Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2009-2010 Targeted Surveys

Targeted Survey Investigating Bacterial Pathogens and Generic *E. coli* in Fresh Leafy Green Vegetables

Canada

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Executive Summary

The Food Safety Action Plan (FSAP) aims to modernize and strengthen Canada's food safety system in order to better protect Canadians from unsafe food and ultimately reduce the occurrence of foodborne illness.

Leafy green vegetables have been reported to be responsible for numerous outbreaks of foodborne illness worldwide. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has ranked leafy green vegetables as the highest priority among fresh fruits and vegetables in terms of microbiological hazards. After harvest, leafy vegetables are only subject to minimal processing (trimming, cutting, sanitizing, washing and packaging) and are often consumed raw. As such, pathogens introduced during any step of production may not only survive but also multiply. The bacterial pathogens *Salmonella* and *Escherichia coli* (*E. coli*) O157:H7 have been related to the majority of the global outbreaks of foodborne illness associated with leafy vegetables. In addition, the bacterial pathogen *Listeria monocytogenes* (*L. monocytogenes*) has been identified as the primary pathogen of concern in ready-to-eat (RTE) foods including fresh-cut RTE leafy vegetables due to its wide distribution in the environment and its ability to grow under refrigeration temperatures.

Considering these factors and their relevance to Canadians, leafy vegetables have been selected as one of the priority commodity groups of fresh fruits and vegetables for enhanced surveillance under the FSAP. During five annual microbiological targeted surveys (2008/09 - 2012/13), over 10,000 leafy vegetable samples will be collected from retail locations and tested for the presence of various pathogens of concern.

This targeted survey (2009/10) focussed on bacterial pathogens of concern and generic *E. coli* (as an indicator of fecal contamination). The main objectives of this survey were to generate baseline surveillance data on:

- the presence and distribution of bacterial pathogens of concern: *E. coli* O157:H7/NM (non-motile) and *Salmonella* species *(*spp.) in whole leafy vegetables and in pre-packaged, fresh-cut, RTE leafy vegetables (hereafter referred to as "fresh-cut leafy vegetables");
- the presence, distribution, and levels of generic *E. coli* in whole and fresh-cut leafy vegetables; and
- the presence, distribution, and levels of the bacterial pathogen *L. monocytogenes* in fresh-cut leafy vegetables.

In this survey, a total of 4250 samples including imported and domestic, conventional and organically grown, whole and fresh-cut leafy vegetables were collected and tested for the

bacterial pathogens *E. coli* O157:H7/NM, *Salmonella* spp. as well as generic *E. coli*. *E. coli* O157:H7 and *E. coli* O157:NM were not detected in any of the samples. *Salmonella* spp. and generic *E. coli* (>100 colony forming units (CFU)/g) were not detected in 99.9% of the samples. A total of five samples (0.1 %) were found to be unsatisfactory: two samples (0.05%) due to the presence of *Salmonella* with the other three samples showing high levels of generic *E. coli* (> 1000 CFU/g). In addition, an elevated level of generic *E. coli* was found in another sample (0.02%). This sample was assessed as investigative and requiring further evaluation, as the *E. coli* counts were elevated but below the unsatisfactory threshold of 1000 CFU/g.

All fresh-cut leafy vegetable samples (1850) were also tested for *L. monocytogenes*. *L. monocytogenes* was not detected in 99.6% of the samples. A total of seven samples (0.4%) were found to be unsatisfactory due to the detection of the pathogen. However, the levels of contamination were below 100 CFU/g when enumeration was performed, a low level generally considered to pose very little risk in this type of food.

To assist in the food safety investigations, pulsed field gel electrophoresis (PFGE) patterns (i.e., DNA typing) of *L. monocytogenes* and *Salmonella,* as well as serotypes of *Salmonella,* were identified from the respective isolates of the positive samples.

All unsatisfactory samples were subject to food safety investigations and appropriate follow-up activities were conducted by the Canadian Food Inspection Agency (CFIA). Two product recalls resulted from the referral of the unsatisfactory samples and subsequent investigations. It is important to note that there were no reported illnesses associated with consumption of any of the products in this survey.

Results of the 2009-10 survey indicate that bacterial pathogens were not detected in the majority of the leafy vegetable samples, including fresh-cut RTE leafy vegetable samples, tested in this survey. A very small fraction of the leafy vegetable samples was found to be contaminated with bacterial pathogens or high levels of generic *E. coli*. The results suggest that not all leafy vegetables in the Canadian market were produced under Good Agricultural Practices (GAPs) and/or Good Manufacturing Practices (GMPs), processed and maintained under sanitary conditions during production and processing, and/or stored at appropriate refrigeration temperatures. The preliminary findings also could be indicative that contamination of leafy vegetables with pathogenic micro-organisms does occur and may be a potential source of foodborne illness in Canada. Results of the three remaining microbiological targeted surveys on leafy vegetables will be released annually upon completion.

1 Introduction

1.1 Food Safety Action Plan

In 2007, the Canadian government launched a five-year initiative in response to a growing number of product recalls and concerns about food safety. This initiative, called the Food and Consumer Safety Action Plan (FCSAP) (1), aims to modernize and strengthen the food safety regulatory system. The FCSAP initiative unites multiple partners in ensuring safe food for Canadians.

The Canadian Food Inspection Agency's (CFIA) Food Safety Action Plan (FSAP) (2) is one element of the government's broader FCSAP initiative. The goal of FSAP is to identify risks in the food supply, limit the possibility of occurrence of these risks, improve import and domestic food controls, and identify food importers and manufacturers.

Within the FSAP, there are twelve main areas of activity, one of which is risk mapping and baseline surveillance. The main objective of this area is to better identify, assess and prioritize potential food safety hazards through risk mapping, information gathering and analysis of foods in the Canadian marketplace. Targeted surveys represent one tool used to test for the presence and level of particular hazards in specific foods.

1.2 Targeted Surveys

Microbiological targeted surveys are used to gather information regarding the potential occurrence of microbiological hazards in defined food commodities. The surveys are designed to focus on priority and/or emerging food hazard issues and to address areas not covered by regular CFIA monitoring activities.

The microbiological targeted surveys aim to establish baseline data on priority microbiological hazards in targeted commodities, primarily fresh fruits and vegetables and imported food ingredients. Based on the priority of microbiological risk associated with particular commodity groups and to account for seasonal variation and other factors, a statistically significant number of leafy green vegetable samples will be collected over the five years of planned FSAP targeted surveys. This work differs from regular CFIA microbiological monitoring activities, which test a limited number of samples of a broad range of commodities for multiple hazards.

To identify food-hazard combinations of greatest potential health risk for the targeted surveys, the CFIA uses a combination of scientific literature, documented outbreaks of foodborne illness, and/or information gathered from the Food Safety Science Committee

(FSSC), a group of Canadian federal, provincial and territorial subject matter experts in the area of food safety (3).

This survey (2009/10) represents part of the collection of over 10,000 leafy green vegetable samples over five years (2008/09 – 2012/13) of microbiological targeted surveys. The first targeted survey (2008/09) considered the bacterial pathogens *E. coli* O157:H7, *Salmonella* and *Shigella*, as well as generic *E. coli* in imported and domestic leafy vegetable samples. This survey focuses on investigating the presence of bacterial pathogens of concern and the presence and levels of generic *E. coli* in imported and domestically produced whole and fresh-cut leafy vegetables available in the Canadian market.

1.3 Codes of Practice, Acts, and Regulations

Food safety standards are developed under the joint FAO/WHO Food Standards Programme. Producers of fresh fruits and vegetables (including leafy vegetables) are encouraged to follow the international codes of practice developed by the Codex Alimentarius Committee, which provide guidance for the safe production of food. Of relevance for this survey are the *Code of Hygienic Practices for Fresh Fruits and Vegetables* (CAC/RCP 53-2003) and the *Recommended International Codes of Practice-General Principles of Food Hygiene* (CAC/RCP 1-1969) (4, 5). These codes address GAPs and GMPs which, when applied, control and reduce the potential for contamination with microbial, chemical, and physical hazards at all stages of production of fresh fruits and vegetables, from primary production to packaging. They outline basic requirements pertaining to environmental hygiene, hygienic production (water, manure, soil biological control, packing, facility and personal hygiene), handling, storage, transportation, maintenance and sanitation.

Fresh fruits and vegetables (including leafy vegetables) available in the Canadian market must comply with the *Food and Drugs Act* (FDA) and the *Food and Drug Regulations* (FDR), which prescribe certain restrictions on the production, importation, sale, composition and content of foods and food products. Section 4(1)a of the FDA prohibits the sale of food contaminated with foodborne pathogens, while sections 4(1)e and 7 prohibit the sale of unsafe food and food produced under unsanitary conditions.
In order to achieve compliance with the FDA and the FDR, the CFIA has developed the *Code of Practice for Minimally Processed Ready-to-Eat Vegetables* (6). This code is intended to provide guidance for the safe manufacturing of minimally processed RTE vegetables consisting of raw vegetables that have been peeled, sliced, chopped or shredded prior to being packaged for sale in Canada.

Fresh fruits and vegetables sold in Canada must also comply with safety requirements of the *Fresh Fruit and Vegetable Regulations* under the *Canada Agricultural Products Act.* These regulations are intended to ensure that fresh fruits and vegetables sold to consumers are safe, wholesome, and properly graded, packaged and labelled. Both the *Fresh Fruit and Vegetable Regulations* and the food-related portions of the FDA and FDR are enforced by the CFIA.

FSAP targeted surveys are conducted for surveillance and not for regulatory purposes. However, bacterial pathogens and/or high levels of generic *E. coli* detected in any samples tested under this survey would trigger food safety investigations, including activities such as follow-up sampling, inspections of facilities, and health risk assessments. Depending on the findings, a recall of the affected product may be recommended and/or implemented.

2 Description of the Hazards and Survey Details

2.1 Microbiological Hazards in Leafy Green Vegetables

The presence of pathogens in leafy vegetables creates a potential risk for foodborne illness as they are often consumed raw. Leafy vegetables have been reported to be responsible for numerous outbreaks of foodborne illnesses worldwide. Due to historical outbreaks associated with leafy vegetables, the potential for contamination during pre-harvest, post-harvest, processing and global distribution along with other evidence, leafy vegetables were identified as a level one (highest) priority in fresh fruits and vegetables in terms of microbiological hazards during a 2007 joint FAO/WHO Expert Meeting (7).

Processing (e.g., cutting, shredding, packaging) and storage of fresh-cut vegetables may provide further opportunities for cross-contamination and potential for growth of bacterial pathogens. For example, cutting releases fluid from the vegetables, which promotes the growth of bacteria (8). Furthermore, inappropriate temperatures during preparation, distribution and/or storage can also encourage the growth of bacteria on RTE leafy vegetables (9, 10).

Production practices can also affect the microbial load of leafy vegetables. For example the use of animal manure has led to concerns about the potential contamination of vegetables with human pathogens such as *E. coli* O157:H7/NM, *Salmonella* spp. and *Shigella* spp. Although proper composting of manure will inactivate pathogens, they can survive for extended periods in improperly composted manure and can subsequently contaminate fresh vegetables grown in soil amended with that manure. Since organic productions are more reliant on the use of manure to fertilize fields, it has been suggested that organic produce may face higher levels of microbial contamination.

This survey included leafy vegetables produced using conventional farming methods and leafy vegetables produced using organic certification standards in order to gather information on whether different practices used for growing produce have an impact on its safety.

2.1.1 Global Foodborne Disease Outbreaks Associated with Leafy Green Vegetables Contaminated with Bacterial Pathogens

From 1998 to 2009, 59 foodborne disease outbreaks associated with leafy vegetables contaminated with bacterial pathogens were reported worldwide, with most of them occurring in North America (information based on data compiled by the Public Health Agency of Canada (PHAC), Table 1 and Appendix B). The frequency of outbreaks associated with *E. coli* O157 or *Salmonella* spp. was higher than the frequency due to other pathogenic bacteria. None of these reported outbreaks were associated with *L. monocytogenes.*

Table 1. Global Foodborne Disease Outbreaks Associated with Leafy Vegetables Contaminated with Bacterial Pathogens (1998-2009)*

Bacterial Pathogens	Outbreaks	
	Number of Outbreaks	Percentage of Outbreaks
E. coli O157	28	47.5
Other pathogenic *E. coli*	4	6.8
Salmonella spp.	18	30.5
Shigella spp.	3	5.1
Campylobacter spp.	3	5.1
Clostridium perfringens	2	3.3
Yersinia pseudotuberculosis	1	1.7
Total	**59**	**100**

* Summarized according to Appendix B

The leafy vegetables implicated in these outbreaks were various types of lettuce (51 outbreaks, 86.4%) and spinach (8 outbreaks, 13.6%). Among these 59 outbreaks associated with leafy vegetables, five occurred in Canada. The identified pathogens were *E. coli* O157:H7 (three outbreaks), *Salmonella* spp. (one outbreak) and *Shigella* spp.(one outbreak).

2.1.2 Bacterial Pathogens in Leafy Green Vegetables

Publicly available information was also used to assess what bacterial pathogens have been identified in microbiological surveys on leafy vegetables carried out in other jurisdictions. While these results may not be directly transferable to Canada, they are considered either to be representative of similar production practices or viewed as good indicators of what might be found.

Surveys were conducted by Ontario Agriculture (2004) (11) and Alberta Agriculture and Rural Development (2007) (12) on provincial fresh produce including leafy vegetables. *Salmonella* spp. were detected in 0.2% (1/530) of Ontario-grown fresh lettuce (11). None was detected in Alberta-grown leafy green samples (187 lettuce and spinach samples) (12). In surveys conducted by the U.S. Food and Drug Administration (US FDA) from 1999 to 2000 on fresh fruits and vegetables (including leafy vegetables), *Salmonella* spp. were found in 0.7% (1/142) of U.S. domestically produced (13) and in 1.7% (2/116) of imported leafy vegetable samples (lettuce) (14). Other surveys on retail fresh fruits and vegetables in Spain (2005-2006) (15) and Brazil (2004) (16) found that *Salmonella* spp. were identified in 1.6% (4/246) and 3.6% (4/111) of leafy vegetable samples (including fresh-cut RTE leafy vegetables), respectively. All of these surveys (except for the one carried out in Brazil) were also tested for *E. coli* O157:H7, which was not found in any of the samples tested.

Available survey data on *L. monocytogenes* in RTE foods also indicate a potential microbiological risk associated with fresh-cut RTE leafy vegetables. *L. monocytogenes* was found in 0.7% (22/2966), 0.9% (2/218), and 0.9% (1/111) of fresh-cut leafy vegetable retail samples in surveys conducted in the U.S. (2000-2001) (17), Spain (2005-2006) (15), and Brazil (2004) (16), respectively. Levels of *L. monocytogenes* contamination in the fresh-cut RTE leafy vegetables samples were generally less than 100 CFU/g, suggesting that a low level of contamination or a limited growth of *L. monocytogenes* occurred. However, one of the 22 positive samples from the U.S. study (17) and one of the two positive samples from the Spanish study (15) were detected at levels greater than 100 CFU/g.

In this survey (2009/10), the bacterial pathogens *E. coli* O157:H7/NM, *Salmonella* spp., and *L. monocytogenes,* as well as generic *E. coli* as an indicator of fecal contamination were targeted.

2.2 Bacterial Pathogens of Concern and Generic *E. coli*

Bacterial pathogens, such as *Salmonella* spp. and *E. coli* O157, are found naturally in the intestines of animals, e.g., poultry and cattle respectively. Most outbreaks associated with these bacterial pathogens are linked to consumption of contaminated food of animal origin (e.g., chicken and beef burger). However, fresh fruits and vegetables have emerged as significant sources of *Salmonella* and *E. coli* O157 related illnesses in the last decade. Fruits and vegetables can be contaminated with these bacterial pathogens in the field by improperly composted manure, contaminated water, wildlife feces, or poor hygienic practices of the farm workers (18).

2.2.1 *Salmonella* spp.

There are over 2,500 serotypes of *Salmonella* spp., many of which are capable of causing human disease, known as salmonellosis.

Salmonellosis is one of the most common foodborne illnesses worldwide. There were approximately 6,000 cases of *Salmonella* infection reported in Canada annually from 2000 to 2004, according to available information from the Public Health Agency of Canada (PHAC) (19). It is believed that the actual number of infections is much higher due to under reporting (in other words, only a fraction of the true number of cases are reported) (19). In the U.S., approximately 30,000 to 40,000 of laboratory-confirmed cases of salmonellosis were reported to the CDC each year between 2000 and 2009 (20). However, it is estimated that 1.4 million cases of *Salmonella* infection occur annually, and that the annual cost associated with these illnesses represents approximately US $2.7 billion (20, 21).

2.2.2 Pathogenic *E. coli*

A few strains of *E. coli* are capable of causing human disease. Based on the disease syndromes and characteristics, there are currently five recognized classes of pathogenic *E. coli* that cause gastroenteritis in humans: enteroaggregative, enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic *E. coli* (22, 23). The enterohemorrhagic *E. coli*, a subset of Verotoxin-producing *E. coli* (VTEC), can produce Shiga-like toxin and cause severe diarrhea. This class of *E. coli* includes the predominant disease-causing *E. coli* O157 (*E. coli* O157:H7 and *E. coli* O157:NM) and other emerging disease-causing non-O157 *E. coli*.

In Canada, 1130 cases of VTEC infections were reported in 2004 and the majority (94%) of the VTEC infections were caused by *E. coli* O157 serotype (19). Similarly, in the U.S., a total of 2348 laboratory-confirmed cases of *E. coli* O157 infections (91%) were

reported in 2005 (24). Under reporting was also existing in reported cases of VTEC infection.

2.2.3 *Listeria monocytogenes*

Listeria spp. are bacteria that are widely distributed in the environment. Of the several species of *Listeria*, *L. monocytogenes* is known to cause an infection in humans called listeriosis (25).

Listeriosis is a relatively uncommon disease but one with potentially serious clinical consequences. There are two forms of listeriosis: non-invasive and invasive. Most people fully recover from non-invasive listeriosis. Invasive listeriosis occurs in people with weakened immune systems. Pregnant women and their unborn babies, newborns, the elderly, and individuals with compromised immune systems are at higher risk. The mortality rate of invasive listeriosis is approximately 30%.

Compared to other bacterial pathogens, *L. monocytogenes* has an abnormally wide range of growth temperatures (i.e., -0.4 to 45ºC) that includes the typical refrigeration temperature of 4°C (25). Therefore, strict refrigeration temperature control is important to limit the growth of *L. monocytogenes* on fresh-cut vegetables during processing, transportation, storage and display at retails (9, 10). The levels of *L. monocytogenes* have been reported to remain constant on fresh-cut RTE vegetables that were stored at 4°C for nine days (9). However, storage at room temperature (25°C) or at a temperature of 10°C increased levels of *L. monocytogenes* on the fresh-cut RTE vegetables (10).

Since *L. monocytogenes* is widely distributed in nature, it is present in a wide variety of foods, including raw vegetables. Likely sources of vegetable contamination include soil, contaminated irrigation water or wash water, decaying vegetation, as well as the processing and packaging environment. Chemical disinfection and irradiation treatment can only partially reduce existing *Listeria* spp. contamination (26, 27). Contaminated fresh-cut vegetables, that are capable of supporting the limited growth of the bacteria at refrigeration temperatures, have been implicated in a few outbreaks of foodborne listeriosis (28).

2.2.4 Generic *E. coli* as an Indicator of Fecal Contamination

Typically, *E. coli* bacteria that inhabit the large intestines of humans and animals are harmless. Due to their regular presence in stools of humans and animals, the occurrence of *E. coli* in foods indicates direct or indirect contamination with fecal matter. Generic *E. coli* counts in fresh produce and other RTE foods indicate GAPs and/or GMPs breakdown or that general cleanliness and sanitary conditions during production, packaging, and storage were inadequate. The presence of generic *E. coli* in foods can

also indicate potential contamination with pathogenic enteric micro-organisms, such as *Salmonella* or *E. coli* O157, that also live in the intestines of infectious humans and animals. It is important to note that the presence of generic *E. coli* in food only implies an increased risk of contamination with pathogenic micro-organisms but does not conclusively indicate that these pathogenic organisms are present.

2.3 Rationale

The overall objective of the FSAP targeted surveys between 2008/09 to 2012/13 is to gather baseline information on the occurrence of pathogens of concern (pathogenic bacteria, viruses and parasites) and indicator bacteria of fecal contamination (generic *E. coli*) in fresh fruits and vegetables, and imported food ingredients available to Canadians at retail. The 2009-10 targeted survey is part of the information collection with a focus on investigating the presence of bacterial pathogens (*E. coli* O157:H7/NM, *Salmonella*, and *L. monocytogenes*) and the presence and levels of generic *E. coli* in imported and domestic, conventional and organic, whole and fresh-cut leafy green vegetables.

Leafy green vegetables have been selected as one of the priority commodity groups of fresh fruits and vegetables for enhanced surveillance under FSAP. The selection was based on a range of factors, including historical outbreaks of foodborne illness and their relevance to Canadians, information gathered from microbiological risk assessment on fresh fruits and vegetables by the FAO/WHO experts (2007) (7) and FSSC (2008) (3).

2.4 Sample Collection

Leafy vegetable samples included whole lettuce (iceberg, romaine, green and red leaf, butter, baby leaf), spinach, escarole endive, Swiss chard, arugula, watercress, and chicory, as well as pre-packaged, fresh-cut lettuce and salad blends or mixes.

All samples were collected from national chain and local/regional grocery stores as well as other conventional retail and natural food stores located across Canada. The number of samples collected in various geographic regions across Canada was based on the relative proportion of the population in the respective regions. Domestic samples were collected during the summer months (June-September). Imported samples were collected primarily in the fall, winter, and spring months.

In this survey, a sample comprised a single sampling unit (e.g., an individual consumer-size package) with a total weight of at least 200 g. Collected samples were required to be shipped under conditions that limited the growth of micro-organisms during transit.

2.5 Sample Distribution

2.5.1 Overview of Samples Collected

A total of 4250 leafy vegetable samples, including whole and fresh-cut leafy vegetables, were collected. The distribution of the leafy vegetable samples by product origin (imported or domestic) and production practices (conventional and organic) is presented in Table 2.

Table 2. Distribution of Leafy Vegetable Samples by Product Origin

Product Origin	Production Practice	Number of Samples	Percentage of Subgroup	Percentage of Total Samples
Imported	**Conventional**	3023	90.9	71.1
	Organic	301	9.1	7.1
	Subtotal	***3324***	***100***	***78.2***
Domestic	**Conventional**	620	67.0	14.6
	Organic	306	33.0	7.2
	Subtotal	***926***	***100***	***21.8***
Total		**4250**		**100**

The majority of the imported samples originated from the U.S. (96.9%); the rest originated from Mexico, the Dominican Republic, and unidentified countries (Table 3).

Table 3. Distribution of Imported Leafy Vegetable Samples by Country of Origin

Country of Origin	Production Practice		Total Samples	
	Conventional	**Organic**		
	Number of Samples	Number of Samples	Number of Samples	Percentage of Samples
Dominican Republic	32	0	32	1.0
Mexico	31	11	42	1.3
United States	2932	289	3221	96.9
Unidentified	28	1	29	0.9
Total	**3023**	**301**	**3324**	**100**

The leafy green vegetable samples consisted of whole leafy vegetable samples (56.5%, 2400/4250) and fresh-cut leafy vegetable samples (43.5%, 1850/4250). Of the fresh-cut leafy vegetable samples, which were additionally tested for *L. monocytogenes*, 93.4% were both imported and conventionally produced.

2.5.2 Sample Distribution by Product Type

More than 14 different types of leafy vegetable samples and five types of fresh-cut leafy vegetable samples were collected for the analyses (Table 4).

Table 4. Distribution of Leafy Vegetable Samples and Fresh-cut Leafy Vegetable Samples by Product Type

Product Type	Leafy Vegetable Samples (whole & fresh-cut)		Fresh-cut Leafy Vegetable Samples	
	Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples
Arugula	95	2.2	0	0
Chicory	27	0.6	0	0
Endive	13	0.3	0	0
Escarole	13	0.3	0	0
Kale	22	0.5	0	0
Lettuce-head	28	0.7	0	0
Lettuce-leaf	301	7.1	0	0
Lettuce-romaine	509	12.0	134	7.2
Lettuce-not specified	993	23.4	754	40.7
Swiss chard	151	3.6	0	0
Spinach	555	13.1	41	2.2
Salad mix	852	20.0	720	38.9
Watercress	13	0.3	0	0
Other*	678	15.9	201	10.9
Total	**4250**	**100.0**	**1850**	**100.0**

*Other refers to leafy vegetable types that are either not specified (e.g. a mix of leafy vegetables) or for which only a small number of samples was collected (e.g. number of samples accounted for less than 0.1%).

2.6 Method Details

All samples were analyzed using the analytical methods published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods (29) (Appendix C). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of fresh fruit and vegetables samples, including leafy green vegetables.

For the detection of *E. coli* O157:H7/NM and *Salmonella*, a two-step procedure was employed. Samples were first screened by polymerase chain reaction (PCR)-based methods. Any presumptive positive results required confirmation by isolation, purification and identification procedures. For the detection of *L. monocytogenes*, samples were set-up using the cultural method for *L. monocytogenes* isolation and confirmation from foods. Enumeration was performed on samples that were confirmed positive.

If any pathogens were detected, these isolates were further characterized by pulsed field gel electrophoresis (PFGE) (i.e., DNA typing) at the CFIA Ottawa Laboratory (Fallow field). Serotyping for *Salmonella* spp. was performed at the *Salmonella* Typing Laboratory, Laboratory for Foodborne Zoonoses, PHAC in Guelph, Ontario.

The count of generic *E. coli* was obtained using the most probable number (MPN) or direct plating procedures.

2.7 Assessment Guidelines

The following assessment criteria were developed for this targeted survey based on the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Food* (30) and associated methods published in Health Canada's *Compendium of Analytical Methods* (29), as well as Health Canada's "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (2004)" (this policy was updated in 2011) (28). The presence or absence of *E. coli* O157:H7/NM, *Salmonella*, or *L. monocytogenes* was determined from a 25 g sample unit drawn from a sample submitted for analysis. A positive result (presence in 25 g) was assessed as unsatisfactory, while a negative result (absence in 25 g) was assessed as satisfactory (Table 5).

Table 5. Assessment Guidelines for Bacterial Pathogens in Leafy Vegetables

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
***E. coli* O157:H7/NM** (MFLP-30 & Supplement 2, and MFLP-80)	Absent in 25 g	Present in 25 g
Salmonella* spp.* (MFLP-29 & MFHPB-20)	Absent in 25 g	Present in 25 g
L. monocytogenes (MFHPB-30 & MFLP-74)	Absent in 25 g	Present in 25 g

* *Compendium of Analytical Methods* (29)

**No criteria have been established by Health Canada at this time for *Salmonella* in fresh fruits and vegetables. However, in the absence of a specified criteria, presence in foods is considered to be a violation of FDA Section 4(1)a and is therefore assessed by the CFIA as unsatisfactory.

A satisfactory assessment for the generic *E. coli* in this survey indicated that the organisms were either not detected in the count, or found at very low levels (i.e., ≤ 100 CFU/g). *E. coli* counts in the range of 100 - 1000 CFU/g were assessed as investigative and may require some form of follow-up activity. For example, further sampling may have been done to verify the levels of generic *E. coli* in the samples in question. Results indicating *E. coli* levels above 1000 CFU/g were assessed as unsatisfactory (Table 6).

Table 6. Assessment Guidelines for Generic *E. coli* in Fresh Leafy Vegetables

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**		
	Satisfactory	**Investigative**	**Unsatisfactory**
Generic *E. coli* (MFHPB-19 & 27)**	≤ 100	100 < x ≤ 1000	> 1000

* *Compendium of Analytical Methods* (29).

** Concentration unit depends on method used. For MFHPB-19 method: MPN/g (Most Probable Number/gram), for MFHPB-27 method: CFU/g (Colony Forming Units/gram).

Unsatisfactory sample assessments were subject to follow-up actions, such as directed follow-up sampling, inspection of establishment, health risk assessment, and/or product action (e.g., product recall).

2.8 Survey Limitations

All samples in this survey were collected at retail. Sampling at retail imposes certain limitations with respect to the traceability of products in the case of positive results. It may not be possible to identify the source of contamination since the samples were collected from bulk or from units pre-packaged at packing facilities or at the retail level.

In addition, to properly assess the compliance of a lot against microbiological standards, five sample units are typically drawn at random from a production lot. However, in this survey, single sample units were collected from partial lots displayed at retail. In cases of positive results, these factors would have to be taken into consideration during the food safety investigations and health risk assessments.

Furthermore, imported samples were collected from available samples at retail without a requirement for the number of samples to be collected per foreign country. In cases of positive results, unsatisfactory rates between countries are not considered to be statistically comparable. Likewise, organic samples were collected based on availability at retail and appeared to be infrequently available. In cases of positive results, unsatisfactory rates between organic versus conventional samples are also not considered to be statistically comparable.

3 Results

3.1 *E. coli* O157:H7/NM, *Salmonella* spp. and Generic *E. coli* in Leafy Vegetable Samples (Whole and Fresh-cut)

E. coli O157:H7 and *E. coli* O157:NM were not detected in any of the leafy vegetable samples in this survey. *Salmonella* spp. and generic *E. coli* were not found in the majority (99.9%, 4244/4250) of leafy vegetable samples (Table 7).

Table 7. Summary of Assessment Results of Leafy Vegetable Samples Analyzed for *E. coli* O157:H7/NM, *Salmonella* spp. and Generic *E. coli*

Product Origin	Production Practice	Number of Samples	Assessment					
			Investigative		Unsatisfactory		Satisfactory	
			Number of Samples	Percent-age of Samples	Number of Samples	Percent-age of Samples	Number of Samples	Percent-age of Samples
Imported	**Conventional**	3023	0	0	3	0.1	3020	99.9
	Organic	301	0	0	1	0.3	300	99.7
	Subtotal	***3324***	***0***	***0***	***4***	***0.1***	***3320***	***99.9***
Domestic	**Conventional**	620	1	0.2	0	0	619	99.8
	Organic	306	0	0	1	0.3	305	99.7
	Subtotal	***926***	***1***	***0.1***	***1***	***0.1***	***924***	***99.8***
Total		**4250**	**1**	**0.02**	**5**	**0.12**	**4244**	**99.9**

A total of five samples (0.1%) were found to be unsatisfactory. Two samples were unsatisfactory due to the presence of *Salmonella* (0.05%, 2/4250) and the other three samples had high levels (>1000 CFU/g) of generic *E. coli* (0.07%, 3/4250) (Table 7). *Salmonella* serotypes *S.* IV:50:z4,z23:- and *S.* IIIb:ROUGH-O:-:- were identified from the *Salmonella* isolates (Table 8).

When considered by production practice, 0.3% (2/607) of organically grown samples and 0.1% (3/3643) of conventionally grown samples (3/3643) were found to be unsatisfactory.

All unsatisfactory samples were subject to food safety investigations and appropriate follow-up activities were conducted by the CFIA. One product recall resulted from the referral of the unsatisfactory samples to the appropriate CFIA programs and subsequent investigations. It is important to note that there were no reported illnesses associated with consumption of any of the products during this survey.

Table 8. Summary of Unsatisfactory Samples

(Analyzed for *E. coli* O157:H7/NM, *Salmonella* spp., and Generic *E. coli*)

Product Origin	Product Type / Production Practice / Country of Origin	Reason for Unsatisfactory Assessment
Imported	Chicory (whole)/ Conventional / USA	*Salmonella* IV:50:z4,z23:-
	Baby spinach (whole) / Conventional / USA	*Salmonella* IIIb:ROUGH-O:-:-
	Romaine lettuce (whole)/ Conventional / USA	generic *E. coli*: 1700 CFU/g
	Romaine leaves (whole) / Organic / USA	generic *E. coli*: 1080 CFU/g
Domestic	Baby spinach (whole) / Organic / Canada	generic *E. coli*: 6100 CFU/g

In addition, an elevated level of generic *E. coli* (980 CFU/g) was found in one sample (0.02%, 1/4250). The sample was assessed as investigative and requiring further evaluation as the *E. coli* counts were elevated but below the unsatisfactory threshold of 1000 CFU/g. The evaluation resulted in no follow-up activities.

3.2 *L. monocytogenes* in Fresh-cut Leafy Vegetable Samples

All of the fresh-cut leafy vegetable samples were tested for *L. monocytogenes* in addition to *E. coli* O157:H7/NM, *Salmonella* spp., and generic *E. coli*. *L. monocytogenes* was not detected in 99.6% (1843/1850) of the fresh-cut samples (Table 9). To assist in the food safety investigations, profiles of pulsed field gel electrophoresis (PFGE) (i.e., DNA typing) were identified from the *L. monocytogenes* isolates.

A total of seven fresh-cut samples were found to be unsatisfactory due to the detection of *L. monocytogenes*. Of the seven samples contaminated with *L. monocytogenes*, levels less than 100 CFU/g were found in four of the samples in which enumerations were performed (Table 10). Enumeration was not performed in the other three samples since the products were at the end of their shelf life when results were obtained. All of the samples positive for *L. monocytogenes* were imported, conventionally grown leafy vegetables. It is notable that the majority of fresh-cut samples (93.4%, 1728/1850) were imported, conventionally grown leafy greens. The unsatisfactory rates between imported and domestic as well as conventional and organically grown samples are not considered to be statistically comparable.

Table 9. Summary of Assessment Results of the Fresh-cut Leafy Vegetable Samples Analyzed for *L. monocytogenes*

Product Origin	Production Practice	Number of Samples	Assessment			
			Unsatisfactory		Satisfactory	
			Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples
Imported	Conventional	1728	7	0.4	1721	99.6
	Organic	45	0	0	45	100
	Subtotal	***1773***	***7***	***0.4***	***1766***	***99.6***
Domestic	Conventional	72	0	0	72	100
	Organic	5	0	0	5	100
	Subtotal	***77***	***0***	***0***	***77***	***100***
Total		**1850**	**7**	**0.4**	**1843**	**99.6**

Table 10. Summary of Unsatisfactory Samples Analyzed for *L. monocytogenes*

Product Type / Production Practice / Country of Origin	Reason for Unsatisfactory Assessment
Salad mix / Conventional / USA	*L. monocytogenes*: detected, < 100 CFU/g
Salad mix / Conventional / USA	*L. monocytogenes*: detected, < 100 CFU/g
Lettuce (cut) / Conventional / USA	*L. monocytogenes*: detected, < 100 CFU/g
Lettuce (iceberg, cut) / Conventional / USA	*L. monocytogenes*: detected, < 100 CFU/g
Lettuce (iceberg, cut) / Conventional / USA	*L. monocytogenes*: detected*
Lettuce (romaine, cut) / Conventional / USA	*L. monocytogenes*: detected*
Salad (Caesar) / Conventional / USA	*L. monocytogenes*: detected*

* Enumeration was not performed for the sample.

All unsatisfactory samples were subject to food safety investigations and appropriate follow-up activities were conducted by the CFIA. One product recall resulted from the referral of the unsatisfactory samples to the appropriate CFIA programs and subsequent

investigations. It is important to note that there were no reported illnesses associated with consumption of any of the products during this survey.

4 Discussion and Conclusion

Surveillance of leafy green vegetables under the FSAP was initiated in fiscal year 2008/09. The bacterial pathogens of concern, *E. coli* O157:H7, *Salmonella*, and *Shigella*, as well as generic *E. coli*, were not found in any of the 601 samples (433 imported, 168 domestic) tested in the 2008/09 leafy green vegetable survey.

In this survey (2009/10), bacterial pathogens *E. coli* O157:H7 and *E. coli* O157:NM were not detected in any of the leafy vegetables (whole and fresh-cut) sampled and tested. *Salmonella* spp. and generic *E. coli* were not detected in 99.9% of the samples; *Salmonella* spp. were identified in 0.05% (2/4250) of the samples and high levels of generic *E. coli* were found in 0.07% (3/4250) of the samples.

Likewise, *L. monocytogenes* was not detected in the majority (99.6%) of the fresh-cut leafy vegetable samples which were additionally tested for this pathogen. Seven samples out of 1850 (0.4%) were positive for *L. monocytogenes*. When enumeration was performed, contamination appeared to occur at low levels (i.e., <100 CFU/g).

Even though the satisfactory rates obtained in this survey are very high, the preliminary results suggest that a small fraction of fresh leafy green vegetables available in the Canadian market in the 2009/10 fiscal year were not produced under GMPs/GAPs, maintained under sanitary conditions and/or stored at appropriate refrigeration temperatures. *Salmonella* was the primary bacterial pathogen observed in whole leafy vegetables, and *L. monocytogenes* was the primary bacterial pathogen observed in fresh-cut RTE leafy vegetables in the 2009/10 survey.

The importance of GAPs/GMPs and sanitary handling along the whole food continuum, including appropriate refrigeration temperatures for storage and sale, remain crucial for minimizing the potential contamination and growth of foodborne pathogens and consequently the risk of foodborne illness.

As part of the five years of microbiological targeted surveys on leafy green vegetables, the 2009/10 targeted survey found that, of the 4250 samples tested for *Salmonella*, *E. coli* O157:H7/NM, and generic *E. coli*, and of the 1850 fresh-cut samples also tested for *L. monocytogenes*:

- Pathogenic bacteria *E. coli* O157:H7 and *E. coli* O157:NM were not detected in any of the samples.
- Pathogenic bacteria *Salmonella* and generic *E. coli* (>100 CFU/g) were not detected in 99.9% (4245/4250) of the samples.
- Pathogenic bacteria *L. monocytogenes* was not detected in 99.6% (1843/1850) of the fresh-cut samples.

- A total of twelve samples were found to be unsatisfactory:
  - *Salmonella* spp. were found in two samples (0.05%, 2/4250);
  - *L. monocytogenes* was detected in seven samples (0.4%, 7/1850), the levels of contamination were below 100 CFU/g when enumeration was performed (4 of the 7 samples were enumerated), a low level posing very little risk in this type of food;
  - High levels of generic *E. coli* (>1000 CFU/g) were found in three samples (0.07%, 3/4250).

- An elevated level of generic *E. coli* (between 100 and 1000 CFU/g) was found in one sample. This sample was assessed as investigative and required further evaluation as the level of *E. coli* was elevated but below the unsatisfactory threshold of 1000 CFU/g. An evaluation of the sample resulted in no follow-up activities.

The CFIA conducted follow-up activities on each of the unsatisfactory samples, including food safety investigations, health risk assessments, directed sampling, review of importation procedures, etc. Two products were recalled as a result of this survey. No reported illnesses were associated with consumption of any of the products.

5 Acknowledgement

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Appendix A: List of Acronyms and Abbreviations

CFIA: Canadian Food Inspection Agency

CDC: Centres for Disease Control and Prevention

CFU: colony forming unit

CFU/g: colony forming units per gram

E. coli: *Escherichia coli*

FAO: Food and Agriculture Organization of the United Nations

FDA: Food and Drug Act

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

HPB/MFHPB: Health Protection Branch/ Microbiology Food Health Protection Branch

L. monocytogenes: *Listeria monocytogenes*

MFLP: Microbiology Food Laboratory Procedures

MPN: Most Probable Number

NM: non-motile

PCR: Polymerase Chain Reaction

PFGE: Pulsed Field Gel Electrophoresis

PHAC: Public Health Agency of Canada

RTE: Ready-to-Eat

***Salmonella* spp.**: *Salmonella* species

spp.: species

US FDA: United States Food and Drug Administration

WHO: World Health Organization

°C: Degree Celsius

g: gram

Appendix B: Global Foodborne Disease Outbreaks Associated With Leafy Green Vegetables Contaminated with Bacterial Pathogens (1998-March 2010) *

Case Number	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized	Number of Deaths
1	1998	April	1999 Int. J. Food. Microbiol 49:103-6	Japan	N/A	*Clostridium perfringens*	Spinach	30		
2	1998	June	CDC	USA	Minnesota	*Campylobacter jejuni*	Lettuce	300		
3	1998	October	Ann. Rheum. Dis. 62(9):866-869, 2003	Finland	Multiple	*Yersinia pseudotuberculosis*	Lettuce, iceberg	38	13	
4	1999	February	CDC	USA	Nebraska	*Escherichia coli* O157:H7	Lettuce, iceberg	72		
5	1999	February	CDC	USA	Nebraska	*Escherichia coli* O157:H9	Lettuce, iceberg	65		
6	1999	September	Epi. & Infect. 132:43-49, 2003	Sweden	N/A	*Escherichia coli* O157	Lettuce	13	2	
7	1999	September	CDC	USA	Multiple	*Escherichia coli* O157	Lettuce, romaine	14		
8	1999	October	CDC	USA	Pennsylvania	*Escherichia coli* O153:H50	Lettuce, romaine	40		
9	1999	October	CDC	USA	Multiple	*Escherichia coli* O157:H7	Lettuce, romaine	46	7	
10	2000		NML, Annual Summary	Canada	Nova Scotia	*Escherichia coli* O157:H7	Spinach	11		
11	2000		CDR Enteric Archives 2001	England	N/A	Campylobacter	Lettuce	18		
12	2000		Clin. Micro. & Infect. 9(8) 839-845, 2003	Multiple	N/A	*Salmonella* Typhimurium DT204b	Lettuce, iceberg	392	61	

Case Number	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized	Number of Deaths
13	2000	May	CDC	USA	Connecticut	*Campylobacter jejuni*	Lettuce	13		
14	2000	August	Epi. & Infect. 130;169-178, 2003	UK	N/A	*Salmonella* Typhimurium DT104	Lettuce	361		
15	2001	May	Infect. Dis. News Brief, 7 Sept 2001	Australia	Queensland	*Salmonella* Bovismorbificans	Lettuce, iceberg	41		
16	2001	May	Infect. Dis. News Brief, 9 Jul 2001	Canada	Multiple	Shigella sonnei	Spinach	31	1	
17	2001	November	Food Safety Network Sept. 18 2006	USA	Texas	*Escherichia coli* O157:H7	Lettuce	20		
18	2001	December	CDC	USA	Virginia	*Clostridium perfringens*	Spinach	33		
19	2002	July	FDA	USA	Washington	*Escherichia coli* O157:H8	Lettuce, romaine	29		
20	2002	November	CDC	USA	Illinois	*Escherichia coli* O157:H7	Lettuce	13		
21	2002	December	Food Safety Network Sept. 18 2006	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	3		
22	2003	September	CDC	USA	California	*Escherichia coli* O157:H7	Lettuce	51		
23	2003	October	CDC	USA	California	*Escherichia coli* O157:H7	Spinach	46	7	1
24	2003	November	CDC	USA	California	*Salmonella* Enteritidis	Lettuce	14		
25	2004	July	CDC	USA	Multiple	*Salmonella* Newport	Lettuce	97		
26	2004	August	New Hampshire Dept. of Health & Human Services	USA	New Hampshire	Salmonella	Lettuce	9		

Case Number	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized	Number of Deaths
27	2004	September	Epi. & Infect. 137(10):1449-1456, 2009	England	N/A	*Salmonella* Newport	Lettuce	677		
28	2004	November	J. Foodborne Pathogens & Dis. 5(2):165-173	Norway	N/A	*Salmonella* Thompson	Lettuce	21		
29	2004	November	Food Safety Network Sept. 18 2006	USA	New Jersey	*Escherichia coli* O157:H7	Lettuce	6		
30	2005		European Food Safety Authority	UK	N/A	*Salmonella* Typhimurium	Lettuce, iceberg	71	0	
31	2005	April	CDC	USA	Oregon	*Salmonella* Paratyphi B var Java	Lettuce	10		
32	2005	May	Eurosurveillance Weekly 10 (44), 2005	Finland	N/A	*Salmonella* Typhimurium DT104	Lettuce	60		
33	2005	August	CDR Weekly Vol. 15 No. 36	England	N/A	*Salmonella* Typhimurium DT104	Lettuce	71		
34	2005	August	Eurosurveillance Weekly 10(9), 2005	Sweden	N/A	*Escherichia coli* O157	Lettuce	135		
35	2005	September	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	34	13	
36	2005	September	Bites (Kansas State)	USA	Multiple	*Escherichia coli* O157:H7	Spinach	204		
37	2006	January	CDC	USA	Oregon	*Shigella sonnei*	Lettuce	35	7	
38	2006		European Food Safety Authority	UK	N/A	*Salmonella* ajioba	Lettuce	153	11	

Case Number	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized	Number of Deaths
39	2006	June	Weber-Morgan Health Dept.	USA	Utah	*Eschericha coli* O121:H19	Lettuce	73		
40	2006	August	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	3		
41	2006	September	CFIA	Canada	Ontario	*Escherichia coli* O157:H7	Lettuce	30	5	
42	2006	October	FSNet Jan 9, 2007	USA	North Carolina	*Escherichia coli*	Lettuce	9	3	
43	2006	November	CDC	USA	Tennessee	*Salmonella* Javiana	Lettuce, iceberg	16	7	
44	2006	November	CDC	USA	New York	*Escherichia coli* O157:H7	Lettuce	20	14	
45	2006	November	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	32		
46	2006	December	CFIA	Canada	Ontario	*Salmonella* Oranienburg	Spinach	3		
47	2006	December	New Jersey Dept. of Health and Senior Services	USA	New Jersey	*Escherichia coli* O157	Lettuce	37		
48	2007	February	CDC	USA	Multiple	*Salmonella* Typhimurium	Lettuce	76	4	
49	2007	March	CDC	USA	Hawaii	*Escherichia coli* O157:H7	Lettuce	8	5	
50	2007	June	CDC	USA	Alabama	*Escherichia coli* O157:H7	Lettuce	26	11	1
51	2007	July	Thu 20 Dec 2007 Eurosurveillance Weekly	Sweden	N/A	*Salmonella* Java	Spinach	172	46	
52	2007	July	CDC	USA	California	*Shigella sonnei*	Lettuce	72	9	
53	2007	September	Eurosurveillance weekly 12(11) 2007	Iceland	N/A	*Escherichia coli* O157	Lettuce, iceberg	9	7	

Case Number	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized	Number of Deaths
54	2007	September	Eurosurveillance 11 Dec. 2008	Netherlands		*Escherichia coli* O157	Lettuce	50		
55	2008	June	Washington Dept. of Health	USA	Washington	*Escherichia coli*	Lettuce	10	2	
56	2008	August	Michigan Dept. of Community Health	USA	Michigan	*Escherichia coli* O157:H7	Lettuce, iceberg	36	8	
57	2008	October	Wellington-Dufferin-Guelph Public Health	Canada	Ontario	*Escherichia coli* O157:H7	Lettuce, romaine	148		
58	2009	July	Public Health Division in Oregon	USA	Multiple	*Salmonella*	Lettuce	124	2	
59	2010	March	CDC	USA	Multiple	*Escherichia coli* O145	Lettuce, romaine	33	12	

* Information in this appendix was prepared by Judy D. Greig, Laboratory for Foodborne Zoonoses , PHAC (Public Health Agency of Canada). The data presented were collected from several sources of information, such as peer-reviewed journals, newspapers, press releases, health units, national laboratory and government websites.

Appendix C: Analytical Methods Used for Microbial Analysis

Bacterial Analysis	**Method Identification Number (Date Issued)**	**Title of Method***
***E. coli* O157:H7/NM**	MFLP-30 (May 2003, Supplement 1 May 2005 & Supplement 2 November 2006)	The Dupont Qualicon Bax® System Method for the Detection of E. Coli O157:H7 in Raw Beef and Fruit Juice
	MFLP-80 (March 2008)	Isolation of *E. coli* O157:H7 or NM in Foods
***Salmonella* spp.**	MFLP-29 (July 2007, modified)	The Qualicon Bax® System Method for the Detection of Salmonella in a Variety of Food and Environmental Samples
	MFHPB-20 (March 2009)	Methods for the Isolation and Identification of *Salmonella* from Foods and Environmental Samples
L. monocytogenes	MFHPB-30 (April 2002)	Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples
	MFLP-74 (January 2001, Supplement March 2002)	Enumeration of *Listeria monocytogenes* in Food
	Appendix L (August 2005)	Confirmation Steps for Methods for The Detection of *Listeria* spp. In Foods And Environmental Samples
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in Foods
	MFHPB-27 (September 1997)	Enumeration of *Escherichia coli* in Foods by the Direct Plating (DP) Method

* *Compendium of Analytical Methods* (29)