8.208 0.4104<br>Total 23 11.658<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.640611 29.60% 19.04% 0.00%<br><br>Means<br>sample N Mean StDev 95% CI<br>cabbage 6 6.5050 0.2153 (5.9595, 7.0505)<br>carrot 6 6.303 0.629 ( 5.758, 6.849)<br>cucumber 6 5.708 0.931 ( 5.163, 6.254)<br>lettuce 6 6.728 0.577 ( 6.183, 7.274)<br><br>Pooled StDev = 0.640611 | Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>sample 4 cabbage, carrot, cucumber, lettuce<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>sample 3 36.54 12.181 6.32 0.003<br>Error 20 38.53 1.927<br>Total 23 75.07<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.38800 48.68% 40.98% 26.09%<br><br>Means<br>sample N Mean StDev 95% CI<br>cabbage 6 3.261 0.507 ( 2.079, 4.443)<br>carrot 6 0.778 1.905 (-0.404, 1.960)<br>cucumber 6 2.342 1.882 ( 1.160, 3.524)<br>lettuce 6 4.106 0.530 ( 2.924, 5.288)<br><br>Pooled StDev = 1.38800 |
| **Tukey Pairwise Comparisons** | **Tukey Pairwise Comparisons** |
| Grouping Information Using the Tukey Method and 95% Confidence<br><br>sample N Mean Grouping<br>lettuce 6 6.728 A<br>cabbage 6 6.5050 A<br>carrot 6 6.303 A<br>cucumber 6 5.708 A<br><br>Means that do not share a letter are significantly different. | Grouping Information Using the Tukey Method and 95% Confidence<br><br>sample N Mean Grouping<br>lettuce 6 4.106 A<br>cabbage 6 3.261 A<br>cucumber 6 2.342 A B<br>carrot 6 0.778 B<br><br>Means that do not share a letter are significantly different. |

## A.8 Comparison of APC and *Listeria* spp. for fresh vegetables in Market D

**APC**

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 4 cabbage, carrot, cucumber, lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 3 22.66 7.553 5.02 0.009
Error 20 30.10 1.505
Total 23 52.76

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.22677 42.95% 34.39% 17.85%

Means
sample N Mean StDev 95% CI
cabbage 6 6.892 0.325 (5.847, 7.936)
carrot 6 6.527 0.578 (5.482, 7.571)
cucumber 6 4.605 2.292 (3.560, 5.650)
lettuce 6 7.013 0.571 (5.969, 8.058)

Pooled StDev = 1.22677

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
lettuce 6 7.013 A
cabbage 6 6.892 A
carrot 6 6.527 A B
cucumber 6 4.605 B

Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 4 cabbage, carrot, cucumber, lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 3 56.07 18.689 7.87 0.001
Error 20 47.51 2.375
Total 23 103.58

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.54126 54.13% 47.25% 33.95%

Means
sample N Mean StDev 95% CI
cabbage 6 2.684 2.120 (1.372,3.997)
carrot 6 0 0 (-1.313,1.313)
cucumber 6 1.799 2.004 (0.486, 3.111)
lettuce 6 4.228 0.996 ( 2.915,5.540)

Pooled StDev = 1.54126

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
lettuce 6 4.228 A
cabbage 6 2.684 A
cucumber 6 1.799 A B
carrot 6 0.000000 B

Means that do not share a letter are significantly different.

## A.9 Total Comparison of APC and *Listeria* spp. for all fresh produce in Retail shops

**APC**

### One-way ANOVA: cfu/g versus from

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
from 16 A cabbage, A carrot, A cucumber, A lettuce, B cabbage, B carrot, B cucumber, B lettuce, C cabbage, C carrot, C cucumber, C lettuce, D cabbage, D carrot, D cucumber, D lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
from 15 135.9 9.058 5.89 0.000
Error 80 123.1 1.538
Total 95 258.9

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.24027 52.47% 43.56% 31.56%

Means
from N Mean StDev 95% CI
A cabbage 6 6.818 0.459 ( 5.811, 7.826)
A carrot 6 6.722 0.875 ( 5.714, 7.729)
A cucumber 6 4.333 2.355 ( 3.326, 5.341)
A lettuce 6 7.113 0.476 ( 6.106, 8.121)
B cabbage 6 6.977 0.448 ( 5.969, 7.984)
B carrot 6 5.902 0.822 ( 4.894, 6.909)
B cucumber 6 2.72 3.02 ( 1.71, 3.73)
B lettuce 6 6.950 0.465 ( 5.942, 7.958)
C cabbage 6 6.5050 0.2153 (5.4974, 7.5126)
C carrot 6 6.303 0.629 ( 5.296, 7.311)
C cucumber 6 5.708 0.931 ( 4.701, 6.716)
C lettuce 6 6.728 0.577 ( 5.721, 7.736)
D cabbage 6 6.892 0.325 ( 5.884, 7.899)
D carrot 6 6.527 0.578 ( 5.519, 7.534)
D cucumber 6 4.605 2.292 ( 3.597, 5.613)
D lettuce 6 7.013 0.571 ( 6.006, 8.021)
Pooled StDev = 1.24027

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
from N Mean Grouping
A lettuce 6 7.113 A
D lettuce 6 7.013 A
B cabbage 6 6.977 A
B lettuce 6 6.950 A

***Listeria* spp.**

### One-way ANOVA: log cfu/g versus from

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
From 16 A cabbage, A carrot, A cucumber, A lettuce, B cabbage, B carrot, B cucumber, B lettuce, C cabbage, C carrot, C cucumber, C lettuce, D cabbage, D carrot, D cucumber, D lettuce

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
From 15 181.3 12.088 5.59 0.000
Error 80 173.1 2.163
Total 95 354.4

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.47085 51.16% 42.01% 29.68%

Means
From N Mean StDev 95% CI
A cabbage 6 1.515 1.675 ( -0.320, 2.710)
A carrot 6 0.557 1.364 ( -0.638, 1.752)
A cucumber 6 3.2 1.867 ( 2.005, 4.395)
A lettuce 6 4.388 0.786 ( 3.193, 5.583)
B cabbage 6 2.643 1.501 ( 1.448, 3.838)
B carrot 6 1.015 1.592 ( -0.180, 2.210)
B cucumber 6 1.517 1.714 ( 0.322, 2.712)
B lettuce 6 4.093 0.953 ( 2.898, 5.288)
C cabbage 6 3.262 0.507 ( 2.067, 4.457)
C carrot 6 0.778 1.907 ( -0.417, 1.973)
C cucumber 6 2.342 1.882 ( 1.147, 3.537)
C lettuce 6 4.105 0.53 ( 2.910, 5.300)
D cabbage 6 2.683 2.119 ( 1.488, 3.878)
D carrot 6 0 0 (-1.195, 1.195)
D cucumber 6 1.8 2.005 ( 0.605, 2.995)
D lettuce 6 4.228 0.995 ( 3.033, 5.423)
Pooled StDev = 1.47085

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
From N Mean Grouping
A lettuce 6 4.388 A
D lettuce 6 4.228 A
C lettuce 6 4.105 A
B lettuce 6 4.093 A

| | | | |
|---|---|---|---|
| D cabbage | 6 | 6.892 | A |
| A cabbage | 6 | 6.818 | A B |
| C lettuce | 6 | 6.728 | A B |
| A carrot | 6 | 6.722 | A B |
| D carrot | 6 | 6.527 | A B |
| C cabbage | 6 | 6.5050 | A B |
| C carrot | 6 | 6.303 | A B |
| B carrot | 6 | 5.902 | A B |
| C cucumber | 6 | 5.708 | A B |
| D cucumber | 6 | 4.605 | A B C |
| A cucumber | 6 | 4.333 | B C |
| B cucumber | 6 | 2.72 | C |

Means that do not share a letter are significantly different.

| | | | |
|---|---|---|---|
| C cabbage | 6 | 3.262 | A B |
| A cucumber | 6 | 3.200 | A B |
| D cabbage | 6 | 2.683 | A B C |
| B cabbage | 6 | 2.643 | A B C |
| C cucumber | 6 | 2.342 | A B C |
| D cucumber | 6 | 1.800 | A B C |
| B cucumber | 6 | 1.517 | A B C |
| A cabbage | 6 | 1.515 | A B C |
| B carrot | 6 | 1.015 | B C |
| C carrot | 6 | 0.778 | B C |
| A carrot | 6 | 0.557 | B C |
| D carrot | 6 | 0.000000 | C |

Means that do not share a letter are significantly different.

# Appendix B

# One-way ANOVA analysis (farms) by Minitab

## B.1 Comparison of APC and *Listeria* spp. for fresh vegetables in Farm A

**APC**

### One-way ANOVA: log cfu/g versus Sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
Sample 5 leek, pakchoi, radish, siliver beet, spring onion

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Farm 4 52.63 13.156 4.00 0.014
Error 21 69.06 3.289
Total 25 121.69

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.81344 43.25% 32.44% 18.21%

Means
Sample N Mean StDev 95% CI
leek 6 5.933 0.475 (4.394, 7.473)
pakchoi 6 6.033 0.706 (4.494, 7.573)
radish 6 2.61 2.88 ( 1.07, 4.15)
siliver beet 2 6.435 0.177 (3.768, 9.102)
spring onion 6 4.265 2.191 (2.725, 5.805)
Pooled StDev = 1.81344

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Sample N Mean Grouping
siliver beet 2 6.435 A B
pakchoi 6 6.033 A
leek 6 5.933 A
spring onion 6 4.265 A B
radish 6 2.61 B
Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus Sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
Sample 5 leek, pakchoi, radish, siliver beet, spring onion

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Sample 4 28.68 7.169 3.07 0.039
Error 21 49.06 2.336
Total 25 77.73

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.52842 36.89% 24.87% 8.27%

Means
Sample N Mean StDev 95% CI
leek 6 2.907 1.441 ( 1.609, 4.204)
pakchoi 6 2.597 1.319 ( 1.299, 3.894)
radish 6 1.112 1.727 (-0.186, 2.409)
siliver beet 2 4.960 0.509 ( 2.712, 7.208)
spring onion 6 1.553 1.721 ( 0.256, 2.851)
Pooled StDev = 1.52842

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
Sample N Mean Grouping
siliver beet 2 4.960 A
leek 6 2.907 A B
pakchoi 6 2.597 A B
spring onion 6 1.553 A B
radish 6 1.112 B
Means that do not share a letter are significantly different.

## B.2 Comparison of APC and *Listeria* spp. for fresh vegetables in Farm B

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus Sample** | **One-way ANOVA: log cfu/g versus Sample** |
| Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Sample 6 cabbage, lettuce, pakchoi, parsley, sliver beet, spinach<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Sample 5 1.987 0.3973 0.79 0.571<br>Error 22 11.133 0.5061<br>Total 27 13.120<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.711382 15.14% 0.00% 0.00%<br><br>Means<br>Sample N Mean StDev 95% CI<br>cabbage 6 6.527 0.977 (5.924, 7.129)<br>lettuce 3 6.340 0.757 (5.488, 7.192)<br>pakchoi 5 6.768 0.259 (6.108, 7.428)<br>parsley 6 6.990 0.654 (6.388, 7.592)<br>sliver beet 2 7.370 0.141 (6.327, 8.413)<br>spinach 6 6.880 0.747 (6.278, 7.482)<br>Pooled StDev = 0.711382 | Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Sample 6 cabbage, lettuce, pakchoi, parsley, sliver beet, spinach<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Sample 5 18.43 3.686 1.76 0.162<br>Error 22 46.00 2.091<br>Total 27 64.42<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.44594 28.60% 12.38% 0.00%<br><br>Means<br>Sample N Mean StDev 95% CI<br>cabbage 6 2.530 2.050 (1.306, 3.754)<br>lettuce 3 1.753 1.519 (0.022, 3.485)<br>pakchoi 5 3.274 1.987 (1.933, 4.615)<br>parsley 6 3.987 0.723 (2.762, 5.211)<br>sliver beet 2 4.630 0.297 (2.510, 6.750)<br>spinach 6 3.770 0.613 (2.546, 4.994)<br>Pooled StDev = 1.44594 |
| **Tukey Pairwise Comparisons** | **Tukey Pairwise Comparisons** |
| Grouping Information Using the Tukey Method and 95% Confidence<br>Sample N Mean Grouping<br>sliver beet 2 7.370 A<br>parsley 6 6.990 A<br>spinach 6 6.880 A<br>pakchoi 5 6.768 A<br>cabbage 6 6.527 A<br>lettuce 3 6.340 A<br>Means that do not share a letter are significantly different. | Grouping Information Using the Tukey Method and 95% Confidence<br>Sample N Mean Grouping<br>sliver beet 2 4.630 A<br>parsley 6 3.987 A<br>spinach 6 3.770 A<br>pakchoi 5 3.274 A<br>cabbage 6 2.530 A<br>lettuce 3 1.753 A<br>Means that do not share a letter are significantly different. |

## B.3 Comparison of APC and *Listeria* spp. for fresh vegetables in Farm C

**APC**

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 6 cabbage, carrot, coriander, leek, parsley, spinach

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 5 22.50 4.501 2.04 0.130
Error 15 33.03 2.202
Total 20 55.54

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.48399 40.52% 20.69% *

Means
sample N Mean StDev 95% CI
cabbage 4 7.265 0.570 ( 5.683, 8.847)
carrot 4 4.71 3.19 ( 3.13, 6.29)
coriander 5 7.306 0.548 ( 5.891, 8.721)
leek 1 7.360 * ( 4.197, 10.523)
parsley 2 7.444 0.402 ( 5.208, 9.681)
spinach 5 7.4040 0.2080 (5.9894, 8.8186)

Pooled StDev = 1.48399

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
parsley 2 7.444 A
spinach 5 7.4040 A
leek 1 7.360 A
coriander 5 7.306 A
cabbage 4 7.265 A
carrot 4 4.71 A

Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 6 cabbage, carrot, coriander, leek, parsley, spinach

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 5 54.601 10.9201 20.93 0.000
Error 17 8.870 0.5218
Total 22 63.471

Model Summary
S R-sq R-sq(adj) R-sq(pred)
0.722329 86.03% 81.91% *

Means
sample N Mean StDev 95% CI
cabbage 4 4.438 0.329 ( 3.676, 5.199)
carrot 4 0.657 1.315 (-0.104, 1.419)
coriander 5 3.092 0.399 ( 2.410, 3.774)
leek 1 2.650 * ( 1.126, 4.174)
parsley 4 4.510 0.207 ( 3.748, 5.272)
spinach 5 5.128 0.805 ( 4.446, 5.810)

Pooled StDev = 0.722329

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
spinach 5 5.128 A
parsley 4 4.510 A B
cabbage 4 4.438 A B
coriander 5 3.092 B
leek 1 2.650 A B C
carrot 4 0.657 C

Means that do not share a letter are significantly different.

## B.4 Total Comparison of APC and *Listeria* spp. for all fresh produce in farms

**APC**

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 17 A leek, A pakchoi, A radish, A sliver beet, A spring onion, B cabbage, B lettuce, B pakchoi, B parsley, B sliver beet, B spinach, C cabbage, C carrot, C coriander, C leek, C parsley, C spinach

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 16 149.6 9.350 4.94 0.000
Error 60 113.6 1.893
Total 76 263.2

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.37589 56.84% 45.33% *

Means
sample N Mean StDev 95% CI
A leek 6 5.933 0.475 (4.810, 7.057)
A pakchoi 6 6.033 0.706 (4.910, 7.157)
A radish 6 2.61 2.88 ( 1.49, 3.73)
A sliver beet 2 6.435 0.177 (4.489, 8.381)
A spring onion 6 4.265 2.191 (3.141, 5.389)
B cabbage 6 6.527 0.977 (5.403, 7.650)
B lettuce 3 6.340 0.757 (4.751, 7.929)
B pakchoi 5 6.768 0.259 (5.537, 7.999)
B parsley 6 6.990 0.654 (5.866, 8.114)
B sliver beet 2 7.370 0.141 (5.424, 9.316)
B spinach 6 6.880 0.747 (5.756, 8.004)
C cabbage 4 7.265 0.570 (5.889, 8.641)
C carrot 4 4.71 3.19 ( 3.34, 6.09)
C coriander 5 7.306 0.548 (6.075, 8.537)
C leek 1 7.360 * (4.608, 10.112)
C parsley 4 7.739 0.416 (6.363, 9.115)
C spinach 5 7.4040 0.2080 (6.1732, 8.6348)

Pooled StDev = 1.37589

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
C parsley 4 7.739 A

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus sample

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
sample 17 A leek, A pakchoi, A radish, A sliver beet, A spring onion, B cabbage, B lettuce, B pakchoi, B parsley, B sliver beet, B spinach, C cabbage, C carrot, C coriander, C leek, C parsley, C spinach

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
sample 16 125.8 7.862 4.54 0.000
Error 60 103.9 1.732
Total 76 229.7

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.31608 54.76% 42.69% *

Means
sample N Mean StDev 95% CI
A leek 6 2.907 1.441 ( 1.832, 3.981)
A pakchoi 6 2.597 1.319 ( 1.522, 3.671)
A radish 6 1.112 1.727 ( 0.037, 2.186)
A sliver beet 2 4.960 0.509 ( 3.099, 6.821)
A spring onion 6 1.553 1.721 ( 0.479, 2.628)
B cabbage 6 2.530 2.050 ( 1.455, 3.605)
B lettuce 3 1.753 1.519 ( 0.233, 3.273)
B pakchoi 5 3.274 1.987 ( 2.097, 4.451)
B parsley 6 3.987 0.723 ( 2.912, 5.061)
B sliver beet 2 4.630 0.297 ( 2.769, 6.491)
B spinach 6 3.770 0.613 ( 2.695, 4.845)
C cabbage 4 4.438 0.329 ( 3.121, 5.754)
C carrot 4 0.657 1.315 (-0.659, 1.974)
C coriander 5 3.092 0.399 ( 1.915, 4.269)
C leek 1 2.650 * ( 0.017, 5.283)
C parsley 4 4.510 0.207 ( 3.194, 5.826)
C spinach 5 5.128 0.805 ( 3.951, 6.305)

Pooled StDev = 1.31608

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample N Mean Grouping
C spinach 5 5.128 A

| | | | |
|---|---|---|---|
| C spinach | 5 | 7.4040 | A |
| B sliver beet | 2 | 7.370 | A B |
| C leek | 1 | 7.360 | A B C D |
| C coriander | 5 | 7.306 | A |
| C cabbage | 4 | 7.265 | A B |
| B parsley | 6 | 6.990 | A B |
| B spinach | 6 | 6.880 | A B |
| B pakchoi | 5 | 6.768 | A B |
| B cabbage | 6 | 6.527 | A B |
| A sliver beet | 2 | 6.435 | A B C D |
| B lettuce | 3 | 6.340 | A B |
| A pakchoi | 6 | 6.033 | A B |
| A leek | 6 | 5.933 | A B |
| C carrot | 4 | 4.71 | A B C D |
| A spring onion | 6 | 4.265 | B D |
| A radish | 6 | 2.61 | C D |

Means that do not share a letter are significantly different.

| | | | |
|---|---|---|---|
| A sliver beet | 2 | 4.960 | A B C |
| B sliver beet | 2 | 4.630 | A B C D E |
| C parsley | 4 | 4.510 | A B |
| C cabbage | 4 | 4.438 | A B |
| B parsley | 6 | 3.987 | A B |
| B spinach | 6 | 3.770 | A B C |
| B pakchoi | 5 | 3.274 | A B C D E |
| C coriander | 5 | 3.092 | A B C D E |
| A leek | 6 | 2.907 | A B C D E |
| C leek | 1 | 2.650 | A B C D E |
| A pakchoi | 6 | 2.597 | A B C D E |
| B cabbage | 6 | 2.530 | A B C D E |
| B lettuce | 3 | 1.753 | A B C D E |
| A spring onion | 6 | 1.553 | B C D E |
| A radish | 6 | 1.112 | C E |
| C carrot | 4 | 0.657 | D E |

Means that do not share a letter are significantly different.

## B.5 Comparison of APC and *Listeria* spp. for soil in farms

| **APC** | ***Listeria*** spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus Farm**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Farm 3 A, B, C<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Farm 2 0.1356 0.06781 0.23 0.800<br>Error 9 2.6717 0.29685<br>Total 11 2.8073<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.544842 4.83% 0.00% 0.00%<br><br>Means<br>Farm N Mean StDev 95% CI<br>A 4 6.755 0.554 (6.139, 7.371)<br>B 4 6.995 0.377 (6.379, 7.611)<br>C 4 6.963 0.664 (6.346, 7.579)<br>Pooled StDev = 0.544842<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>Farm N Mean Grouping<br>B 4 6.995 A<br>C 4 6.963 A<br>A 4 6.755 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus Farm**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br><br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Farm 3 A, B, C<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Farm 2 1.2412 0.6206 5.71 0.025<br>Error 9 0.9781 0.1087<br>Total 11 2.2193<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.329655 55.93% 46.14% 21.65%<br><br>Means<br>Farm N Mean StDev 95% CI<br>A 4 4.672 0.365 (4.300, 5.045)<br>B 4 4.298 0.308 (3.925, 4.670)<br>C 4 3.885 0.313 (3.512, 4.258)<br>Pooled StDev = 0.329655<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>Farm N Mean Grouping<br>A 4 4.672 A<br>B 4 4.298 A B<br>C 4 3.885 B<br>Means that do not share a letter are significantly different. |

# Appendix C

# One-way ANOVA analysis (individual comparison) by Minitab

## C.1 Comparison of APC and *Listeria* spp. for cabbage

**APC**

### One-way ANOVA: log cfu/g versus From

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
From 6 Farm B, Farm C, Market A, Market B, Market C, Market D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
From 5 2.249 0.4498 1.40 0.258
Error 26 8.367 0.3218
Total 31 10.616

Model Summary
S R-sq R-sq(adj) R-sq(pred)
0.567283 21.19% 6.03% 0.00%

Means
From N Mean StDev 95% CI
Farm B 4 6.405 1.235 ( 5.822, 6.988)
Farm C 4 7.265 0.570 ( 6.682, 7.848)
Market A 6 6.818 0.459 ( 6.342, 7.294)
Market B 6 6.977 0.448 ( 6.501, 7.453)
Market C 6 6.5050 0.2153 (6.0290, 6.9810)
Market D 6 6.892 0.325 ( 6.416, 7.368)
Pooled StDev = 0.567283

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

From N Mean Grouping
Farm C 4 7.265 A
Market B 6 6.977 A
Market D 6 6.892 A
Market A 6 6.818 A
Market C 6 6.5050 A
Farm B 4 6.405 A

Means that do not share a letter are significantly different.

***Listeria*** **spp.**

### One-way ANOVA: log cfu/g versus From

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Rows unused 12
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
From 6 Farm B, Farm C, Market A, Market B, Market C, Market D

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
From 5 22.50 4.500 1.79 0.147
Error 28 70.37 2.513
Total 33 92.86

Model Summary
S R-sq R-sq(adj) R-sq(pred)
1.58528 24.23% 10.70% 0.00%

Means
From N Mean StDev 95% CI
Farm B 6 2.530 2.050 (1.204, 3.856)
Farm C 4 4.438 0.329 (2.814, 6.061)
Market A 6 1.515 1.675 (0.189, 2.841)
Market B 6 2.643 1.501 (1.318, 3.969)
Market C 6 3.262 0.507 (1.936, 4.587)
Market D 6 2.683 2.119 (1.358, 4.009)
Pooled StDev = 1.58528

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

From N Mean Grouping
Farm C 4 4.438 A
Market C 6 3.262 A
Market D 6 2.683 A
Market B 6 2.643 A
Farm B 6 2.530 A
Market A 6 1.515 A

Means that do not share a letter are significantly different.

## C.2 Comparison of APC and *Listeria* spp. for carrot

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 5 Farm C, Market A, Market B, Market C, Market D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 4 11.57 2.894 1.61 0.206<br>Error 23 41.38 1.799<br>Total 27 52.95<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.34125 21.86% 8.27% 0.00%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm C 4 4.71 3.19 ( 3.33, 6.10)<br>Market A 6 6.722 0.875 (5.589, 7.854)<br>Market B 6 5.902 0.822 (4.769, 7.034)<br>Market C 6 6.303 0.629 (5.171, 7.436)<br>Market D 6 6.527 0.578 (5.394, 7.659)<br>Pooled StDev = 1.34125<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Market A 6 6.722 A<br>Market D 6 6.527 A<br>Market C 6 6.303 A<br>Market B 6 5.902 A<br>Farm C 4 4.71 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 5 Farm C, Market A, Market B, Market C, Market D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 4 3.408 0.8521 0.43 0.784<br>Error 23 45.333 1.9710<br>Total 27 48.741<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.40392 6.99% 0.00% 0.00%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm C 4 0.657 1.315 (-0.795, 2.110)<br>Market A 6 0.557 1.364 (-0.629, 1.742)<br>Market B 6 1.015 1.592 (-0.171, 2.201)<br>Market C 6 0.778 1.907 (-0.407, 1.964)<br>Market D 6 0 0 (-1.186, 1.186)<br>Pooled StDev = 1.40392<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Market B 6 1.015 A<br>Market C 6 0.778 A<br>Farm C 4 0.657 A<br>Market A 6 0.557 A<br>Market D 6 0.000000 A<br>Means that do not share a letter are significantly different. |

## C.3 Comparison of APC and *Listeria* spp. for lettuce

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 5 Farm B, Market A, Market B, Market C, Market D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 4 1.475 0.3688 1.22 0.331<br>Error 22 6.654 0.3025<br>Total 26 8.129<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.549957 18.15% 3.27% 0.00%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm B 3 6.340 0.757 (5.682, 6.998)<br>Market A 6 7.113 0.476 (6.648, 7.579)<br>Market B 6 6.950 0.465 (6.484, 7.416)<br>Market C 6 6.728 0.577 (6.263, 7.194)<br>Market D 6 7.013 0.571 (6.548, 7.479)<br>Pooled StDev = 0.549957<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Market A 6 7.113 A<br>Market D 6 7.013 A<br>Market B 6 6.950 A<br>Market C 6 6.728 A<br>Farm B 3 6.340 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 5 Farm B, Market A, Market B, Market C, Market D<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 4 16.35 4.0880 4.84 0.006<br>Error 22 18.60 0.8452<br>Total 26 34.95<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.919365 46.79% 37.12% 12.69%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm B 3 1.753 1.519 (0.653, 2.854)<br>Market A 6 4.388 0.786 (3.610, 5.167)<br>Market B 6 4.093 0.953 (3.315, 4.872)<br>Market C 6 4.105 0.530 (3.327, 4.883)<br>Market D 6 4.228 0.995 (3.450, 5.007)<br>Pooled StDev = 0.919365<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Market A 6 4.388 A<br>Market D 6 4.228 A<br>Market C 6 4.105 A<br>Market B 6 4.093 A<br>Farm B 3 1.753 B<br>Means that do not share a letter are significantly different. |

## C.4 Comparison of APC and *Listeria* spp. for pakchoi

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 2 Farm A, Farm B<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 1 1.472 1.4720 4.80 0.056<br>Error 9 2.762 0.3069<br>Total 10 4.234<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.553977 34.77% 27.52% 5.29%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm A 6 6.033 0.706 (5.522, 6.545)<br>Farm B 5 6.768 0.259 (6.208, 7.328)<br>Pooled StDev = 0.553977<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Farm B 5 6.768 A<br>Farm A 6 6.033 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus From**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>From 2 Farm A, Farm B<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>From 1 1.251 1.251 0.46 0.515<br>Error 9 24.490 2.721<br>Total 10 25.741<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>1.64959 4.86% 0.00% 0.00%<br><br>Means<br>From N Mean StDev 95% CI<br>Farm A 6 2.597 1.319 (1.073, 4.120)<br>Farm B 5 3.274 1.987 (1.605, 4.943)<br>Pooled StDev = 1.64959<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br>From N Mean Grouping<br>Farm B 5 3.274 A<br>Farm A 6 2.597 A<br>Means that do not share a letter are significantly different. |

## C.5 Comparison of APC and *Listeria* spp. for spinach

**APC**

### One-way ANOVA: log cfu/g versus From

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
From 2 Farm B, Farm C

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
From 1 0.7488 0.7488 2.27 0.166
Error 9 2.9639 0.3293
Total 10 3.7128

Model Summary
S R-sq R-sq(adj) R-sq(pred)
0.573868 20.17% 11.30% 0.00%

Means
From N Mean StDev 95% CI
Farm B 6 6.880 0.747 ( 6.350, 7.410)
Farm C 5 7.4040 0.2080 (6.8234, 7.9846)
Pooled StDev = 0.573868

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
From N Mean Grouping
Farm C 5 7.4040 A
Farm B 6 6.880 A
Means that do not share a letter are significantly different.

***Listeria*** spp.

### One-way ANOVA: log cfu/g versus From

Method
Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05
Equal variances were assumed for the analysis.

Factor Information
Factor Levels Values
From 2 Farm B, Farm C

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
From 1 5.030 5.0295 10.13 0.011
Error 9 4.466 0.4963
Total 10 9.496

Model Summary
S R-sq R-sq(adj) R-sq(pred)
0.704468 52.96% 47.74% 28.93%

Means
From N Mean StDev 95% CI
Farm B 6 3.770 0.613 (3.119, 4.421)
Farm C 5 5.128 0.805 (4.415, 5.841)
Pooled StDev = 0.704468

### Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence
From N Mean Grouping
Farm C 5 5.128 A
Farm B 6 3.770 B
Means that do not share a letter are significantly different.

## C.6 Comparison of APC and *Listeria* spp. for parsley

| APC | *Listeria* spp. |
|---|---|
| **One-way ANOVA: log cfu/g versus Farm**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Farm 2 B, C<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Farm 1 1.341 1.3410 4.05 0.079<br>Error 8 2.651 0.3314<br>Total 9 3.992<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.575703 33.59% 25.29% 0.01%<br><br>Means<br>Farm N Mean StDev 95% CI<br>B 6 6.990 0.654 (6.448, 7.532)<br>C 4 7.738 0.414 (7.074, 8.401)<br><br>Pooled StDev = 0.575703<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br><br>Farm N Mean Grouping<br>C 4 7.738 A<br>B 6 6.990 A<br>Means that do not share a letter are significantly different. | **One-way ANOVA: log cfu/g versus Farm**<br>Method<br>Null hypothesis All means are equal<br>Alternative hypothesis At least one mean is different<br>Significance level α = 0.05<br>Equal variances were assumed for the analysis.<br><br>Factor Information<br>Factor Levels Values<br>Farm 2 B, C<br><br>Analysis of Variance<br>Source DF Adj SS Adj MS F-Value P-Value<br>Farm 1 0.6573 0.6573 1.92 0.203<br>Error 8 2.7405 0.3426<br>Total 9 3.3978<br><br>Model Summary<br>S R-sq R-sq(adj) R-sq(pred)<br>0.585292 19.34% 9.26% 0.00%<br><br>Means<br>Farm N Mean StDev 95% CI<br>B 6 3.987 0.723 (3.436, 4.538)<br>C 4 4.510 0.207 (3.835, 5.185)<br><br>Pooled StDev = 0.585292<br><br>**Tukey Pairwise Comparisons**<br>Grouping Information Using the Tukey Method and 95% Confidence<br><br>Farm N Mean Grouping<br>C 4 4.510 A<br>B 6 3.987 A<br>Means that do not share a letter are significantly different. |

# Appendix D

## Data analysis from 3M Molecular Detection

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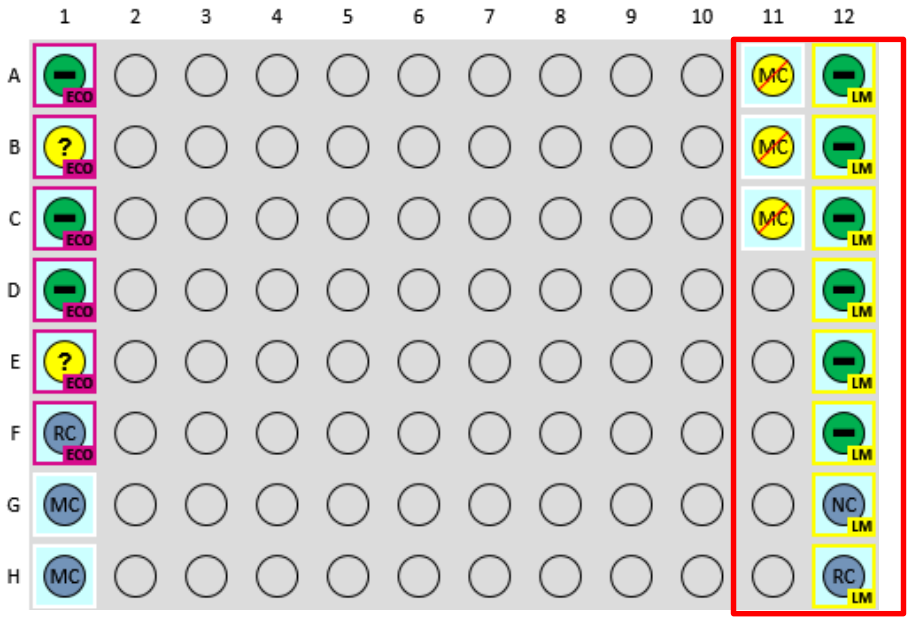
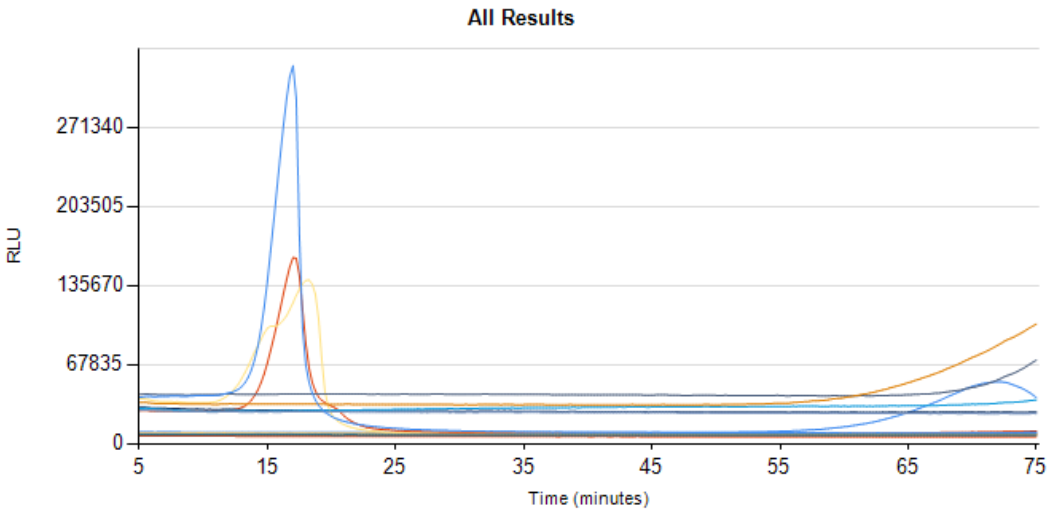



| Well ID | Sample ID | Assay Type | Well Type | Kit Lot Number | Result |
|---|---|---|---|---|---|
| A11 | Carrot | Matrix Control | Matrix Control | 050213 MC 1 | Inhibited |
| B11 | Cabbage | Matrix Control | Matrix Control | 050213 MC 1 | Inhibited |
| C11 | Cucumber | Matrix Control | Matrix Control | 050213 MC 1 | Inhibited |
| A12 | *L. monocytogenes* | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B12 | Cucumber Sunday Market | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C12 | Cabbage Sunday Market | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D12 | Carrot Sunday Market | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E12 | Cucumber enriched Sunday M | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F12 | Leek | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G12 | | *L. monocytogenes* | Negative Control | 2014-07 EA | Valid |
| H12 | | *L. monocytogenes* | Reagent Control | 2014-07 EA | Valid |


All Results
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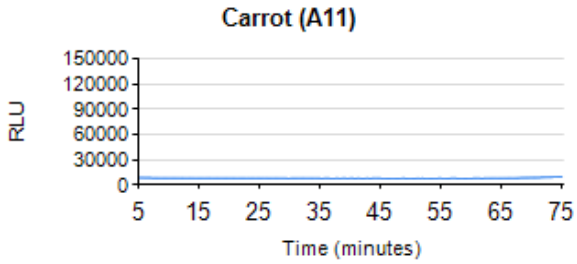
203505
135670
67835
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


Carrot (A11)
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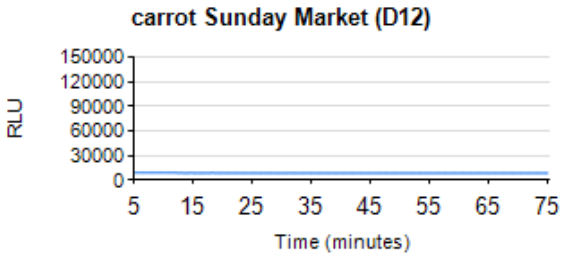
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


carrot Sunday Market (D12)
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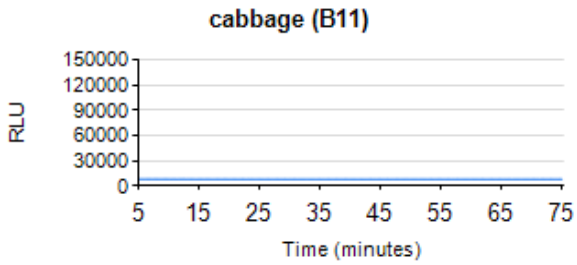
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


cabbage (B11)
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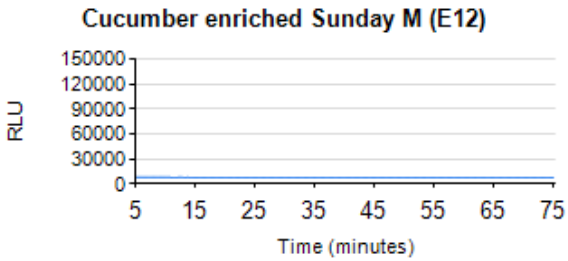
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


Cucumber enriched Sunday M (E12)
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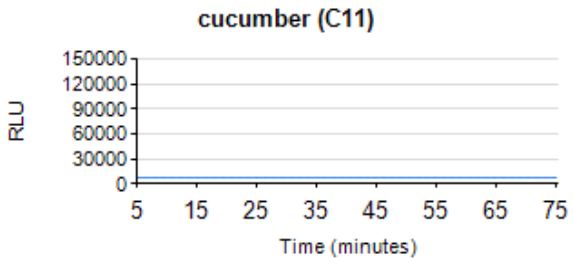
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


cucumber (C11)
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60000
30000
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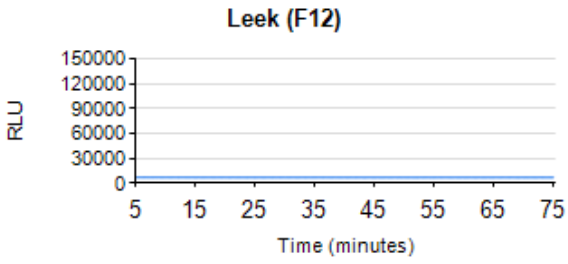
RLU
5
15
25
35
45
55
65
75
Time (minutes)


Leek (F12)
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RLU
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25
35
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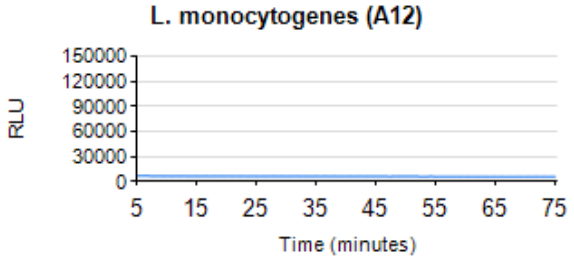
55
65
75
Time (minutes)


L. monocytogenes (A12)
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0
RLU
5
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25
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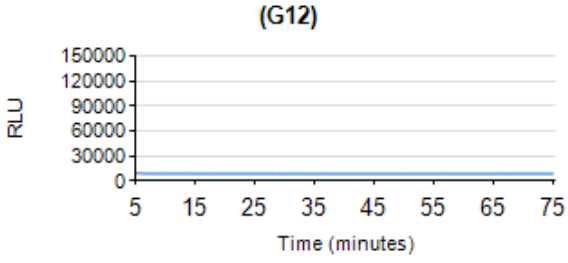
75
Time (minutes)


(G12)
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90000
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RLU
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15
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35
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55
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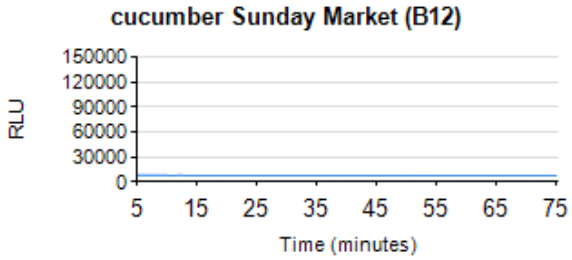
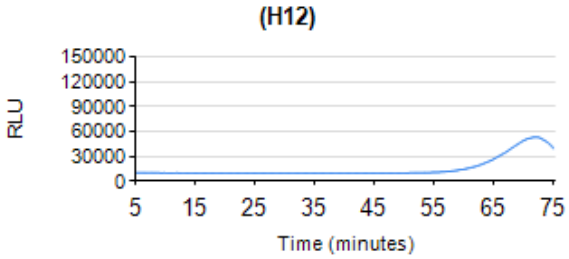
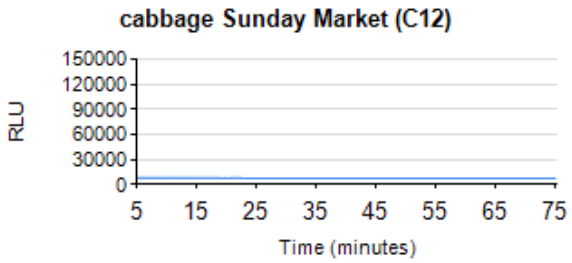
75
Time (minutes)

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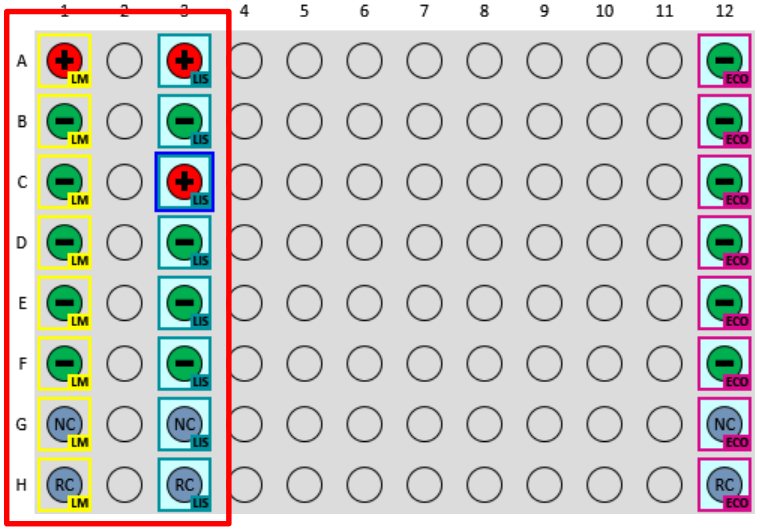
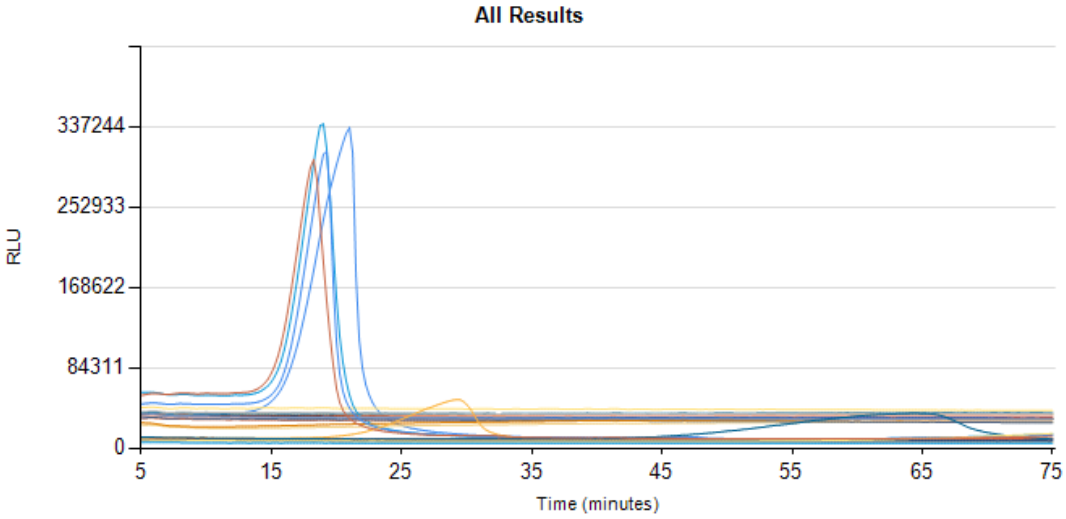
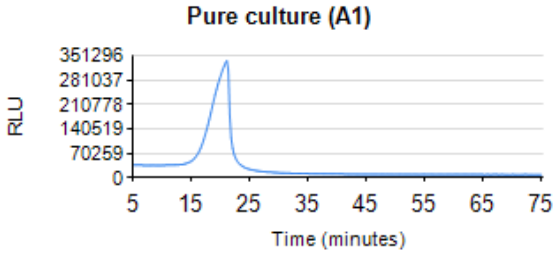
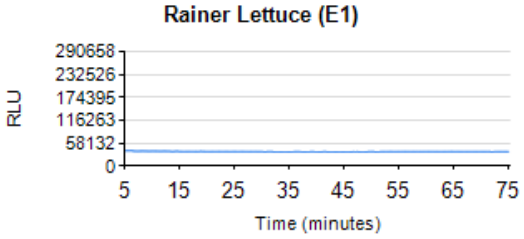


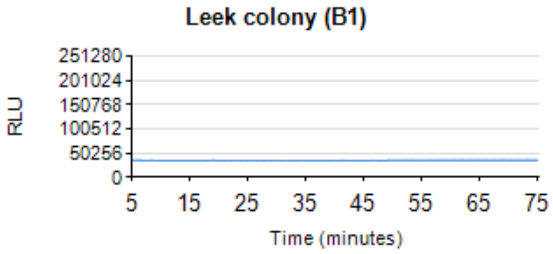

| Well ID | Sample ID | Assay Type | Well Type | Kit Lot Number | Result |
|---|---|---|---|---|---|
| A1 | Pure culture | *L. monocytogenes* | Sample | 2014-07 EA | Positive |
| B1 | Leek colony | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C1 | Rainer Soil | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D1 | Rainer Parsley | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E1 | Rainer Lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F1 | Rainer Spinach | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G1 | | *L. monocytogenes* | Negative Control | 2014-07 EA | Valid |
| H1 | | *L. monocytogenes* | Reagent Control | 2014-07 EA | Valid |
| A3 | Pure culture | *Listeria* | Sample | 2013-09 EB | Positive |
| B3 | Leek colony | *Listeria* | Sample | 2013-09 EB | Negative |
| C3 | Rainer Soil | *Listeria* | Sample | 2013-09 EB | Positive |
| D3 | Rainer Parsley | *Listeria* | Sample | 2013-09 EB | Negative |

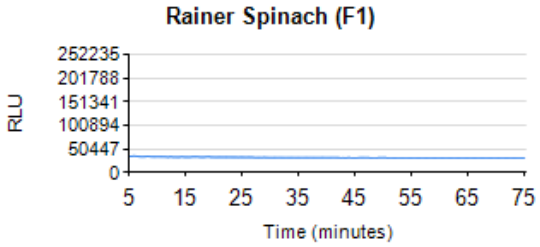
| | | | | | |
|---|---|---|---|---|---|
| E3 | Rainer Lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| F3 | Rainer Spinach | *Listeria* | Sample | 2013-09 EB | Negative |
| G3 | | *Listeria* | Negative Control | 2013-09 EB | Valid |
| H3 | | *Listeria* | Reagent Control | 2013-09 EB | Valid |

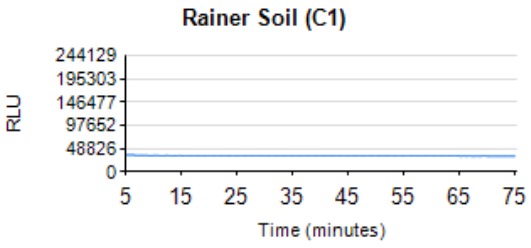


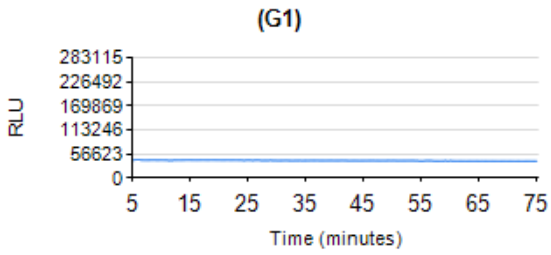



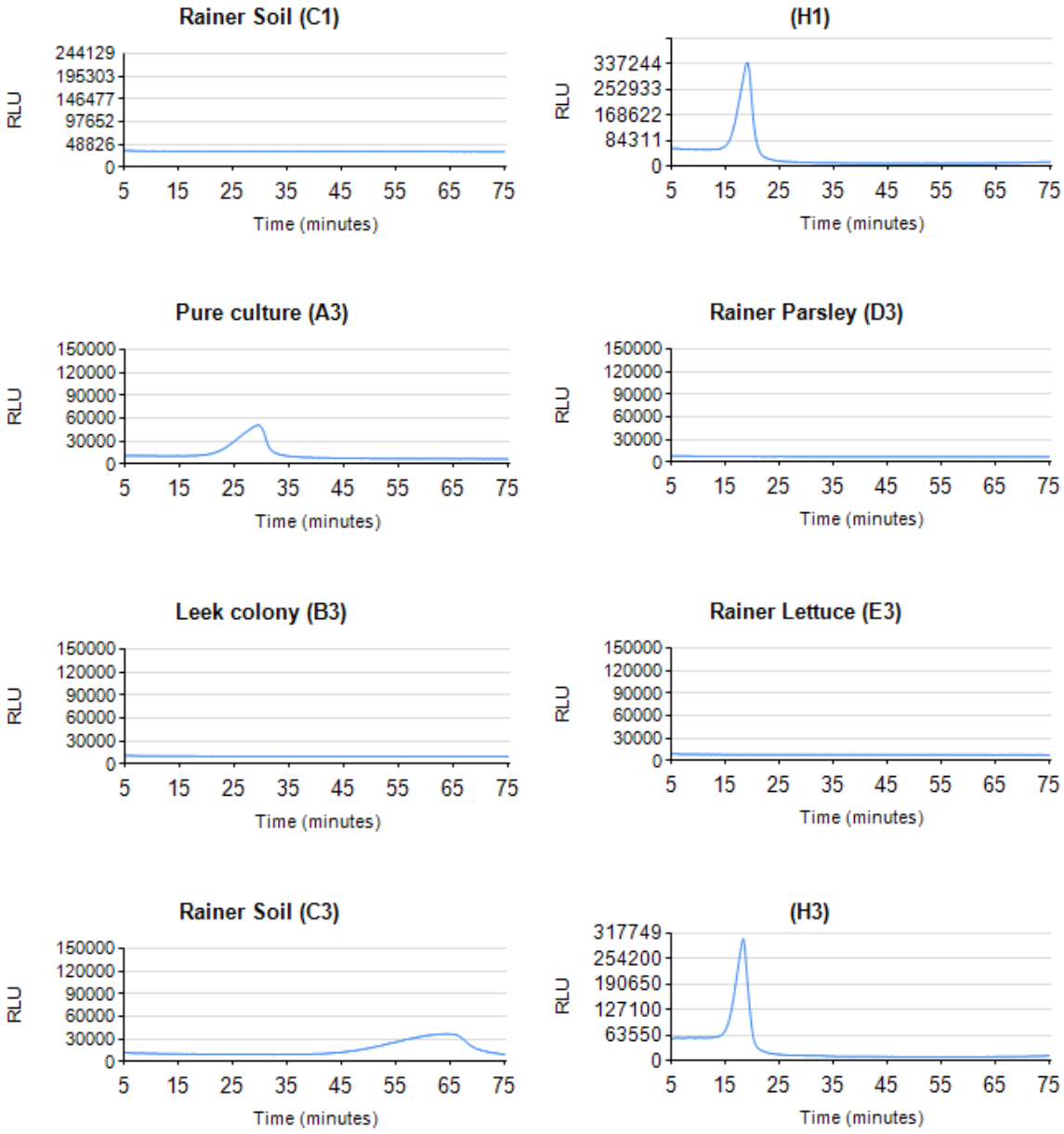



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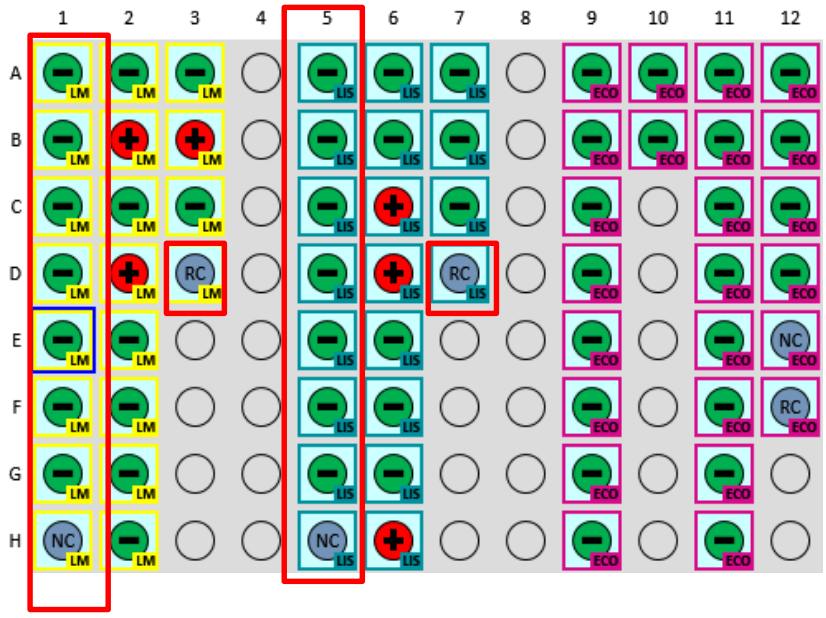
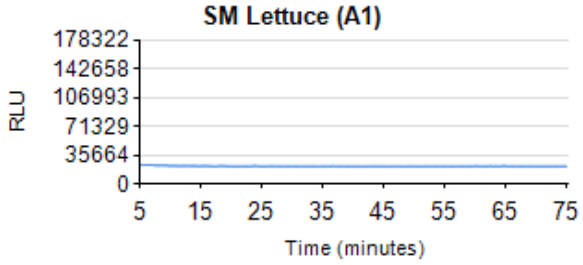
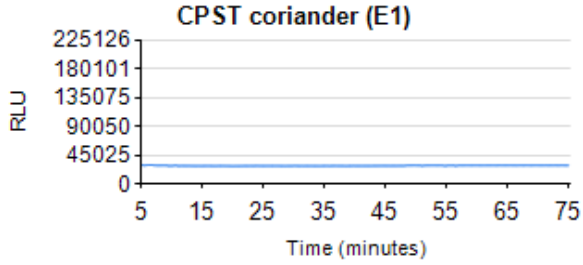
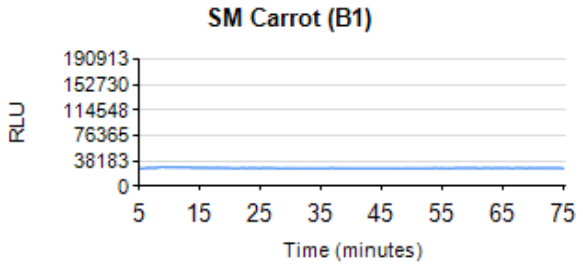
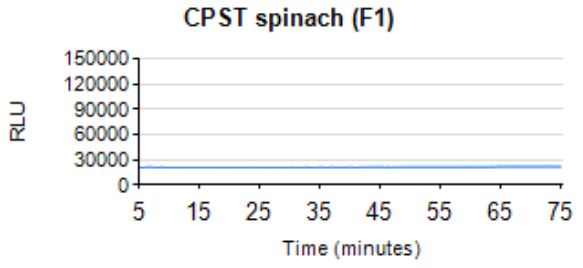


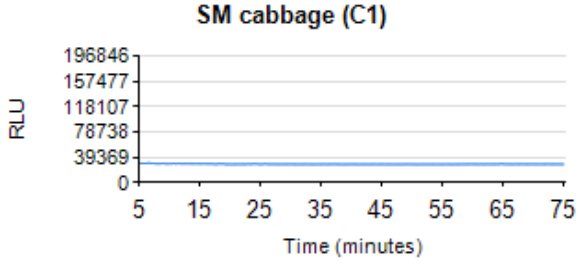
| Well ID | Sample ID | Assay Type | Well Type | Kit Lot Number | Result |
|---|---|---|---|---|---|
| A1 | SM Lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B1 | SM Carrot | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C1 | SM cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D1 | SM Cucumber | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E1 | CPST coriander | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F1 | CPST spinach | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G1 | CPST soil 2 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| H1 | | *L. monocytogenes* | Negative Control | 2014-07 EA | Valid |
| D3 | | *L. monocytogenes* | Reagent Control | 2014-07 EA | Valid |
| A5 | SM Lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| B5 | SM Carrot | *Listeria* | Sample | 2013-09 EB | Negative |
| C5 | SM cabbage | *Listeria* | Sample | 2013-09 EB | Negative |
| D5 | SM Cucumber | *Listeria* | Sample | 2013-09 EB | Negative |
| E5 | CPST coriander | *Listeria* | Sample | 2013-09 EB | Negative |
| F5 | CPST spinach | *Listeria* | Sample | 2013-09 EB | Negative |
| G5 | CPST soil | *Listeria* | Sample | 2013-09 EB | Negative |
| H5 | | *Listeria* | Negative Control | 2013-09 EB | Valid |
| D7 | | *Listeria* | Reagent Control | 2013-09 EB | Valid |

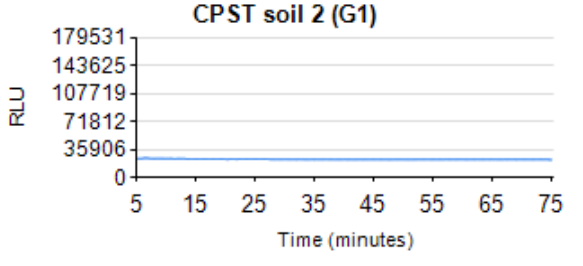


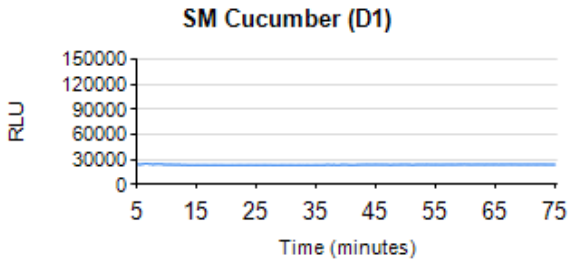



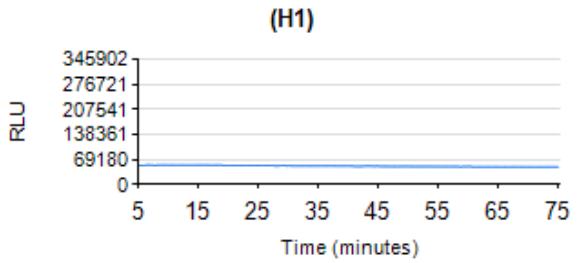



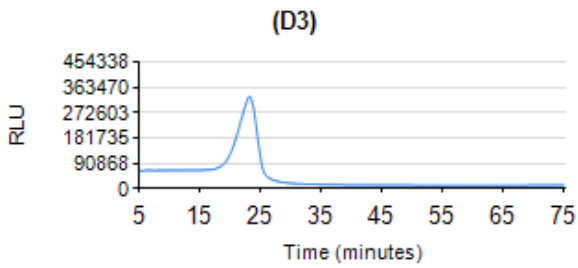



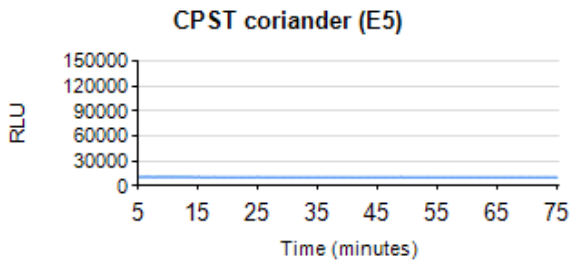



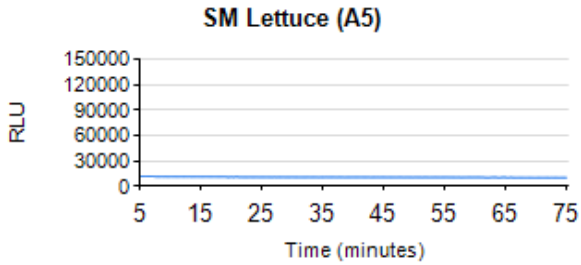



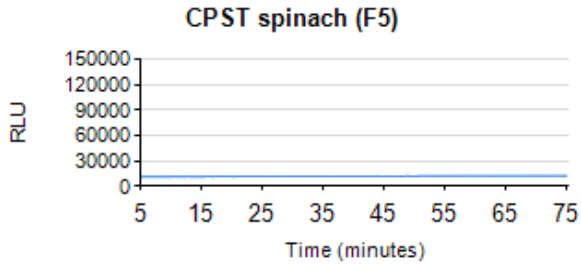


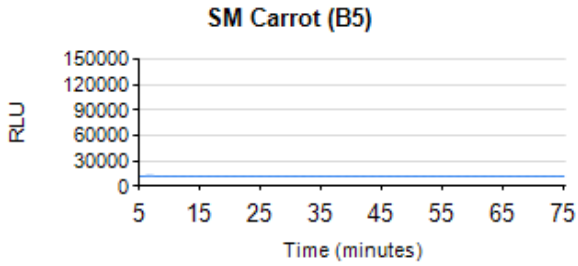



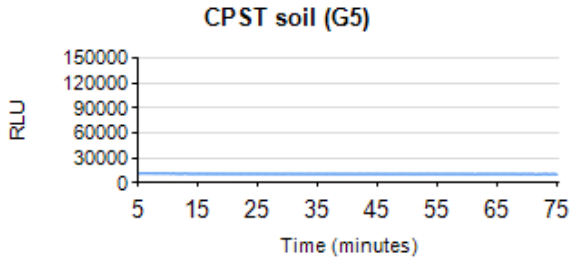



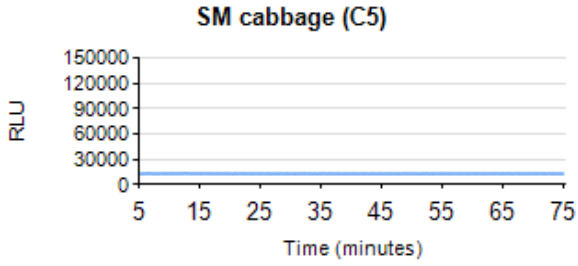



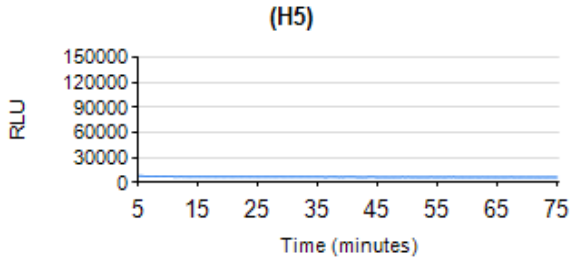



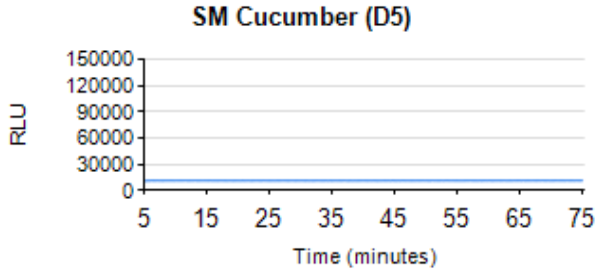



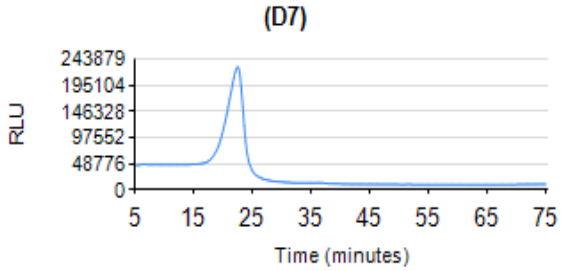

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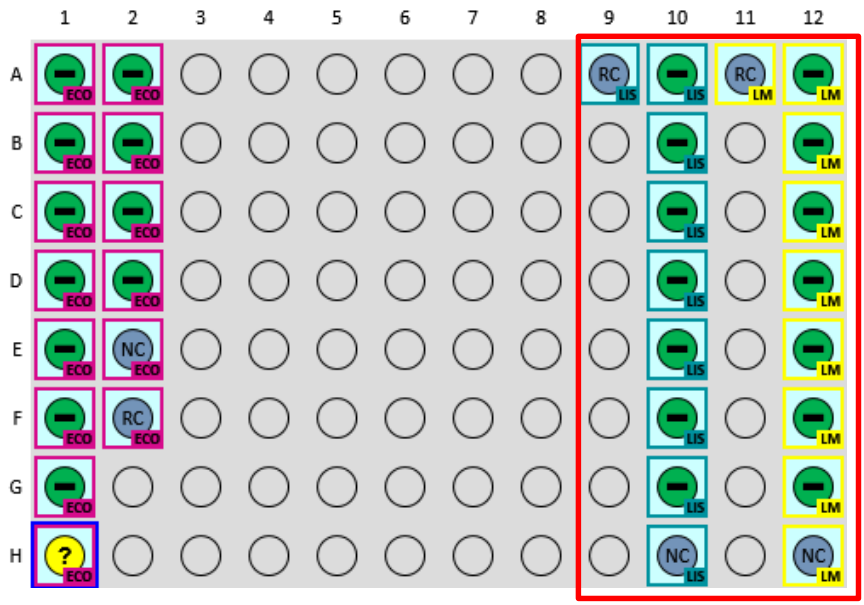
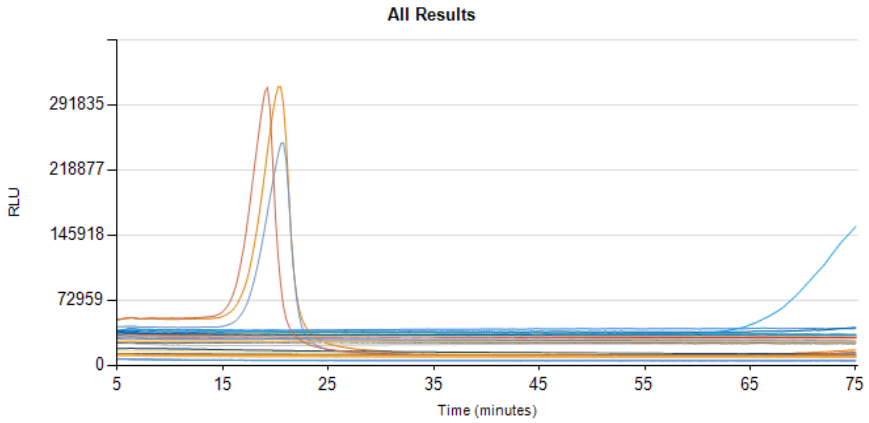
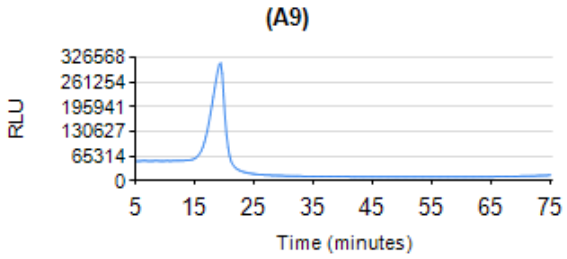
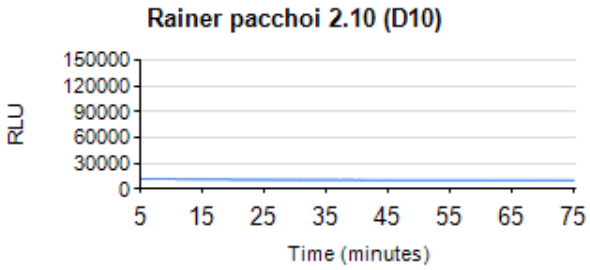
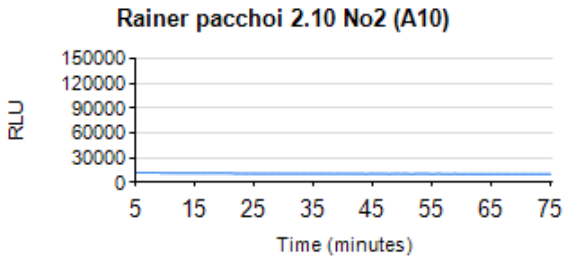


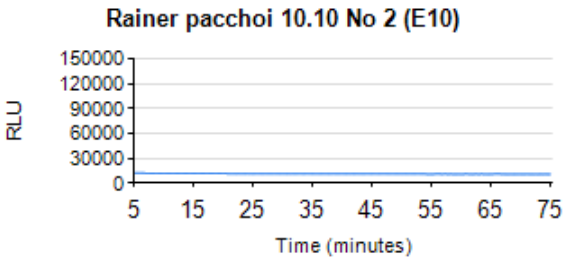

| Well ID | Sample ID | Assay Type | Well Type | Kit Lot Number | Result |
|---|---|---|---|---|---|
| A9 | | *Listeria* | Reagent Control | 2013-09 EB | Valid |
| A10 | Rainer pacchoi 2.10 No2 | *Listeria* | Sample | 2013-09 EB | Negative |
| B10 | Rainer pacchoi 10.10 No 2 | *Listeria* | Sample | 2013-09 EB | Negative |
| C10 | Rainer pacchoi 10.10 No1 | *Listeria* | Sample | 2013-09 EB | Negative |
| D10 | Rainer pacchoi 2.10 | *Listeria* | Sample | 2013-09 EB | Negative |
| E10 | Rainer pacchoi 10.10 No 2 | *Listeria* | Sample | 2013-09 EB | Negative |
| F10 | SM Lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| G10 | SM Carrot | *Listeria* | Sample | 2013-09 EB | Negative |
| H10 | | *Listeria* | Negative Control | 2013-09 EB | Valid |
| A11 | | *L. monocytogenes* | Reagent Control | 2014-07 EA | Valid |
| A12 | Rainer pacchoi 2.10 No1 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B12 | Rainer pacchoi 10.10 No 2 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C12 | Rainer pacchoi 10.10 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D12 | Allen pacchoi 2.10 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E12 | Rainer pacchoi 10.10 No2 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F12 | SM Lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G12 | SM Carrot | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| H12 | | *L. monocytogenes* | Negative Control | 2014-07 EA | Valid |

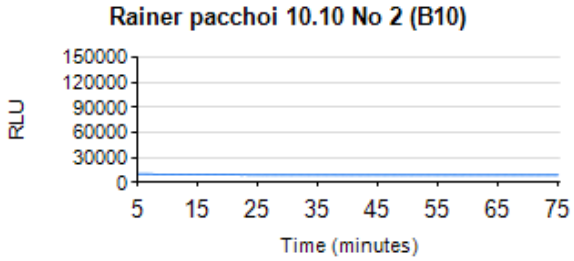

All Results
291835
218877
145918
72959
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

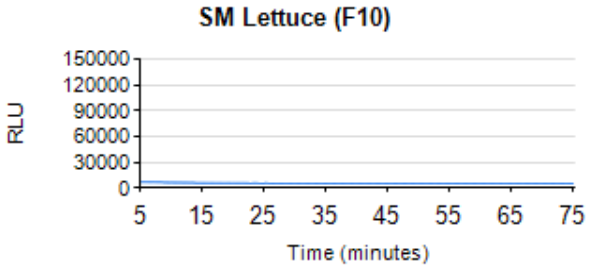

(A9)
326568
261254
195941
130627
65314
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

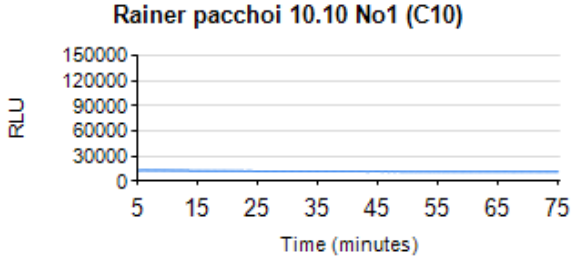

Rainer pacchoi 2.10 (D10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

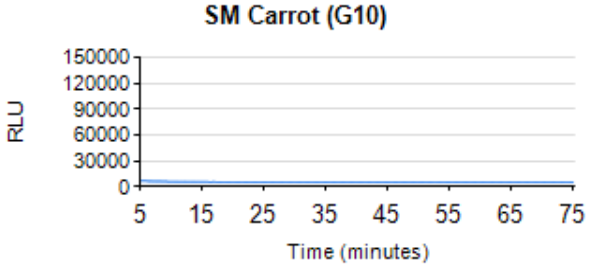

Rainer pacchoi 2.10 No2 (A10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

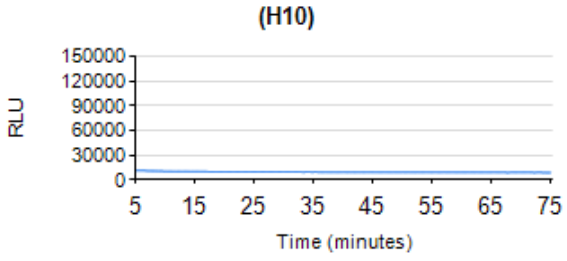

Rainer pacchoi 10.10 No 2 (E10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

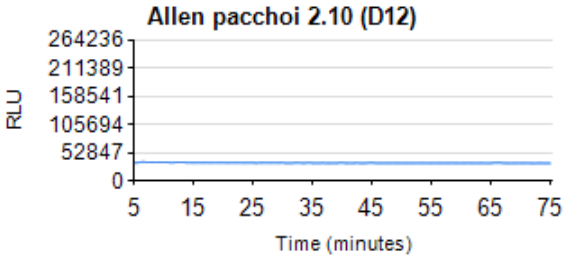

Rainer pacchoi 10.10 No 2 (B10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

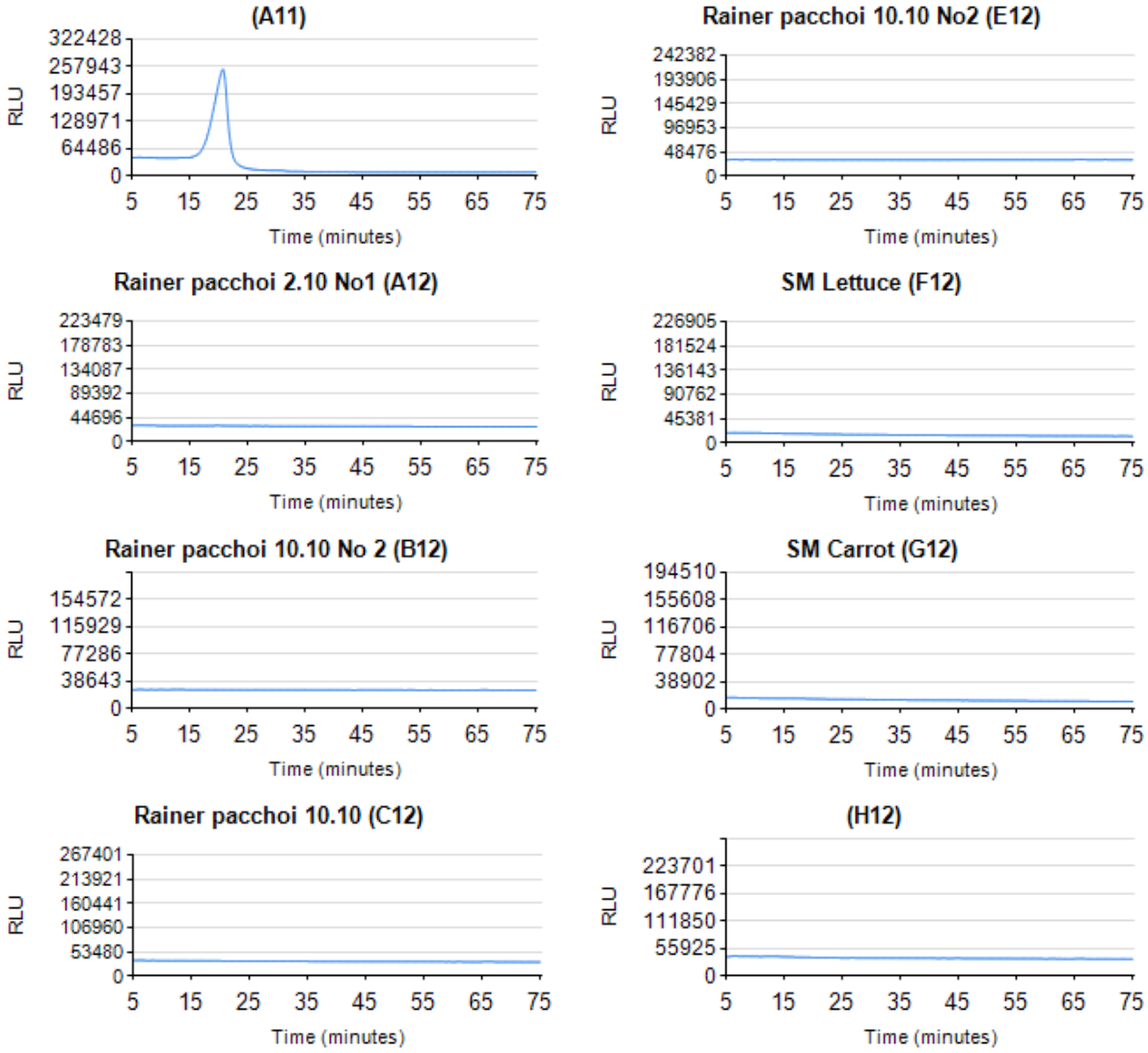

SM Lettuce (F10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


Rainer pacchoi 10.10 No1 (C10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


SM Carrot (G10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


(H10)
150000
120000
90000
60000
30000
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)


Allen pacchoi 2.10 (D12)
264236
211389
158541
105694
52847
0
RLU
5
15
25
35
45
55
65
75
Time (minutes)

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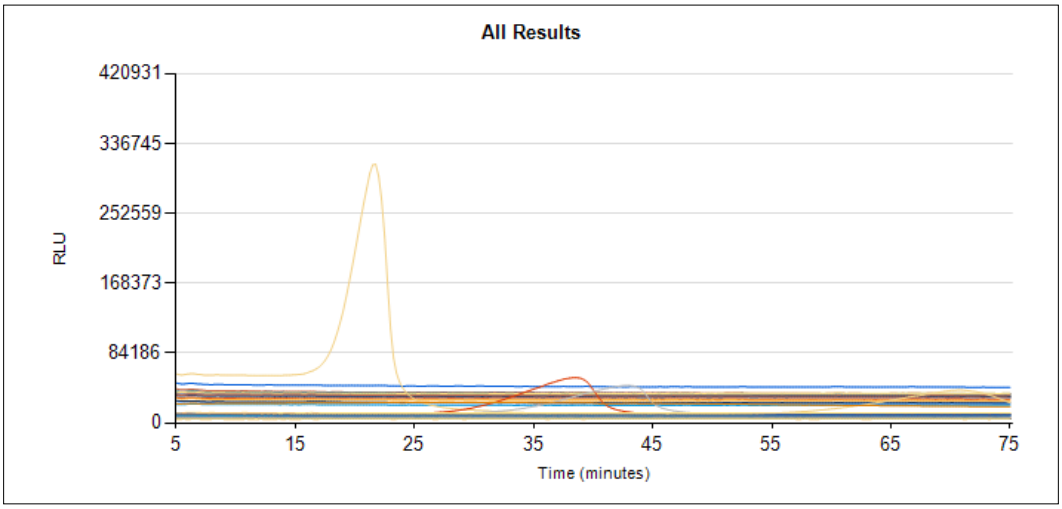
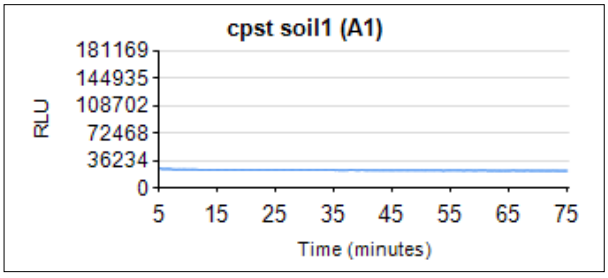
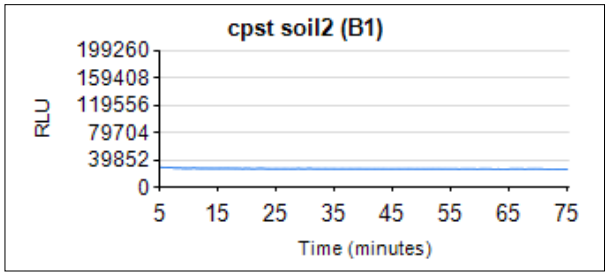
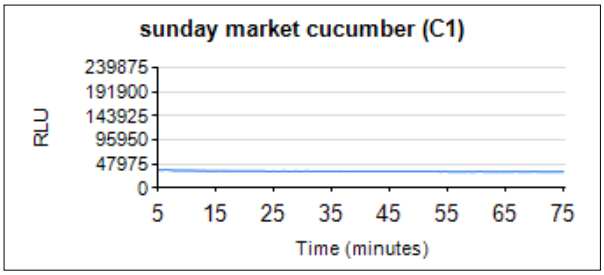


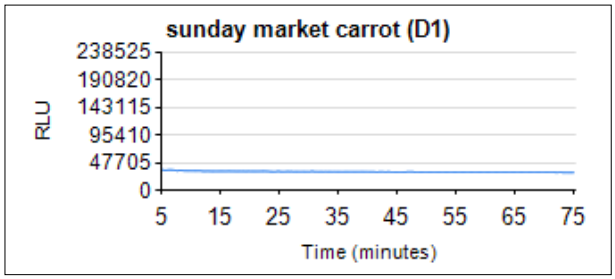

| Well ID | Sample ID | Assay Type | Well Type | Kit Lot Number | Result |
|---|---|---|---|---|---|
| A1 | Cpst soil1 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B1 | Cpst soil2 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C1 | Sunday market cucumber | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D1 | Sunday market carrot | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E1 | Sunday market cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F1 | Sunday market lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G1 | Unwashed allen spring onion | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| H1 | Unwashed grower market lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| A2 | Cpst leek | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B2 | Unwashed Sunday market lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C2 | Unwashed Sunday market cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D2 | Unwashed grower market cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E2 | Unwashed Rainer Spinach | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F2 | CM carrot | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G2 | CM cucumber | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| H2 | CM cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| A3 | CM lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| B3 | GM carrot | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| C3 | GM cucumber | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| D3 | GM cabbage | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| E3 | GM lettuce | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| F3 | Allen soil 1 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| G3 | Rainer soil 2 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |
| H3 | Allen leek Week 5 | *L. monocytogenes* | Sample | 2014-07 EA | Negative |

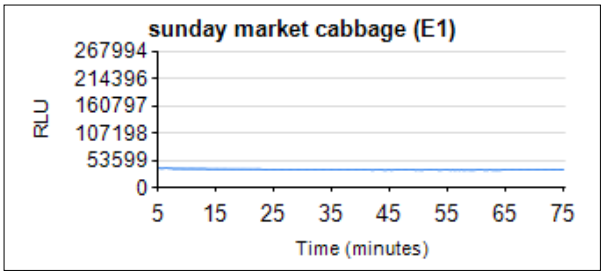
| | | | | | |
|---|---|---|---|---|---|
| A4 | | *L. monocytogenes* | Negative Control | 2014-07 EA | Valid |
| B4 | | *L. monocytogenes* | Reagent Control | 2014-07 EA | Valid |
| A7 | Cpst soil 1 | *Listeria* | Sample | 2013-09 EB | Negative |
| B7 | Cpst soil 2 | *Listeria* | Sample | 2013-09 EB | Negative |
| C7 | Sunday market cucumber | *Listeria* | Sample | 2013-09 EB | Negative |
| D7 | Sunday market carrot | *Listeria* | Sample | 2013-09 EB | Negative |
| E7 | Sunday market cabbage | *Listeria* | Sample | 2013-09 EB | Negative |
| F7 | Sunday market lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| G7 | Unwashed allen spring onion | *Listeria* | Sample | 2013-09 EB | Positive |
| H7 | Unwashed grower market lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| A8 | Cpst leek | *Listeria* | Sample | 2013-09 EB | Positive |
| B8 | Sunday market lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| C8 | Unwashed Sunday market cabbage | *Listeria* | Sample | 2013-09 EB | Negative |
| D8 | Unwashed grower market cabbage | *Listeria* | Sample | 2013-09 EB | Negative |
| E8 | Unwashed Rainer spinach | *Listeria* | Sample | 2013-09 EB | Negative |
| F8 | CM carrot | *Listeria* | Sample | 2013-09 EB | Negative |
| G8 | CM cucumber | *Listeria* | Sample | 2013-09 EB | Positive |
| H8 | CM cabbage | *Listeria* | Sample | 2013-09 EB | Negative |
| A9 | CM lettuce | *Listeria* | Sample | 2013-09 EB | Negative |
| B9 | GM carrot | *Listeria* | Sample | 2013-09 EB | Negative |
| C9 | GM cucumber | *Listeria* | Sample | 2013-09 EB | Error |
| D9 | GM cabbage | *Listeria* | Sample | 2013-09 EB | Error |
| E9 | GM lettuce | *Listeria* | Sample | 2013-09 EB | Error |
| F9 | Allen soil 1 | *Listeria* | Sample | 2013-09 EB | Error |
| G9 | Rainer soil 2 | *Listeria* | Sample | 2013-09 EB | Error |
| H9 | Allen leek Week 5 | *Listeria* | Sample | 2013-09 EB | Error |
| A10 | | *Listeria* | Negative Control | 2013-09 EB | Error |
| B10 | | *Listeria* | Reagent Control | 2013-09 EB | Error |

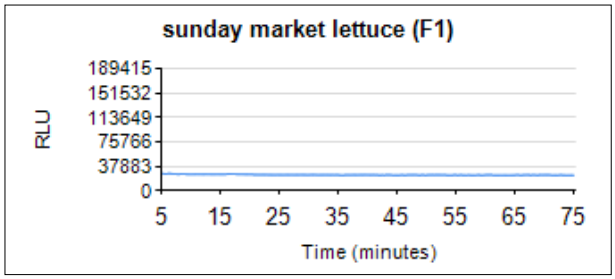


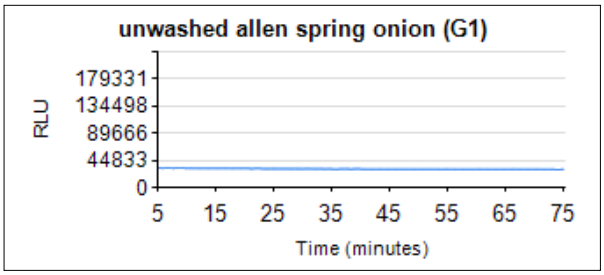



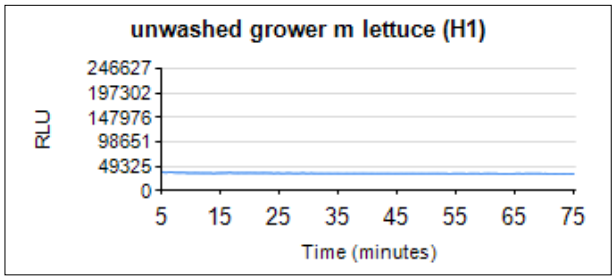

sunday market cucumber (C1)
RLU
239875
191900
143925
95950
47975
0
5 15 25 35 45 55 65 75
Time (minutes)

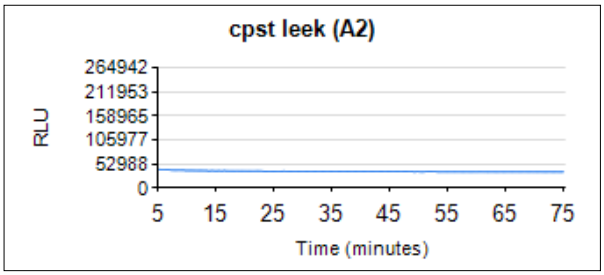

sunday market carrot (D1)
RLU
238525
190820
143115
95410
47705
0
5 15 25 35 45 55 65 75
Time (minutes)

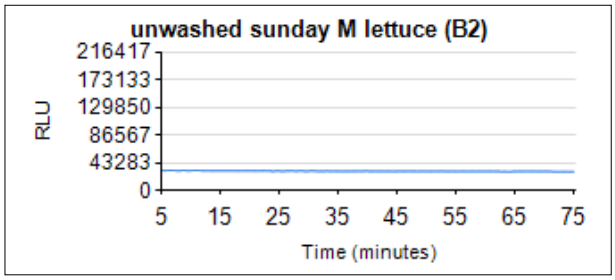

sunday market cabbage (E1)
RLU
267994
214396
160797
107198
53599
0
5 15 25 35 45 55 65 75
Time (minutes)

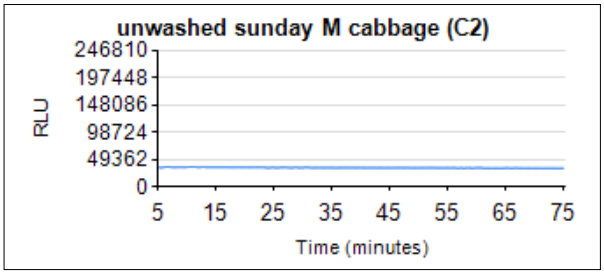

sunday market lettuce (F1)
RLU
189415
151532
113649
75766
37883
0
5 15 25 35 45 55 65 75
Time (minutes)

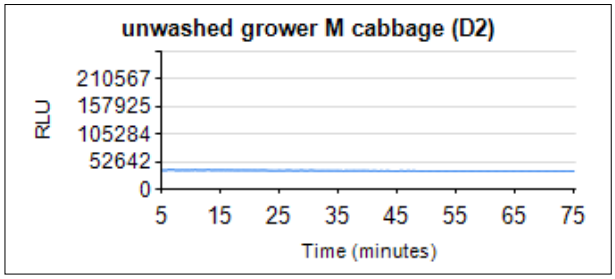

unwashed allen spring onion (G1)
RLU
179331
134498
89666
44833
0
5 15 25 35 45 55 65 75
Time (minutes)

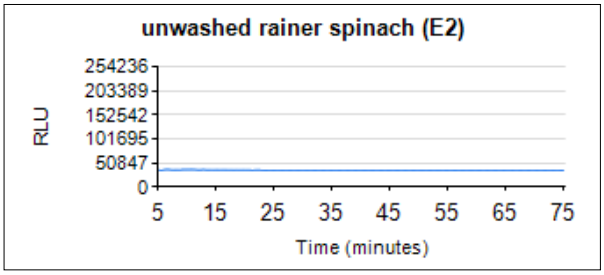

unwashed grower m lettuce (H1)
RLU
246627
197302
147976
98651
49325
0
5 15 25 35 45 55 65 75
Time (minutes)

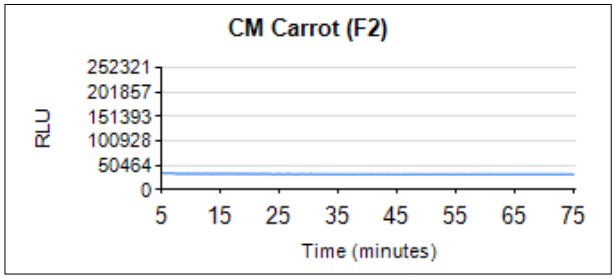

cpst leek (A2)
RLU
264942
211953
158965
105977
52988
0
5 15 25 35 45 55 65 75
Time (minutes)

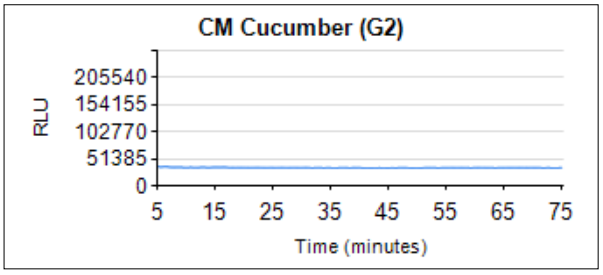

unwashed sunday M lettuce (B2)
RLU
216417
173133
129850
86567
43283
0
5 15 25 35 45 55 65 75
Time (minutes)

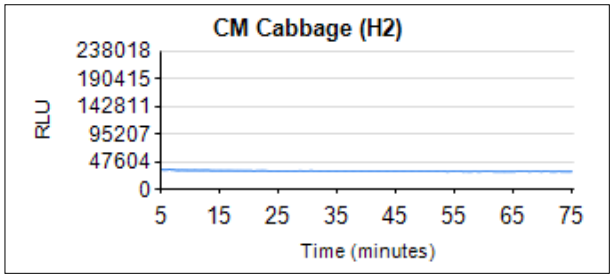

unwashed sunday M cabbage (C2)
RLU
246810
197448
148086
98724
49362
0
5 15 25 35 45 55 65 75
Time (minutes)

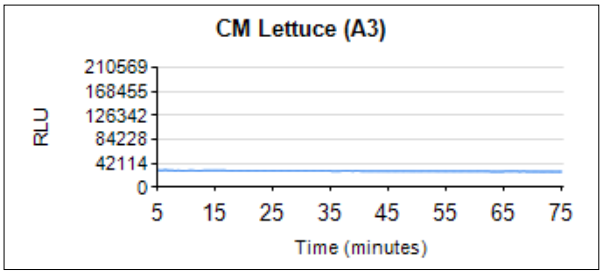

unwashed grower M cabbage (D2)
RLU
210567
157925
105284
52642
0
5 15 25 35 45 55 65 75
Time (minutes)

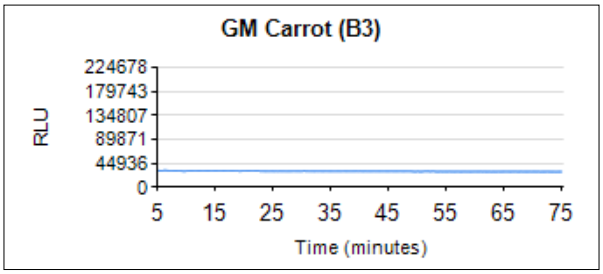

unwashed rainer spinach (E2)
RLU
254236
203389
152542
101695
50847
0
5 15 25 35 45 55 65 75
Time (minutes)

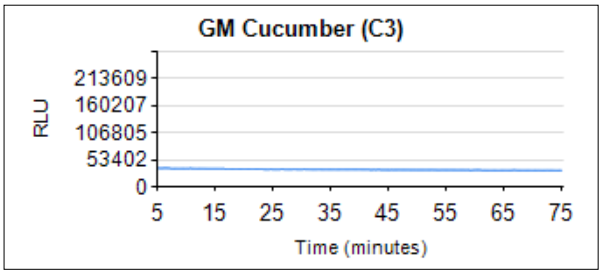

CM Carrot (F2)
RLU
252321
201857
151393
100928
50464
0
5 15 25 35 45 55 65 75
Time (minutes)

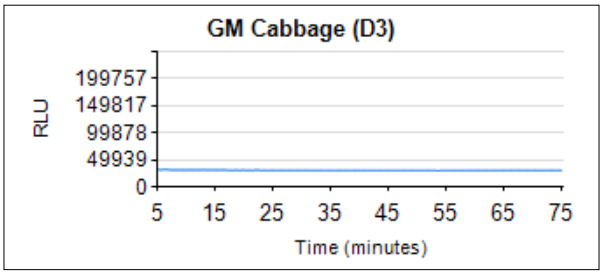

CM Cucumber (G2)
RLU
205540
154155
102770
51385
0
5 15 25 35 45 55 65 75
Time (minutes)

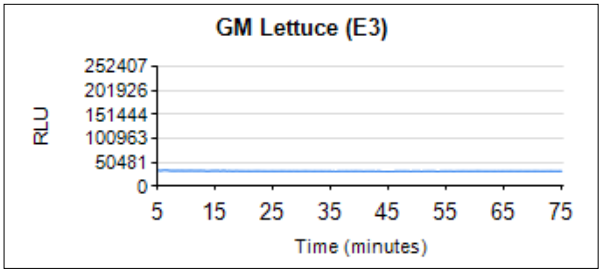

CM Cabbage (H2)
RLU
238018
190415
142811
95207
47604
0
5 15 25 35 45 55 65 75
Time (minutes)

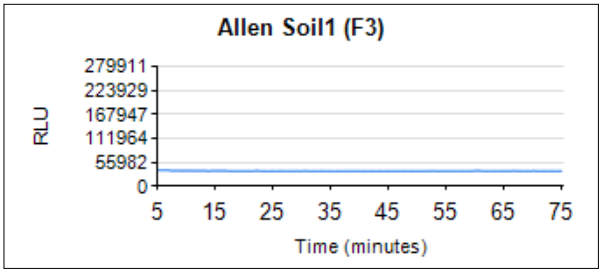

CM Lettuce (A3)
RLU
210569
168455
126342
84228
42114
0
5 15 25 35 45 55 65 75
Time (minutes)

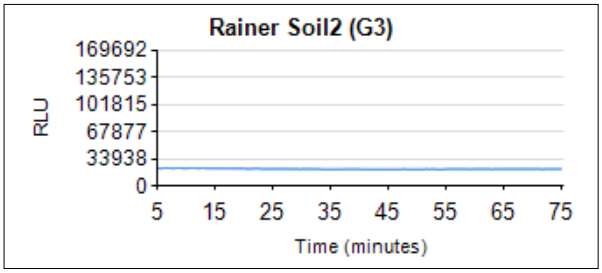

GM Carrot (B3)
RLU
224678
179743
134807
89871
44936
0
5 15 25 35 45 55 65 75
Time (minutes)

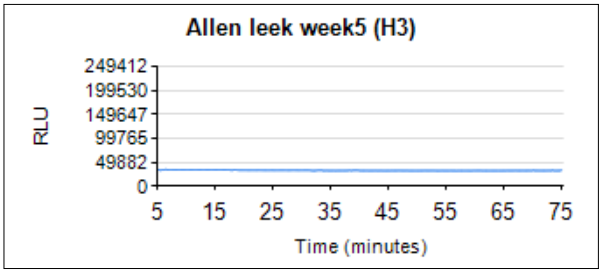

GM Cucumber (C3)
RLU
213609
160207
106805
53402
0
5 15 25 35 45 55 65 75
Time (minutes)

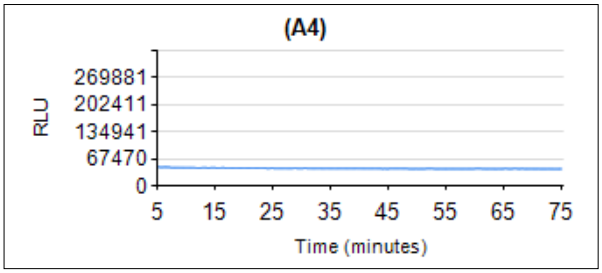

GM Cabbage (D3)
RLU
199757
149817
99878
49939
0
5 15 25 35 45 55 65 75
Time (minutes)

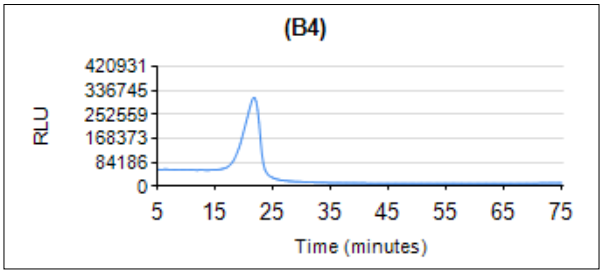

GM Lettuce (E3)
RLU
252407
201926
151444
100963
50481
0
5 15 25 35 45 55 65 75
Time (minutes)

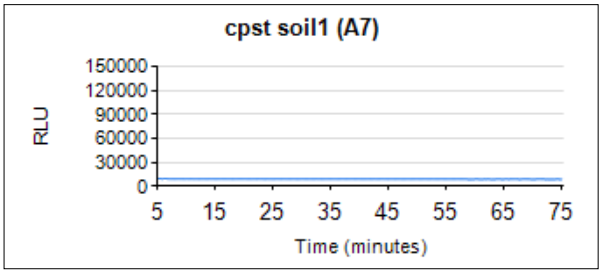

Allen Soil1 (F3)
RLU
279911
223929
167947
111964
55982
0
5 15 25 35 45 55 65 75
Time (minutes)

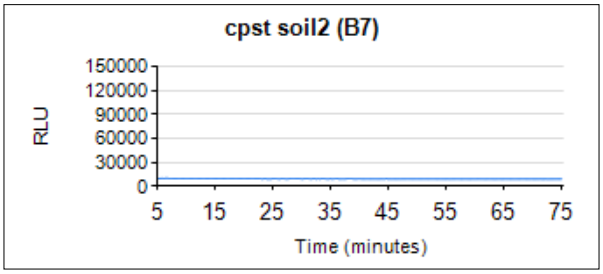

Rainer Soil2 (G3)
RLU
169692
135753
101815
67877
33938
0
5 15 25 35 45 55 65 75
Time (minutes)

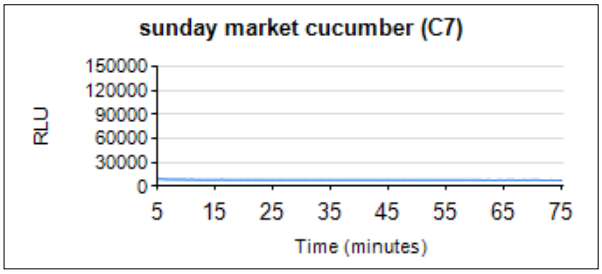

Allen leek week5 (H3)
RLU
249412
199530
149647
99765
49882
0
5 15 25 35 45 55 65 75
Time (minutes)

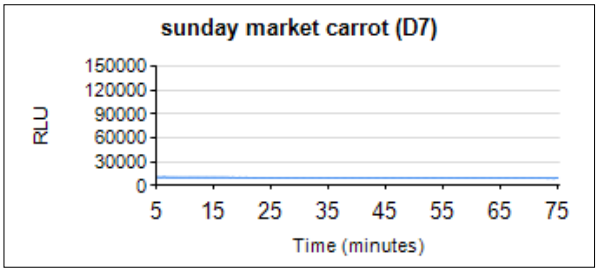

(A4)
RLU
269881
202411
134941
67470
0
5 15 25 35 45 55 65 75
Time (minutes)

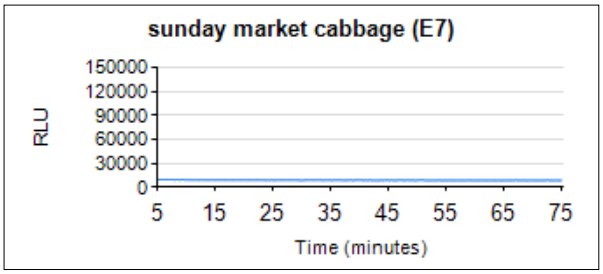

(B4)
RLU
420931
336745
252559
168373
84186
0
5 15 25 35 45 55 65 75
Time (minutes)

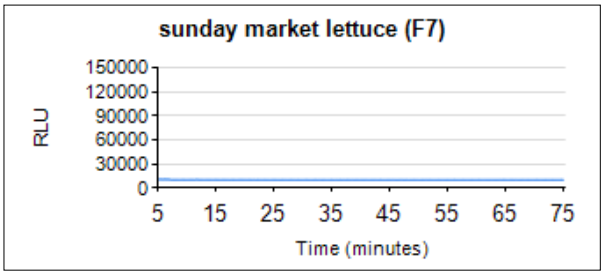

cpst soil1 (A7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

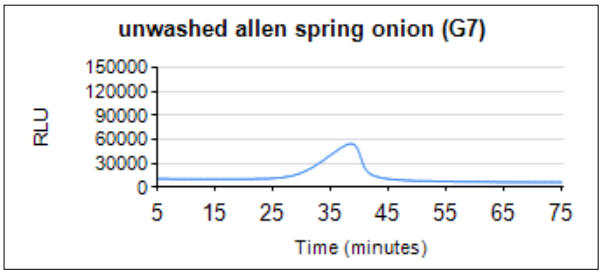

cpst soil2 (B7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

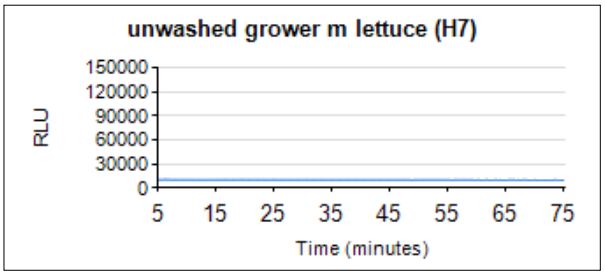

sunday market cucumber (C7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

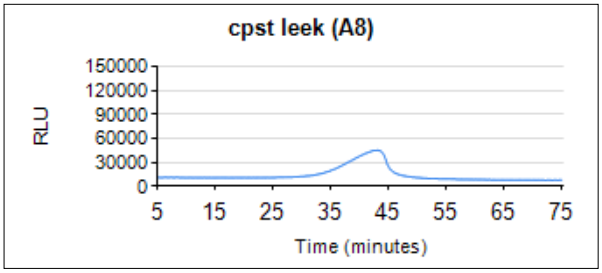

sunday market carrot (D7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

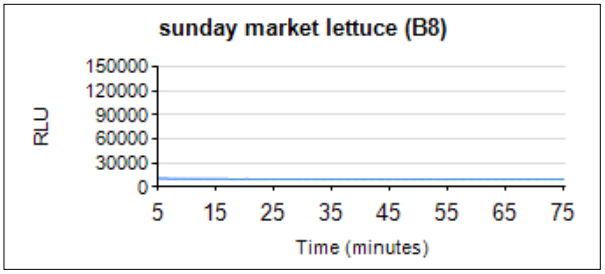

sunday market cabbage (E7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

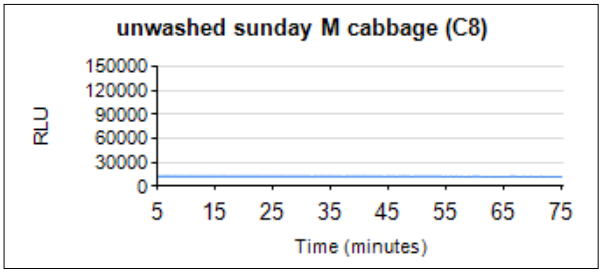

sunday market lettuce (F7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

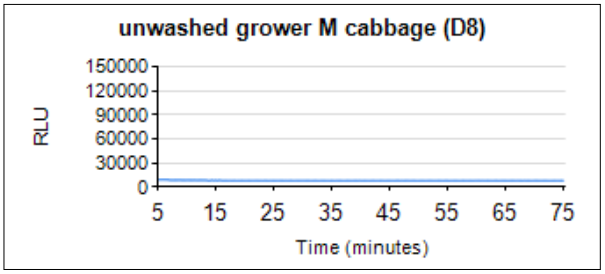

unwashed allen spring onion (G7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)

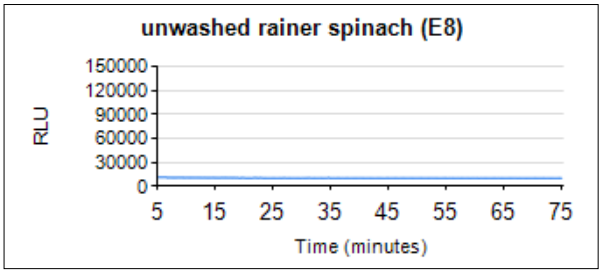

unwashed grower m lettuce (H7)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


cpst leek (A8)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


sunday market lettuce (B8)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


unwashed sunday M cabbage (C8)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


unwashed grower M cabbage (D8)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


unwashed rainer spinach (E8)
RLU
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60000
30000
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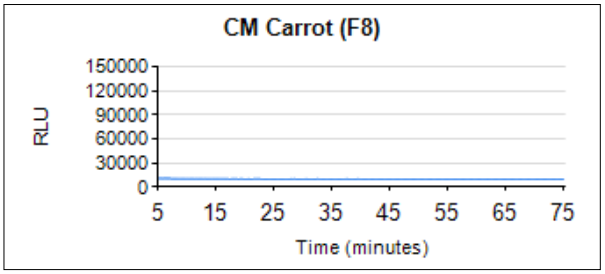
Time (minutes)


CM Carrot (F8)
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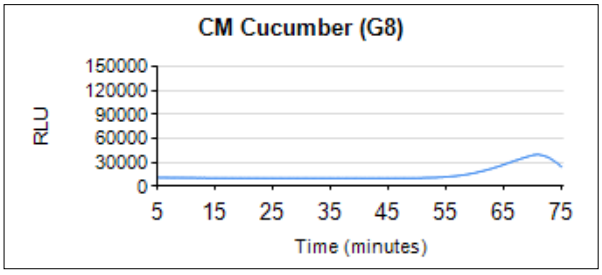
Time (minutes)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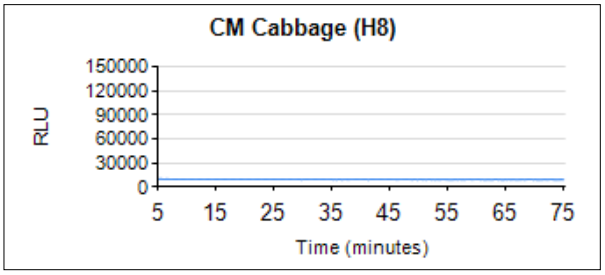
Time (minutes)


CM Cabbage (H8)
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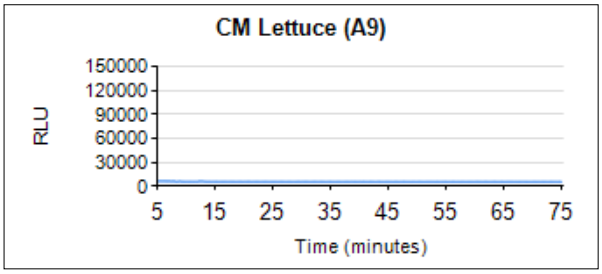
Time (minutes)


CM Lettuce (A9)
RLU
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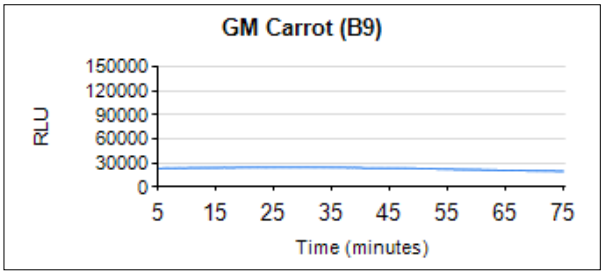
Time (minutes)


GM Carrot (B9)
RLU
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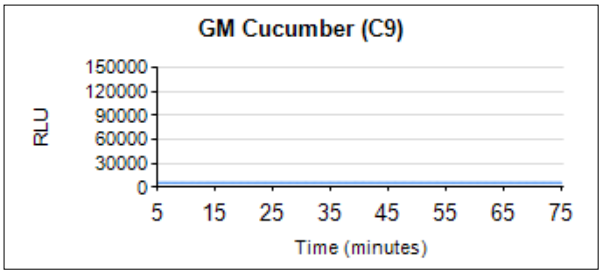
Time (minutes)


GM Cucumber (C9)
RLU
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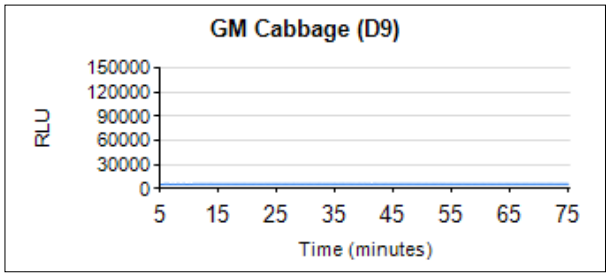
Time (minutes)


GM Cabbage (D9)
RLU
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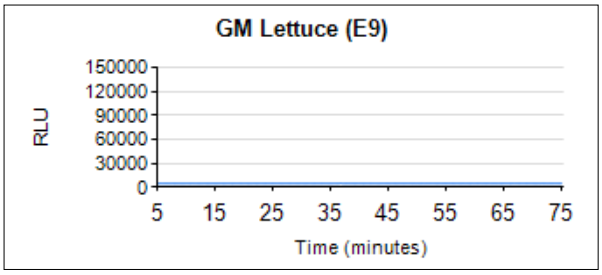
Time (minutes)


GM Lettuce (E9)
RLU
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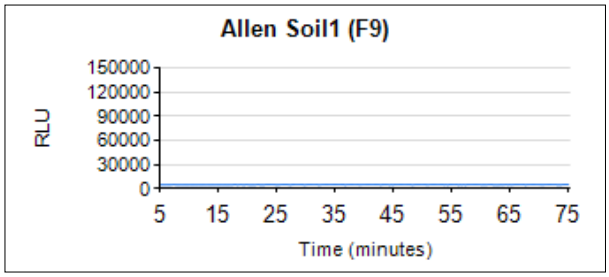
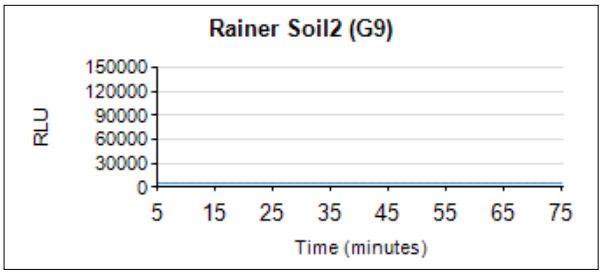
Time (minutes)


Allen Soil1 (F9)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


Rainer Soil2 (G9)
RLU
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60000
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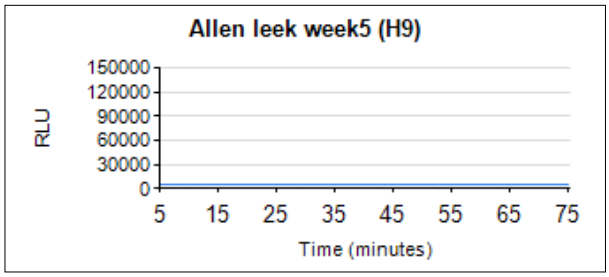
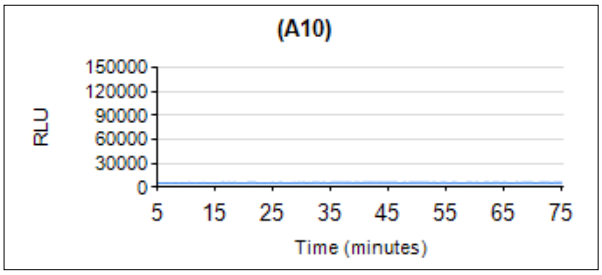
Time (minutes)


Allen leek week5 (H9)
RLU
150000
120000
90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)


(A10)
RLU
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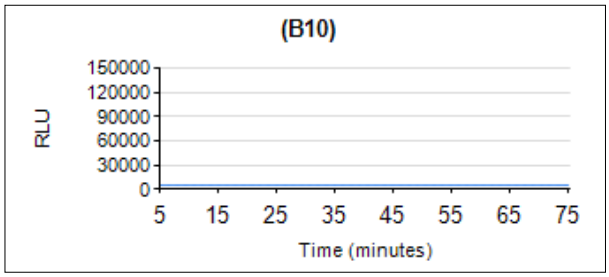
Time (minutes)


(B10)
RLU
150000
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90000
60000
30000
0
5 15 25 35 45 55 65 75
Time (minutes)