

# **Microbiological survey of fresh horticultural produce in Australia, 2005–2007**

**A national survey conducted under the Coordinated Food Survey Plan with participation by food regulatory agencies in ACT, NSW, NT, Qld, SA and Tas**

**April 2010**

## Executive summary

Food Standards Australian New Zealand (FSANZ) was the lead agency for a national coordinated survey of the prevalence of microbiological contamination in fresh horticultural produce. The survey collected samples from three points in the horticultural produce supply chain: from the field, at the farm gate and at retail; with the exception of seed sprouts which were collected prior to germination, at the end of the production line and at retail.

Six states and territories across Australia (ACT, NSW, NT, QLD, SA and TAS) were involved in this survey. The survey was undertaken as part of the Coordinated Food Survey Plan of the Implementation Sub-Committee (ISC) of the Food Regulation Standing Committee. A total of 369 samples were analysed, comprised of 134 lettuce samples, 113 seed sprout samples, 105 strawberry samples, 15 parsley samples, and 2 basil samples. The samples were collected during the period of October 2005 to July 2007 and analysed for the presence of *Escherichia coli*, verocytotoxin producing *E. coli* (VTEC) or specifically for *E. coli* O157:H7, *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp. and faecal coliforms.

The presence of pathogenic bacteria on the fresh produce sampled was very low – no pathogens were detected on the majority samples. However, VTEC was detected on one seed sprout sample at the end of production and on one field parsley sample. *L. monocytogenes* was detected on two farm gate and two retail strawberry samples. *Salmonella* was detected on one field strawberry sample.

While limited by the sample size, this survey provides a snap shot of the microbiological contamination of selected fresh horticultural produce at the time of sampling (2005–2007). It confirms that infrequent contamination of fresh produce with potentially harmful bacteria can occur, reiterating the importance for industry and consumers to follow general advice on the safe production, preparation and handling (e.g. washing and refrigeration) of these products.

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## Introduction

Fresh fruit and vegetables are an important component of the Australian diet. In 1998/1999 Australians consumed 135kg of fruit and 162kg of vegetables per person per year. This was a 20% increase in fruit consumption and an 8% increase in vegetable consumption from 1988/1989. Consumption is expected to have increased further since 1998/1999 due to national programs promoting fruit and vegetable daily intake (DAFF, 2009; Department of Health WA, 2005).

Outbreaks of foodborne illness have been attributed to consumption of fruit and vegetables in Australia (see Attachment 1) and around the world. The incidence of foodborne illness associated with fresh produce is increasing. In the United States the number of reported outbreaks attributed to fresh produce has greatly increased from only 1% of foodborne disease cases in the 1970's to 12% by the 1990's (Sivapalasingam *et al.*, 2004). This increase may be due to: improved epidemiology, surveillance and detection methodology; changes in production, processing and distribution practices in the fresh horticulture industry; and/or the presence and prevalence of emerging pathogens (Bassett and McClure, 2008). The increases seen in fruit and vegetable consumption may also be a contributing factor to this identified rise in outbreaks.

The *Australia New Zealand Food Standards Code* does not prescribe microbiological limits for the commodities analysed in this study other than a requirement for the absence of *Salmonella* in 25g for cultured seeds and grains (bean sprouts, alfalfa etc) (Standard 1.6.1). The FSANZ guidelines for the microbiological examination of ready-to-eat foods view the presence of pathogenic strains of *Escherichia coli* and *Salmonella* spp. and  $\geq 10^2$  cfu/g<sup>1</sup> of *Listeria monocytogenes* as potentially hazardous. It should be noted that these guidelines do not cover 'raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer'. However, the commodities in this study do not require hulling or peeling and may/may not be washed prior to consumption. As such, the FSANZ guidelines are relevant to the commodities examined in this study and suggest acceptable levels of microorganisms in fresh produce.

## Survey Objective

Food Standards Australian New Zealand (FSANZ) led this national Australian survey of microbiological contamination of selected fresh horticultural produce under the Implementation Sub-Committee (ISC) Coordinated Food Survey Plan. The aim of this survey was to gather information on the prevalence and level of contamination of fresh horticultural produce through the farm-to-table food chain in Australia.

This survey was presented and agreed to at the ISC Workshop in Adelaide in May 2004. The survey was placed on the ISC Coordinated Food Survey Plan and this 3 year rolling plan was endorsed by ISC in July 2004. The survey was planned and conducted in accordance with the goals of the Coordinated Food Survey Plan set out

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<sup>1</sup> cfu/g (colony forming units per gram)

in the document 'Protocol and Strategy for the Establishment and Operation of a Coordinated Food Survey Plan'.

This survey will support other activities coordinated through the ISC Workplan. Under component 1 of the ISC workplan this survey relates to the project on enhancing linkages between human, food and animal surveillance. Under component 6 of the ISC workplan, the survey will inform the development of an implementation plan for the production of safe seed sprouts, and the development of risk management measures for seed sprouts. This involves identifying hazards throughout the chain and implementing control measures to maximise public health and safety.

## **National Coordinated Survey Under ISC**

On 30 October 2003 the Food Regulation Standing Committee's Implementation Sub-Committee (ISC) agreed to the development of a 'Coordinated Food Survey Plan' for the Australian jurisdictions, food regulatory partners and New Zealand. This was in recognition that there were significant advantages in implementing agreed national survey priorities in a prospective and coordinated manner. ISC agreed to the conduct of a nationally coordinated microbial survey of fresh horticultural produce in Australia during 2005-2007.

## **Methodology**

The participating jurisdictions in the survey were:

- Australian Capital Territory Department of Health
- New South Wales Food Authority
- Northern Territory Department of Health and Families
- Queensland Department of Health
- South Australia Department of Health
- Tasmania Department of Health and Human Services

The survey analysed a total of 369 samples of fresh horticultural produce including 134 lettuce samples, 113 seed sprout samples, 105 strawberry samples, 15 parsley samples, and 2 basil samples. Samples were collected during the period October 2005 to July 2007.

Three sampling points were investigated in this survey:

- In the field (before harvesting, sampled where the produce was grown)
- At the farm gate (sampled after the produce had been harvested)
- At retail (sampled at retail stores)

The exception was seed sprouts, which were sampled as:

- Seeds prior to germination
- Sprouts at the end of production line
- Sprouts at retail sale

Some samples were also collected of sprout irrigation water.

All samples were bagged separately, stored below 7°C and transported directly to the laboratory for microbiological testing. Australian Standard methods (where available) were used to test for the presence of *E. coli*, *E. coli* O157:H7, verocytotoxin producing *E. coli* (VTEC), *Listeria* spp., *L. monocytogenes*, *Salmonella* spp. and faecal coliforms. Faecal coliforms and *E. coli* were also enumerated. Samples were collected by participating jurisdictions and the data was collated by FSANZ. See Attachment 2 for the detailed sampling instructions used in the survey, including the preferred analytical test methods.

Some samples were tested for the presence of the VTEC toxin rather than specifically for *E. coli* O157:H7 (*E. coli* O157:H7 is a VTEC organism). This data has been presented separately as samples tested for *E. coli* O157:H7 and samples tested for VTEC. As not all samples were tested for each pathogen, the number of samples tested is reported in the data table.

## Results

**Table 1: Summary of the Microbiological Status of Australian Horticultural Products Sampled**

Results are provided as: number of positive samples (number of samples tested)

| Produce                                 |                              | <i>E. coli</i> (MPN $\geq$ 3/g) | <i>E. coli</i> O157:H7 | VTEC            | <i>Salmonella</i> | <i>Listeria</i>  | <i>L. monocytogenes</i> |
|---|------------------------------|---------------------------------|------------------------|-----------------|-------------------|------------------|-------------------------|
| <b>Lettuce</b>                          | Field                        | 0 (n=37)                        | 0 (n=28)               | 0 (n=9)         | 0 (n=37)          | 0 (n=31)         | 0 (n=37)                |
|   | Farm gate                    | 0 (n=19)                        | 0 (n=19)               | NT              | 0 (n=19)          | 0 (n=10)         | 0 (n=19)                |
|   | Retail                       | 4 (n=78)                        | 0 (n=60)               | 0 (n=9)         | 0 (n=78)          | 0 (n=72)         | 0 (n=78)                |
|   | <b>Total</b>                 | <b>4 (n=134)</b>                | <b>0 (n=107)</b>       | <b>0 (n=18)</b> | <b>0 (n=134)</b>  | <b>0 (n=113)</b> | <b>0 (n=134)</b>        |
| <b>Seed sprouts</b>                     | Seeds prior to germination   | 0 (n=13)                        | 0 (n=4)                | 0 (n=9)         | 0 (n=13)          | 0 (n=13)         | 0 (n=13)                |
|   | Sprouts at end of production | 4 (n=34)                        | 0 (n=24)               | 1 (n=10)        | 0 (n=34)          | 0 (n=24)         | 0 (n=34)                |
|   | Retail                       | 3 (n=54)                        | 0 (n=37)               | 0 (n=8)         | 0 (n=54)          | 2 (n=48)         | 0 (n=54)                |
|   | Water                        | 2 (n=12)                        | 0 (n=1)                | NT              | 0 (n=3)           | 0 (n=3)          | 0 (n=3)                 |
|   | <b>Total</b>                 | <b>9 (n=113)</b>                | <b>0 (n=66)</b>        | <b>1 (n=27)</b> | <b>0 (n=104)</b>  | <b>2 (n=88)</b>  | <b>0 (n=104)</b>        |
| <b>Strawberries</b>                     | Field                        | 5 (n=31)                        | 0 (n=28)               | 0 (n=3)         | 1 (n=31)          | 0 (n=27)         | 0 (n=31)                |
|   | Farm gate                    | 2 (n=22)                        | 0 (n=19)               | 0 (n=3)         | 0 (n=22)          | 2 (n=19)         | 2 (n=22)                |
|   | Retail                       | 2 (n=52)                        | 0 (n=49)               | 0 (n=3)         | 0 (n=52)          | 2 (n=48)         | 2 (n=52)                |
|   | <b>Total</b>                 | <b>9 (n=105)</b>                | <b>0 (n=96)</b>        | <b>0 (n=9)</b>  | <b>1 (n=105)</b>  | <b>4 (n=94)</b>  | <b>4 (n=105)</b>        |
| <b>Parsley</b>                          | Field                        | 0 (n=3)                         | NT                     | 1 (n=3)         | 0 (n=3)           | 0 (n=3)          | 0 (n=3)                 |
|   | Farm gate                    | 0 (n=3)                         | NT                     | 0 (n=3)         | 0 (n=3)           | 0 (n=3)          | 0 (n=3)                 |
|   | Retail                       | 1 (n=9)                         | 0 (n=3)                | 0 (n=3)         | 0 (n=9)           | 0 (n=6)          | 0 (n=9)                 |
|   | <b>Total</b>                 | <b>1 (n=15)</b>                 | <b>0 (n=3)</b>         | <b>1 (n=9)</b>  | <b>0 (n=15)</b>   | <b>0 (n=12)</b>  | <b>0 (n=15)</b>         |
| <b>Basil</b>                            | Retail                       | 1 (n=2)                         | 0 (n=1)                | NT              | 0 (n=2)           | 0 (n=2)          | 0 (n=2)                 |
|   | <b>Total</b>                 | <b>1 (n=2)</b>                  | <b>0 (n=1)</b>         | <b>NT</b>       | <b>0 (n=2)</b>    | <b>0 (n=2)</b>   | <b>0 (n=2)</b>          |
| <b>Total number of positive samples</b> |                              | <b>24 (n=369)</b>               | <b>0 (n=273)</b>       | <b>2 (n=63)</b> | <b>1 (n=360)</b>  | <b>6 (n=309)</b> | <b>4 (n=360)</b>        |

MPN (Most Probable Number); NT (not tested); n (number of samples tested)

## **Lettuce**

A total of 134 lettuce samples were tested for microbiological contamination. Various types of lettuce were sampled: butter (n=15), coral (n=18), cos (n=19), iceberg (n=26), mignonette (n=14), oak (n=24) and others (n=18).

There were no pathogens detected on the lettuce samples and generic *E. coli* was only detected in retail samples (see Table 1). Generic *E. coli* was detected in four samples with levels between 3 - >1100 MPN/g.

## **Seed Sprouts**

A total of 113 seed sprout samples were collected, including 12 sprout irrigation water samples. Various types of seed sprouts were sampled: alfalfa (n=40), bean (n=7), broccoli (n=3), mung bean (n=22), onion (n=5), pea (n=2), sprout mix (n=7) and snow pea (n=15). Irrigation water was collected from a range of seed sprouts: alfalfa (n=5), bean (n=3), broccoli (n=1), mung bean (n=1), onion (n=1) and sprout mix (n=1).

One sample of broccoli sprouts sampled at the end of the production line had detectable levels of VTEC and two samples of snow pea sprouts taken at retail had detectable levels of *Listeria* spp. (no *L. monocytogenes* was detected). No *Salmonella* spp. were detected in any of the seed sprout samples tested. Generic *E. coli* was detected in nine samples with levels between 3 – 7900 MPN/g.

## **Strawberries**

A total of 105 strawberry samples were tested for microbial contamination. Pathogens and generic *E. coli* were detected in samples taken from all stages of production: in the field, at the farm gate and at retail (see Table 1).

*Salmonella* Warrigul was detected in one field strawberry sample; this sample also had an elevated generic *E. coli* level. *L. monocytogenes* was detected in four of the strawberry samples. Two of these samples were collected at the farm gate and they were both from the same packing shed. The other two *L. monocytogenes* positive samples were taken at retail and were from a common grower. *Listeria* enumeration was performed on the four positive samples, with <100 cfu/g *Listeria* spp. detected for all four samples. No *E. coli* O157:H7 or VTEC were detected in any of the strawberry samples. Generic *E. coli* was detected in nine samples with levels between 3 - >1600 MPN/g.

## **Parsley**

A total of 15 parsley samples were collected at various stages during production. VTEC was detected in one curly parsley sample from the field and generic *E. coli* was detected in one retail sample at a level of 3.6 MPN/g. No *Listeria* or *Salmonella* were detected in any of the samples (see Table 1).

## ***Basil***

Only two samples of basil were tested for microbiological contamination, both taken at retail (see Table 1). Generic *E. coli* was detected in one sample at a level of 23 MPN/g.

## **Summary of Results**

The microbiological quality of 369 fresh horticultural produce samples was assessed for the presence of *E. coli* (including VTEC and specifically *E. coli* O157:H7), *Listeria* (including *L. monocytogenes*), *Salmonella* and faecal coliforms.

Two VTEC positive samples (seed sprout and parsley), four *L. monocytogenes* positive samples (all strawberries) and one *Salmonella* positive sample (strawberries) were identified in this study. For strawberries, pathogenic contamination was found in samples taken from all stages along the produce supply chain. For seed sprouts, pathogens were detected after sprouting and at retail. No pathogen contamination was detected for lettuce.

## **Discussion**

### ***Foodborne Illness Associated with Fresh Horticultural Produce***

Contaminated fresh horticultural produce has been identified as a vehicle for human foodborne illness. Pathogenic bacteria can survive for extended periods on fresh produce and some fresh produce support bacterial growth (NACMCF, 1999). As fresh produce is generally not cooked prior to consumption, contaminated produce presents a food safety risk to consumers.

In the period from 1991 to 2007 there were 15 outbreaks in Australia associated with the consumption of fresh produce (see Attachment 1 for further details). Numerous international outbreaks have also been reported involving fresh produce, including lettuce, raspberries, spinach, spring onions and tomatoes (Bassett and McClure, 2008; Heaton and Jones, 2008).

### ***Microbiological Contamination of Fresh Horticultural Produce***

While limited by the sample size, this survey provides a snap shot of the microbiological contamination of selected fresh horticultural produce at the time of sampling (2005 – 2007). The low level of prevalence indicates that microbial contamination with pathogenic organisms, whilst uncommon, is a possibility in the Australian market.

This survey identified the presence of VTEC in seed sprouts and parsley and *L. monocytogenes* and *Salmonella* in strawberries. The presence of enteric pathogens such as VTEC and *Salmonella* could result from contamination in the field (from manure, contaminated water, livestock, wild animals and birds) or harvesting (from cross-contamination of equipment or farm workers). Both pathogenic *E. coli*

and *Salmonella* have been associated with recent outbreaks due to contaminated fresh produce (Bassett and McClure, 2008; Heaton and Jones, 2008). *Listeria* is a ubiquitous environmental contaminant found on/in plants, animals, insects, humans, soil and water sources (Sutherland *et al.*, 2003). However, the significance of its presence on fresh produce is unclear. A cluster of cases of listeriosis due to consumption of fruit salad has been reported (NSW Health, 2000), however the contamination may have occurred during processing.

### **Control Measures**

Temperature control, washing and use of sanitising agents such as chlorine are standard practice in the horticultural industry. However these procedures often have limited efficacy, especially when the organism may be attached by a bio-film and/or internalised via broken or cut tissue (Beuchat, 1996; Beuchat and Ryu, 1997; Harris *et al.*, 2003; Johnston *et al.*, 2005; Solomon *et al.*, 2002). Other mitigation strategies have been suggested to reduce the likelihood of fresh horticultural produce contamination. These include UV treatment, bacteriophages, irradiation, modified atmosphere packaging and gas flushing with carbon dioxide (Bialka and Demirci, 2007; Mahmoud and Linton, 2008; Niemira, 2007; Pao *et al.*, 2004; USDA, 2008). However, a one-size-fits-all solution is difficult to obtain due to each fruit and vegetable possessing different characteristics and inherent risks.

Other studies looking at pathogens in horticultural produce both in Australia and overseas suggests that appropriate control measures by industry may include good agricultural and manufacturing practices, minimising cross contamination opportunities and controlling the temperature of fresh horticultural produce. Also, consumers should be advised to wash all fresh produce prior to consumption. It is recommended that consumers follow the FSANZ *Listeria* factsheet<sup>2</sup> when consuming fresh horticultural produce.

### **Limitations of the Survey**

Various Australian microbiological surveys of fresh produce have previously been undertaken (see Attachment 4). These earlier surveys identified a low prevalence of pathogens with the majority of sampling only performed at retail. This current survey sampled at three different points along the horticulture produce chain. Unfortunately the same samples were not traced through the supply chain; instead, random samples were taken at the different sampling points. Therefore if a sample was found to be positive for a pathogen at one point it was not possible to trace the sample back and find the initial point of contamination. Also, due to the low sample numbers (n=3) it could not be determined if the VTEC positive curly parsley sample was an abnormality. This result does provide evidence suggesting that parsley has the potential to be contaminated with VTEC.

Of great importance to the horticultural industry, but not tested in this study, is the potential for viruses to contaminate fresh produce. Viruses such as Norovirus and Hepatitis A can potentially be transmitted to fresh produce through contaminated

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<sup>2</sup> <http://www.foodstandards.gov.au/newsroom/factsheets/factsheets2005/listeriacommonlyaske3115.cfm>

water and handling and have been implicated in past foodborne illness outbreaks associated with fresh produce (Seymour and Appleton, 2001).

## **Follow-up Action**

Overall, this small survey revealed that microbiological contamination in fresh horticultural products available in Australia is low. However, infrequent contamination with VTEC, *L. monocytogenes* and *Salmonella* was observed in some produce.

As samples were collected and analysed in each participating jurisdiction, any follow-up action was conducted by the relevant jurisdiction in which the sample was collected. The follow-up action involved contacting the farmer or producer involved (where possible) so they could review their production and handling practices.

With the *Salmonella* positive strawberry sample, manure used during strawberry production was implicated as the likely source. Further testing carried out on strawberries grown with manure did not detect any *Salmonella*. Since the sampling period, the production system has been changed and manure is no longer used, which will alleviate this problem in the future. The *Salmonella* positive batch of strawberries was used in jam as this involved heat treatment. This heat treatment would kill any *Salmonella* present on the strawberries, rendering the product safe for human consumption.

Following on from the VTEC positive seed sprout sample, jurisdictional requirements for sprout producers were revised. These revised requirements include performing a pre-screening test on all batches of seeds in which spent irrigation water from a test bath of seeds must be free from *Salmonella*. A follow-up survey was performed by the jurisdiction on seed sprouts and this survey did not detect VTEC in any samples.

Since this survey was undertaken a number of measures have been put in place by the fresh produce industry and jurisdictions to enhance the microbiological safety of these products. More recent surveys of particular sectors of this industry have found even lower levels of microbiological contamination.

## **Conclusion**

The results from this survey provide an overview of the prevalence of pathogen contamination of fresh horticultural produce (represented by lettuce, seed sprouts, strawberries, parsley and basil) in Australia during the sampling period of 2005–2007. Whilst the number of samples taken was limited and the detection of contamination in these samples infrequent, this survey indicates that pathogen contamination of fresh produce can occur. The factors that contribute to fresh produce contamination would need to be further investigated when risk management measures are considered.

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## **Attachments**

**Attachment 1:** Reported Foodborne Outbreaks Associated with Consumption of Fresh Horticultural Produce in Australia (1991 to 2007)

**Attachment 2:** Sampling Instructions Given to Participants

**Attachment 3:** Sampling Used in the Survey

**Attachment 4:** Other Australian Surveillance Activities Examining the Microbiological Quality of Fresh Horticultural Produce

**Attachment 1: Reported Foodborne Outbreaks Associated with Consumption of Fresh Horticultural Produce in Australia (1991 to 2007)**

| Year      | Product                               | Pathogen                         | Cases (death) | Location    | Contributing factor   | Reference  |
|-----------|---------------------------------------|----------------------------------|---------------|-------------|---|--|
| 1991      | Orange Juice                          | Norwalk-like virus               | >4000         | Multi-state | Unpasteurised, suspect plumbing connections   | (Food Science Australia and Minter Ellison Consulting, 2002; Lester <i>et al.</i> , 1991)) |
| 1995      | Cucumber                              | <i>Campylobacter</i> spp.        | 78            | SA          | Cross contamination, self-serve salad bar   | (Kirk <i>et al.</i> , 1997)  |
| 1997-1999 | Fruit salad                           | <i>L. monocytogenes</i>          | 9 (6)         | NSW         |   | (NSW Health, 2000)   |
| 1998      | Cold salad                            | Unknown                          | 26            | NSW         |   | (Food Science Australia and Minter Ellison Consulting, 2002)                               |
| 1998      | Salad                                 | Unknown                          | 29            | NSW         | Salad not refrigerated, poor hygiene, food handler ill  | (Food Science Australia and Minter Ellison Consulting, 2002)                               |
| 1998      | Semi-dried tomatoes with fresh garlic | <i>S. Virchow</i> PT8            | 32(1)         | NSW         | Fresh garlic imported from China, tomatoes dried at a temperature inadequate for <i>Salmonella</i> activation | (Bennett <i>et al.</i> , 2003)   |
| 1999      | Orange juice                          | <i>S. Typhimurium</i> PT 135a    | 533           | Multi-state | Unpasteurised   | (Anon, 1999; Food Science Australia and Minter Ellison Consulting, 2002)                   |
| 2001      | Lettuce                               | <i>S. Bovismorbificans</i> PT 32 | 36            | QLD         | Contaminated lettuce shredder   | (Stafford, 2002)   |
| 2004      | Red onions (sliced)                   | <i>S. Typhimurium</i> 12a        | 28            | VIC         |   | (OzFoodNet, 2004)  |
| 2005-2006 | Alfalfa                               | <i>S. Oranienburg</i>            | 126           | WA          |   | (OzFoodNet, 2006)  |
| 2006      | Bean shoots (suspected)               | <i>S. Saintpaul</i>              | 11            | VIC         |   | (OzFoodNet, 2006)  |
| 2006      | Alfalfa                               | <i>S. Oranienburg</i>            | 15            | VIC         |   | (OzFoodNet, 2007)  |
| 2006      | Cantaloupe                            | <i>S. Saintpaul</i>              | 79            | Multi-state |   | (Munnoch <i>et al.</i> , 2009, OzFoodNet, 2007))   |
| 2006      | Paw paw                               | <i>S. Litchfield</i>             | 17            | Multi-state |   | (OzFoodNet, 2007)  |
| 2007      | Baby corn                             | <i>Shigella sonnei</i>           | 55            | QLD         | Imported from Thailand  | (Lewis <i>et al.</i> , 2009)   |

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## Attachment 2: Sampling Instructions Given to Participants

### Proposed sampling plan for the horticulture survey

| <b>SEED SPROUT</b>  |   |   |   |              |
|---------------------|---|---|---|--------------|
| <b>Jurisdiction</b> | <b>Sampling Point 1</b><br>(The seed harvested)<br>Sample no. | <b>Sampling Point 2</b><br>(The sprout at end of<br>production process)<br>Sample no. | <b>Sampling Point 3</b><br>(Sprout at retail)<br>Sample no. | <b>Total</b> |
| Qld                 | 24 (8 x 3 varieties)  | 18 (6 x 3 varieties)  | 18 (6 x 3 varieties)  | 60           |
| NSW                 | 12 (4 x 3 varieties)  | 9 (3 x 3 varieties)   | 9 (3 x 3 varieties)   | 30           |
| Vic                 | 12 (4 x 3 varieties)  | 15 (5 x 3 varieties)  | 12 (4 x 3 varieties)  | 39           |
| SA                  | 12 (4 x 3 varieties)  | 15 (5 x 3 varieties)  | 12 (4 x 3 varieties)  | 39           |
| ACT                 | NA (Not Available)  | NA  | 18 (6 x 3 varieties)  | 18           |
| NT                  | NA  | NA  | 24 (8 x 3 varieties)  | 24           |
| Tas                 | NA  | NA  | 15 (5 x 3 varieties)  | 15           |
| <b>Total</b>        | 60  | 57  | 108   | 225          |

| <b>WHOLE LETTUCE</b> |   |   |  |              |
|----------------------|---|---|--|--------------|
| <b>Jurisdiction</b>  | <b>Sampling Point 1</b><br>(lettuce in the field)<br>Sample no. | <b>Sampling Point 2</b><br>(lettuce at the farm gate)<br>Sample no. | <b>Sampling Point 3</b><br>(lettuce at retail)<br>Sample no. | <b>Total</b> |
| Qld                  | 9 (3 x 3 varieties)   | 6 (2 x 3 varieties)   | 6 (2 x 3 varieties)  | 21           |
| NSW                  | 27 (9 x 3 varieties)  | 24 (8 x 3 varieties)  | 24 (8 x 3 varieties)   | 75           |
| Vic                  | 12 (4 x 3 varieties)  | 15 (5 x 3 varieties)  | 12 (4 x 3 varieties)   | 39           |
| SA                   | 9 (3 x 3 varieties)   | 9 (3 x 3 varieties)   | 12 (4 x 3 varieties)   | 30           |
| ACT                  | NA  | NA  | 21 (7 x 3 varieties)   | 21           |
| NT                   | 9 (3 x 3 varieties)   | -   | 6 (2 x 3 varieties)  | 15           |
| Tas                  | 12 (4 x 3 varieties)  | 6 (2 x 3 varieties)   | 6 (2 x 3 varieties)  | 24           |
| <b>Total</b>         | 78  | 60  | 87   | 225          |

| <b>FRESH HERB - PARSLEY</b> |   |   |  |              |
|-----------------------------|---|---|--|--------------|
| <b>Jurisdiction</b>         | <b>Sampling Point 1</b><br>(parsley in the field)<br>Sample no. | <b>Sampling Point 2</b><br>(parsley at the farm gate)<br>Sample no. | <b>Sampling Point 3</b><br>(parsley at retail)<br>Sample no. | <b>Total</b> |
| Qld                         | NA  | NA  | 12   | 12           |
| NSW                         | 3   | 3   | 3  | 9            |
| Vic                         | 8   | 8   | 8  | 24           |
| SA                          | NA  | NA  | -  | -            |
| ACT                         | NA  | NA  | 12   | 12           |
| NT                          | 3   | 3   | 3  | 9            |
| Tas                         | 3   | 3   | 3  | 9            |
| <b>Total</b>                | 17  | 17  | 41   | 75           |

| <b>FRESH STRAWBERRIES</b> |  |  |   |              |
|---------------------------|--|--|---|--------------|
| <b>Jurisdiction</b>       | <b>Sampling Point 1</b><br>(strawberries in the field)<br>Sample no. | <b>Sampling Point 2</b><br>(strawberries at farm gate)<br>Sample no. | <b>Sampling Point 3</b><br>(strawberries at retail)<br>Sample no. | <b>Total</b> |
| Qld                       | 6  | 6  | 9   | 21           |
| NSW                       | 3  | 3  | 3   | 9            |
| Vic                       | 4  | 5  | 8   | 17           |
| SA                        | 7  | 7  | 14  | 28           |
| ACT                       | NA   | NA   | -   | -            |
| NT                        | NA   | NA   | -   | -            |
| Tas                       | -  | -  | -   | -            |
| <b>Total</b>              | 20   | 21   | 34  | 75           |

**Key:**

NA Not available  
- Not to be tested

**Instructions given to participants:**

The sampling plan above outlines the proposed sample collection for jurisdictions involved in the Horticulture Survey. There are 3 separate sampling points at which samples should be collected. These sampling points are not consistent for each horticulture item. i.e. sampling point 1 and 2 for seed sprouts are not consistent with the sampling points for the three other produce items.

Each sample number in the above table represents the number of samples that need to be collected for each produce variety. Jurisdictions will be required to collect **3** different varieties for each sample number for **seed sprouts and whole lettuce only (identified by x 3 varieties in the table above)**. Jurisdictions will only need **1** variety for parsley and also for strawberries.

e.g. For Qld at Sampling Point 1: Qld will be required to collect 8 samples from 3 different seed sprout varieties. This equates to 24 samples.

Microbiological tests will need to be performed on each sample from each variety of produce at each sampling point i.e. each **sample** of seed sprouts purchased at sampling point 1 from Qld will need to be tested for all five microorganisms. (= 8 samples x 3 varieties x 5 micro-organisms = 120). The proposed microorganism tests to be tested for at each sampling point, in order of priority are:

1. *E. coli*
2. *Salmonella* spp (down to serovar)
3. *L. monocytogenes*
4. EHEC
5. Total Plate Count

\*: The five microbiological tests above represent the minimal number of microbiological tests in the survey. The actual number of microbiological tests is estimated to be twice the number indicated above due to the likely analysis of *E. coli*

O157:H7 and *E. coli* O111:H<sup>-</sup> under EHEC and *Salmonella* analysis down to serovars.

For sampling point 3, 'at retail', it is advised that jurisdictions collect samples that are both imported and domestically produced. This will allow for the comparison of microorganisms, where applicable, between Australian grown and internationally grown produce items.

Where possible, it would also be advantageous for jurisdictions that have to sample the same produce item over all 3 sampling points, that the **same** sample batch be followed through all sampling points. This will hopefully make it possible to determine where contamination of microorganisms is occurring.

Also, please try to ensure that retail samples are purchased in as many different stores/markets as possible.

Collection, transport, storage and handling of samples need to ensure cross contamination is minimised. Disposable plastic gloves and disposable breathable or perforated plastic bags must be used in sample collection. Bare hand should not be in contact with the samples. Samples collected and stored in sample bags need to be placed in an esky where the temperature is kept below 7 degree C at the sample collection and during sample transport. Once transported to a laboratory or storage place, samples should be stored in a refrigerated environment with temperature maintained as close to 0 degree C as possible. Contamination due to condensation should be avoided during transport and storage. The duration of sample storage between sample collection and actual analysis should be minimised to less than 5 days.

The following information is useful as part of the instruction to sample collectors and laboratory personnel:

**Considerations when examining raw fruits and vegetables for the presence and populations of pathogenic microorganisms (FDA):**

- Procedure for sampling
- Location of source (field, packing shed, processing plant, retail location, food service, home)
- Number and size of samples
- Distribution of samples in test lot
- Protection of samples for transport to laboratory
- Handling samples between collection and analysis
- Protection against cross-contamination
- Temperature between selection and analysis of sample
- Time between selection and analysis of samples
- Processing samples
- Weight or number of pieces to represent samples
- Area or portion to be tested (whole piece, skin only, diced, cut)
- Selection of wash fluid or diluent
- Ratio of produce to wash fluid or diluent
- Temperature of produce and wash fluid or diluent

- Soaked or not soaked before processing
- Type of processing (washing, rubbing, stomaching, homogenizing, macerating, blending)
- Time of processing
- Culturing techniques
- Enrichment and/or direct plating
- Composition and volume of enrichment broth
- Composition of direct plating medium
- Pour-plate or surface plate Incubation temperature and time
- Confirmation procedures

**The following analytical test methods are recommended for the survey:**

**1. E. coli**

AS 5013.15-2004 Food microbiology - microbiology - General guidance for the enumeration of presumptive *Escherichia coli* - Most probable number technique

**2. Salmonella spp.**

AS 5013.10-2004 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.

And

AS 5013.5-2004 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony count technique at 30°C.

**3. L. monocytogenes**

Detection and enumeration of *Listeria monocytogenes* in foods (Bacteriological Analytical Manual online) - US FDA and Centre for Food Safety & Applied Nutrition  
<<http://www.cfsan.fda.gov/~ebam/bam-10.html>>

Or

ISO 11290-2 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method

**4. EHEC (*E. coli* O157:H7 only)**

Diarrheagenic *Escherichia coli* (Bacteriological Analytical Manual online) - US FDA and Centre for Food Safety & Applied Nutrition  
<http://www.cfsan.fda.gov/~ebam/bam-4.html>

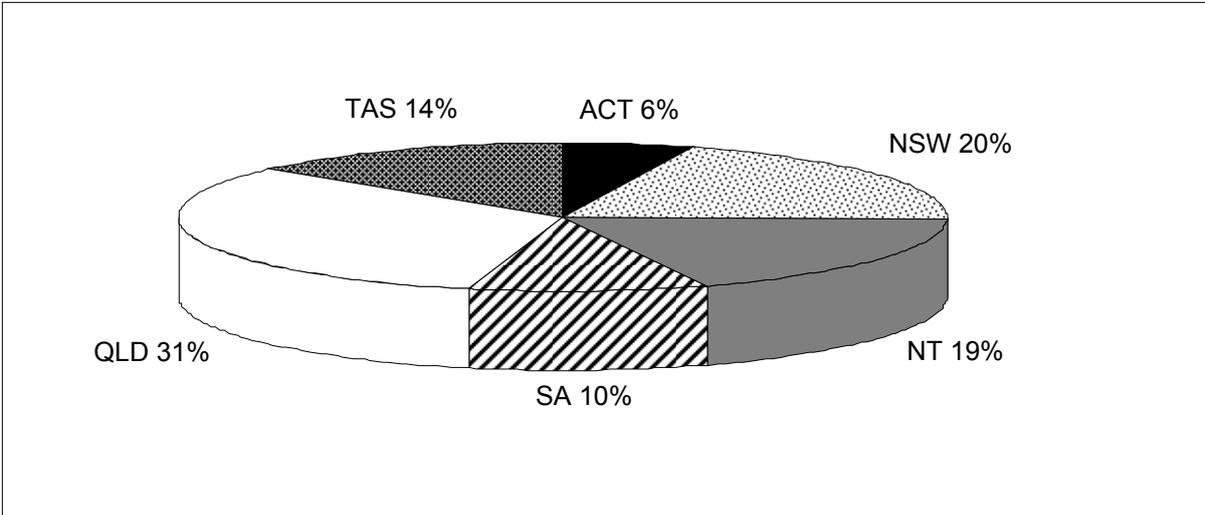
**5. Total Plate Count**

AS 5013.1-2004 Food microbiology - Examination for specific organisms - Standard plate count,

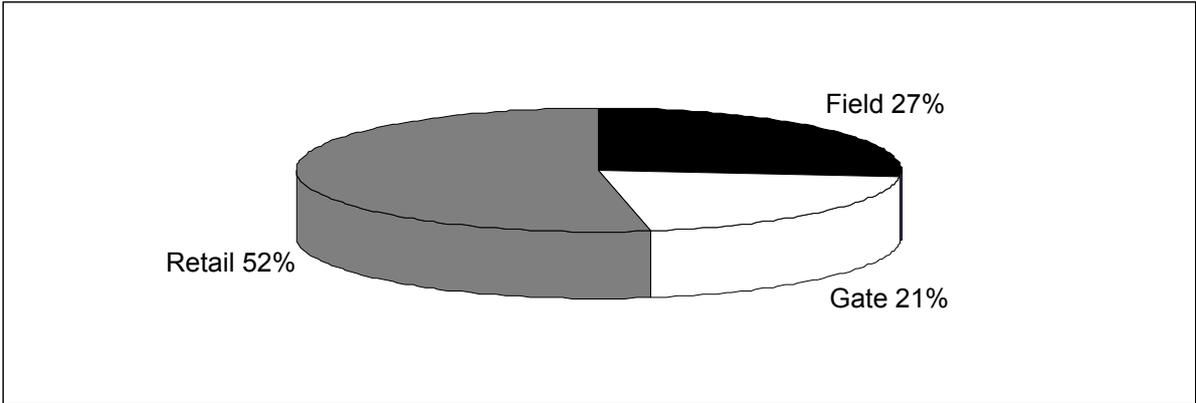
And

AS 5013.5-2004 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony count technique at 30°C.

**Attachment 3: Sampling Used in the Survey**



**Figure A: Collection of samples by state**



**Figure B: Collection of samples by location**

**Attachment 4: Other Australian Surveillance Activities Examining the Microbiological Quality of Fresh Horticultural Produce**

| <b>Year</b>                             | <b>Jurisdiction</b>                 | <b>Subject of the survey</b>                                      | <b>Reference</b>                |
|---|-------------------------------------|---|---------------------------------|
| 2008                                    | NSW Food Authority                  | Microbiological quality of sprouts                                | (NSW Food Authority, 2008)      |
| 2007                                    | OzFoodNet – Northern Territory      | Food sampling at fresh produce markets                            | Personal communication          |
| 2007                                    | SA Department of Health             | Product sampling, including fresh produce                         | Personal communication          |
| 2006                                    | NSW Food Authority                  | Fresh cut microbiology survey                                     | Personal communication          |
| 2006                                    | VIC Department of Health            | Microbiological quality of salad vegetables                       | (DPI VIC, 2006)                 |
| 2005                                    | WA Department of Health             | Microbiological quality of retail fruit and vegetables            | (Department of Health WA, 2005) |
| Apr–Jun 2001                            | ACT (ACT Health Protection Service) | Microbiological quality of seed sprouts                           | (Millard and Rockliff, 2001)    |
| Jan–Mar 2000                            | WA (WA Food monitoring program)     | Microbiological quality of sprouts                                | (Department of Health WA, 2002) |
| 1999                                    | Queensland (Food watch Queensland)  | Microbiological quality of ready-to-eat fruit and vegetables      | (QLD Health, 1999)              |
| 1999                                    | Queensland (Food watch Queensland)  | <i>Salmonella</i> in alfalfa sprouts and sprout seeds             | (QLD Health, 1999)              |
| Jan-Jun 95<br>Oct-Dec 96<br>July-Sep 98 | ACT (ACT Health Protection Service) | Microbial quality of self serve salad bars                        | (Millard, 1998)                 |
| Oct 97-May98                            |                                     | Psychrotrophic bacterial pathogens in minimally processed lettuce | (Szabo et al 2000)              |

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