Ministry for Primary Industries
Manatū Ahu Matua

A Microbiological Survey of Seed Sprouts (and Shoots) Available in New Zealand

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Scientific Interpretative Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

A Microbiological Survey of Seed Sprouts (and Shoots) Available in New Zealand
ESR Report 14060

Seed sprouts are a staple ingredient in Asia where they are typically eaten cooked. However in New Zealand and other countries, seed sprouts and shoots are consumed raw and minimally processed as consumers seek an increasingly healthy lifestyle. Seed sprouts are considered a high risk product in terms of bacterial contamination as the conditions used for germination are near optimal for the growth of pathogenic bacteria, the product is sold whole, and is often consumed without further processing or treatment. In the last 40 years, there have been a number of outbreaks of foodborne disease associated with the consumption of raw seed sprouts, predominantly due to contamination with Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and occasionally *Listeria monocytogenes*.

The Australia New Zealand Food Standards Code Standard 1.6.1 Microbiological Limits for Food that includes microbiological criteria for cultured seeds and grains (bean sprouts, alfalfa etc.) specifies criteria for *Salmonella* and *L. monocytogenes* in these foods. However, there has been no information available on the microbiological status of seed sprouts and shoots available in New Zealand.

The Ministry for Primary Industries (MPI) commissioned a microbiological survey of ready-to-eat packaged seed sprouts and shoots, available at supermarkets, independent sellers and farmer's markets in New Zealand. The survey was undertaken between April and August 2014. Fifty different batches of seed sprouts and shoots were purchased, and the samples were analysed for levels of *Escherichia coli* and mesophilic aerobic microflora. Five units of each of the 50 batches of seed sprouts and shoots were mixed to form a composite sample and were analysed for the presence of *Salmonella* spp., *Listeria* spp., *Listeria monocytogenes* and shiga toxin-producing *Escherichia coli* (STEC). When any of these microorganisms were detected, the individual subsamples were further tested to enumerate the concentrations of *Listeria* spp., *L. monocytogenes* and *Salmonella* spp. *L. monocytogenes* was detected in a single composite sample of a batch of sunflower seed sprouts at levels <100 CFU/g. *Listeria* spp. (excluding *L. monocytogenes*) was detected in six composite samples (6/50) of seed sprouts and shoots at levels <100 CFU/g. STEC was not detected in any of the composite samples and the concentration of generic E. coli in the majority of individual subsamples (218/222) was <3 MPN/g *Salmonella Adelaide* was detected in 2 out of 5 individual subsamples from 1 composite sample of alfalfa sprouts and snow pea shoots with counts of 0.04 MPN/g.

MPI informed businesses whose products tested positive for *Listeria* spp. (including *L. monocytogenes*) and they were provided with guidance to improve their operation. Based on the detection of *L. monocytogenes* and *S. Adelaide* in a small number of individual subsamples and composite samples, it can be concluded that some ready-to-eat seed sprouts and shoots available in New Zealand do not conform to the microbiological limits set out in the Australia New Zealand Food Standards Code Standard 1.6.1.

The results from this survey will be used to inform the implementation of the Food Act 2014 under which seed sprout producers will be required to manage the associated risks. MPI will provide guidance to assist businesses in this process.

Client report FW 14060

A MICROBIOLOGICAL SURVEY OF SEED SPROUTS (AND SHOOTS) AVAILABLE IN NEW ZEALAND

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A MICROBIOLOGICAL SURVEY OF SEED SPROUTS (AND SHOOTS) AVAILABLE IN NEW ZEALAND

Prepared for the Ministry for Primary Industries under project MFS/12/8 - Food Consultation, as part of overall contract for scientific services

Client report no. FW 14060

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June 2015

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SUMMARY

A quantitative microbiological survey of ready-to-eat packaged seed sprouts and shoots, available from supermarkets, independent sellers and farmer's markets in New Zealand, was carried out between April and August 2014. Fifty different lots/batches of various types of seed sprouts and shoots were purchased. Between 2 and 5 subsamples of each of the 50 samples were then tested for the concentrations of *Escherichia coli* and mesophilic aerobic microflora. Fifty composite samples (each from the same batch of seed sprouts and shoots) were tested for the presence or absence of *Salmonella* spp., *Listeria* spp., *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC). When any of the composite samples were positive for *Listeria* spp. or *Salmonella* spp. each of the individual subsamples was tested to enumerate the concentrations of *Listeria* spp., *L. monocytogenes* and *Salmonella* spp.

L. monocytogenes was detected in one composite sample of sunflower seed sprouts at a concentration of <100 CFU/g. *Listeria* spp. (excluding *L. monocytogenes*) were detected in six composite samples (6/50) of seed sprouts and shoots. *L. innocua* was detected in three different composite samples of soybean sprouts, one composite sample of chickpea sprouts, and one composite sample of alfalfa and broccoli sprouts, all at a concentration of <100 CFU/g. *L. seeligeri* was detected in one composite sample of soybean sprouts at a concentration of <100 CFU/g. STEC was not detected in any of the composite samples. The concentration of generic *E. coli* in the majority of individual subsamples (218/222) was <3 MPN/g. The composite sample set of sunflower seed sprouts that tested positive for *L. monocytogenes* also contained 3 out of 5 individual subsamples that had *E. coli* counts of 4, 4 and 9 MPN/g respectively while one of the composite sets of soybean sprouts that tested positive for *L. innocua* also contained 1 out of 5 individual subsamples that had an *E. coli* count of 23 MPN/g. *Salmonella* Adelaide was detected in 2 out of 5 individual subsamples from 1 composite set of alfalfa sprouts and snow pea shoots with counts of 0.04 MPN/g. Based on the detection of *L. monocytogenes* and *S.* Adelaide in a small number of individual subsamples and composite samples, it can be concluded that some ready-to-eat seed sprouts and shoots available in New Zealand do not conform with the microbiological limits set out in the Australia New Zealand Food Standards code 1.6.1 (http://www.comlaw.gov.au/Details/F2014C01189) (accessed February 2015)

1 INTRODUCTION

Ready-to-eat seed sprouts are a staple ingredient in Asia (where they are typically cooked as opposed to eaten raw) and across the globe due to their health benefits, low cost of production and enhancement of culinary dishes (Warriner and Smal 2014). Seed sprouts are a good source of protein with a high mineral content. Some sprouts are also believed to have anti-cholesterol properties (Kim et al. 2012) and others are believed to contain anti-cancer compounds (Gawlik-Dziki et al. 2014). In recent years, the consumption of raw, minimally processed fruits and vegetables including seed sprouts and shoots has gained popularity as consumers seek an increasingly healthy lifestyle, alongside the uptake of raw and paleo diets (http://en.wikipedia.org/wiki/Paleolithic_diet).

In the last 40 years however, there have been numerous outbreaks of foodborne disease associated with the consumption of seed sprouts, predominantly due to contamination with Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and occasionally *Listeria monocytogenes* (Yang et al. 2013; Michino et al. 1999; FSANZ 2011; Little et al. 2004; Dechet et al. 2014). The majority of reported outbreaks have occurred in North America, where between 1995 and 2010 there were more cases of illness (>2000) directly linked to seed sprouts than any other vegetable (Yang et al. 2013). However, the two largest reported outbreaks were in Japan in 1996 (Michino et al. 1999) and Germany (followed a month later by a parallel outbreak in France) in 2011 (Scharlach et al. 2013; Rubino et al. 2011). In Japan there were more than 6,000 cases of *E. coli* O157:H7 infection associated with consumption of radish sprouts, while in Germany (and France) there were at least 3,855 cases of *E. coli* (STEC) O104:H4 infection linked to consumption of sprouts (possibly fenugreek). One of the intriguing factors in the German outbreak was that only 25% of individuals infected recalled eating seed sprouts and it was only when the meals were studied in detail that it became apparent that seed sprouts had been included as garnishes to meals and salads (Buchholz et al. 2011). *E. coli* O157:H7 is however very infectious with only a low dose of 10-100 cells being required to cause foodborne illness (Wang and Doyle 1998).

Seed sprouts are considered a high risk product in terms of bacterial contamination as the germination conditions used are near optimal for pathogen proliferation, the product is sold intact and perishable and is often consumed without further processing or treatment (Yang et al. 2013). Furthermore, there are no industry standard procedures, applied in New Zealand, for the decontamination of seed sprouts by seed suppliers or processors (FSANZ 2011). In New Zealand, there are however strict regulations governing the microbiological limits of particular

pathogenic bacteria in seed sprouts, and samples must comply with criteria set down in the Australia New Zealand Food Standards Code 1.6.1 (http://www.comlaw.gov.au/Details/F2014C01189; http://www.foodstandards.gov.au/publications/Documents/Guidance%20on%20the%20application%20of%20limits%20for%20Listeria%20monocytogenes%20FINAL.pdf) (accessed February 2015). In particular, clause 6, sub clauses 2 and 3 state that for fresh-cut and packaged horticultural produce which do not receive a listericidal process during manufacture, and in which the growth of *L. monocytogenes* could occur, the pathogen must not be detected in 25g of any of five samples analysed throughout their expected shelf life. Validation documentation is required as evidence to demonstrate that a product does not support the growth of *L. monocytogenes* or that growth is limited. No such validation has yet been undertaken for seed sprouts within Australasia. A previous microbiological survey of retail fresh produce within New Zealand included the sampling of seed sprouts (plant type not specified) (McIntyre and Cornelius 2009). In this report up to 20% of the seed sprout samples tested (depending on which microbiological reference criteria were applied to analysing the results) were considered either marginal or unsatisfactory in terms of the level of *E. coli* and faecal coliforms.

Numerous studies have evaluated the effect of seed treatments or direct treatments of sprouts on reducing or eliminating pathogenic bacteria from the product. Specific chemical, biological, heat or pressure treatments have been shown to reduce the burden of *Salmonella* and *E. coli* by > 8 $\log_{10}$ CFU/g however most seed sprout disinfection regimes have been demonstrated to reduce the risk of contamination (by several log reductions) but not to completely eliminate the risk of human infection (Dechet et al. 2014). For this reason, the United States Food and Drug Administration (FDA) published a proposed regulation on Standards for Produce Safety which included a regime for disinfecting seeds and a requirement for testing spent sprout irrigation water for pathogens (FDA 2013). If a pathogen is present in the seed there is a high likelihood of proliferation as the warm humid environment optimal for seed sprout production is also ideal for bacterial growth. For example, *S.* Stanley increased by 2.5 logs during the first 24 hours of germination on contaminated alfalfa seeds (Jaquette et al. 1996), while *E. coli* O157:H7 inoculated onto radish seeds increased by 4-5 logs during 24 hours of sprouting (Hara-Kudo et al. 1997). In addition to germination, seed sprout production is susceptible to contamination during the production process both pre and postharvest (Yang et al. 2013). Pre-harvest contamination may result from poor quality irrigation water and soil, proximity of the field to animal rearing facilities or wild animals such as deer grazing on crops (Pachepsky et al. 2011;

Guan and Holley 2003), while post-harvest contamination may occur during transport or storage.

The Ministry for Primary Industries (MPI) is currently developing guidelines to assist growers minimise the microbiological hazards associated with the production and harvesting of seed sprouts and shoots in New Zealand. The Food Act (2014) http://www.legislation.govt.nz/act/public/2014/0032/latest/DLM2995811.html?search=ta_act_F_ac%40ainf%40anif_an%40bn%40rn_25_a&p=4 (accessed February 2015) details risk-based measures already in place to achieve the safety and suitability of food for the consumer but is not specifically aimed at operators and producers of seed sprouts. The aim of this survey was therefore to provide an indication of the microbial quality of these products, available at both retail and farmer's markets, and to help support the decision-making process about whether additional controls need to be put in place to sufficiently manage *Listeria* spp., STECs and *Salmonella*. Information in this report will assist MPI with the development and implementation of pathogen risk management strategies, and also provide information about possible sources of foodborne illness in New Zealand.

2 MATERIALS AND METHODS

2.1 Sampling Programme

Seed sprouts and shoots were obtained through direct purchase or online/phone ordering from retail outlets including supermarket chains, independent sellers and farmer's markets. Every attempt was made to ensure that the products originated from separate suppliers to reduce the chance of sampling multiple brands from the same supplier. Sampling was designed to cover products available across New Zealand. Seed sprouts and shoots were obtained from retail outlets in Kerikeri, Auckland, Tauranga, Wellington, Christchurch and Dunedin. Wheat grass, watercress, cress and hydroponically-grown micro-leaf vegetables were excluded from the survey.

Sampling and testing occurred between April and August 2014 and was carried out in two Rounds. Round 1 sampling took place between April 2014 and June 2014 and consisted of 28 samples comprising 124 subsamples. Of these, 5 samples were from supermarkets; 8 samples were from independent sellers and 15 samples were from farmer's markets. Round 2 sampling was carried out between July 2014 and August 2014 and consisted of 22 samples comprising 98 subsamples. Of these, 7 samples were from supermarkets; 7 samples were from independent sellers and 8 samples were from farmer's markets Samples were purchased and stored under the processor's stated storage conditions (4°C +/- 3°C) until bacteriological analysis commenced within +/- 2 days of the processor's stated best-before/use-by date. If purchased within Christchurch, samples were transported to ESR (Christchurch Science Centre (CSC)) within no more than one hour of purchase. If purchased in another city, samples were held under refrigeration and transported by overnight or next-day courier in a chilly bin with ice-replacement packaging, to maintain the temperature at 4°C $\pm$ 3°C during transit to ESR CSC. Sample temperatures and package integrity were checked upon receipt at CSC prior to the assignation of a unique 'Public Health Laboratory' identification number. All samples were stored under refrigeration at ESR until processing and any un-used samples were stored in Whirl-Pak bags in a freezer until the end of the survey.

2.2 Sample preparation

Fifty lots or samples of sprouts were purchased and tested. Samples were tested within +/- two days of the manufacturer's stated expiry/best-before date. If no expiry/best-before date was specified on the packaging, an assumed time frame of four days post purchase was adhered to

prior to laboratory analyses. Between 2 and 5 subsamples of each lot or batch were created. The testing procedure required 200 grams of sprouts for each of these subsamples. Where the product was available in large sizes only (e.g. 1 kg), one bag was required with five separate samples taken from diametrically opposite corners and the centre of the bag respectively. Where the product was available in smaller pack sizes, these were tested individually or combined to create the 200 gram minimum sample size. Where multiple bags of sample were used, care was taken to ensure that they all had the same best-before/use-by date. Due to insufficient product availability, for some samples, only 2 subsamples were created.

The outer surface of each package was disinfected with 70% isopropyl alcohol before cutting open using sterilized scissors or a scalpel. To assemble individual samples, a sufficient number of separate packages, or 200g from each of five sampling positions within a larger pack size, were transferred into a large stomacher bag, using sterile tongs, and gently mixed. Portions of the mixed samples were then weighed out to be used for individual and composite pathogen analyses. The remainder of each sample was stored at 4°C for re-testing if required, then stored frozen, in Whirl-Pak bags and secondary packaging, for the duration of the survey. Sample enrichments, for STEC analyses, were also stored frozen until the end of the survey. Metadata on the sampling process and product (shelf-life, best-before date or use-by date, packed-on date, origin of products, type of packaging etc.) were recorded. A photograph of each individual package was also taken.

2.3 Microbiological Methods

Product samples were tested using the methods stated in Table 1 and detailed in Appendices 1, 2 and 3.

E. coli enumerations and aerobic plate counts (APC) were performed on all 222 subsamples. A composite of each of the fifty samples (created by recombining 25 grams from each of the subsamples) was also tested for the presence/absence of *Listeria* spp., *L. monocytogenes*, S*almonella*, and STECs (specifically serogroups O26, O45, O103, O111, O121, O145 and O157).

When any of the composite samples were positive for *Listeria* or *Salmonella*, each of the individual subsamples was tested to enumerate *Listeria* spp., *L. monocytogenes* and *Salmonella*.

Further testing of individual subsamples was undertaken when the presence/absence testing on composite samples yielded positive results. Culturally and biochemically-confirmed isolates of pathogens were further confirmed by serotyping (*Salmonella* spp., *L. monocytogenes*), and pulsed field gel electrophoresis (PFGE) for *L. monocytogenes* (Graves and Swaminathan 2001).

Confirmed results for the detection of any pathogen analysis were communicated immediately to MPI via an official laboratory report.

Table 1. Methods used for the microbiological analyses of seed sprouts and shoots

Microorganism	Type of test	Method used	Method name/source
L. monocytogenes, *Listeria* spp.	Presence/Absence	ISO 11290-1:1996/Amd.1:2004	Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method"(Appendix 2)
Salmonella spp.	Presence/Absence	ISO 6579:2002	Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Appendix 2)
STECs	Presence/Absence	Assurance GDS MPX Top 7 STEC detection system	Using BIOCONTROL Assurance GDS machine and methodology (Appendix 3)
L. monocytogenes, *Listeria* spp.	Enumeration	ISO 11290-2:1998/Amd.1:2004	Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method (Appendix 2)
Salmonella	Enumeration	ISO:7218:2007 ISO 6579:2002	Most Probable Number (Appendix 2)
E. coli	Enumeration	APHA (Most Probable Number (MPN) method)	American Public Health Association (APHA 2001, 4th Edition) method, Chapter: 'Enterobacteriaceae, Coliforms, and *Escherichia coli* as Quality and Safety Indicators', sections 8.7/8.8/8.9 (Appendix 2)
Aerobic plate count (APC), mesophilic	Enumeration	ISO 4833:2003	Microbiology of food and animal feeding stuff – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C (Appendix 2)

2.4 Data analysis

To compare APCs from products bought from different retail outlets, the data was grouped by retail type (farmers market, independent store and supermarket). The APC data for these retail groups were compared graphically using box plots generated using the R statistical package (R Core Team, 2012 http://www.R-project.org/) (accessed February 2015).

3 RESULTS

3.1 Sample location and type

A total of 50 different samples of ready-to-eat seed sprouts and shoots products were obtained from across New Zealand from farmer's markets, independent stores and supermarkets (Table 2). To yield sufficient sample for testing, 146 packages of sprout were purchased, each with between 50 and 1,000 grams of product.

Table 2. Number of samples taken by location and retail outlet type

	Farmer's market	Independent Store	Supermarket
Kerikeri	2	-	-
Auckland	2	4	3
Tauranga	1	-	-
Wellington	16	2	2
Christchurch	Not found on sale	6	7
Dunedin	2	3	-
Total	**23**	**15**	**12**

The type of ready-to-eat seed sprouts and shoots sampled depended on their availability on the day of sampling. Half of the samples collected were mung bean sprouts, with the full range of shoots and sprouts sampled listed in Table 3.

Table 3. Number of samples taken by sprout or shoot variety and retail outlet type

	Farmer's market	Independent Store	Supermarket
Alfalfa & broccoli sprouts	-	-	1
Alfalfa & onion sprouts	-	-	1
Alfalfa sprouts	4	1	-
Alfalfa sprouts & snow peas shoots	-	-	2
Alfalfa sprouts, snow peas shoots, mixed beans	-	-	1
Broccoli combo	-	-	1
Chickpea sprouts	-	-	2
Crunch combo (sprout type not specified)	1	-	-
Green bean sprouts	-	1	-
Lentil, azuki and pea shoots	-	-	1
Mung bean sprouts	17	6	2
Radish sprouts	-	-	1
Soybean sprouts	-	7	-
Sunflower seed sprouts	1	-	-
Total	**23**	**15**	**12**

3.2 Microbial analyses

Of the 50 composite samples, one (2%) tested positive for *S.* Adelaide, one (2%) tested positive for *L. monocytogenes* and six (12%) samples tested positive for other *Listeria* species. STEC was not detected in any of the samples.

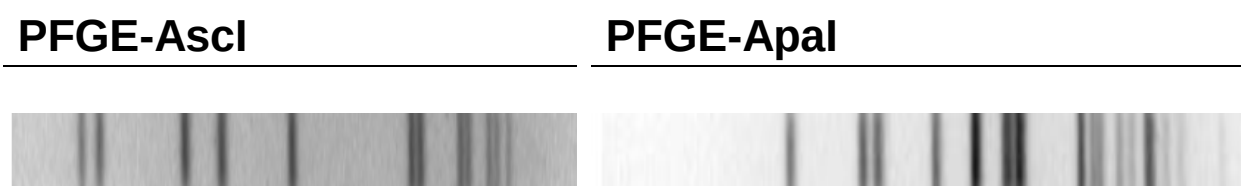
The details of the positive samples for *S.* Adelaide and *Listeria* spp. are given in Table 4. Positive samples came from all of the three retail sources, in three major cities (Auckland, Christchurch and Dunedin) and covered a range of different plant types.

Table 4. Microbial analysis results for *Salmonella*, *L. monocytogenes* and/or *Listeria* spp. positive samples

Brand	Type	Retail outlet	Sampling date	Best before / best by date	Genus	Subsample counts	*E. coli* MPN/g	APC range CFU/g
A	Alfalfa sprouts and snow pea shoots	Supermarket	29/7/2014	31/7/2014	*S.* Adelaide	2 of 0.04 MPN/g 3 of < 0.03 MPN/g	all <3	3.8 - 4.3 $\times 10^7$
B	Sunflower seed	Farmer's market	17/6/2014	19/6/2014	*L. monocytogenes* O1/2	all < 100 CFU/g	2 of <3 2 of 4 1 of 9	2.4 - 5.7 $\times 10^6$
C	Soybean	Independent	21/7/2014	22/7/2014	*L. seeligeri*	all < 100 CFU/g	all <3	1.5 - 1.9 $\times 10^7$
D	Soybean	Independent	14/5/2014	12/5/2014	*L. innocua*	all < 100 CFU/g	all <3	1.0 - 1.3 $\times 10^7$
	Soybean	Independent	2/9/2014	4/9/2014	*L. innocua*	all < 100 CFU/g	1 of 23 4 of <3	6.7 - 9.3 $\times 10^6$
E	Chickpeas	Supermarket	4/6/2014	3/6/2014	*L. innocua*	all < 100 CFU/g	all <3	1.0 - 1.9 $\times 10^8$
	Chickpeas	Supermarket	29/7/2014	3/8/2014	*L. innocua*	all < 100 CFU/g	all <3	1.4 - 2.7 $\times 10^8$
	Alfalfa and broccoli	Supermarket	19/8/2014	21/8/2014	*L. innocua*	all < 100 CFU/g	all <3	5.2 - 7.8 $\times 10^8$

The *L. monocytogenes* isolate was serotyped as O1/2, and had the PFGE pattern Asc0060:Apa0124a (Figure 1). This PFGE pattern is closely-related (but not indistinguishable) to Asc0060:Apa0124 which is a pulsotype from human isolates that is not common in New Zealand.

Figure 1. PFGE pattern of the *L. monocytogenes* isolate



Enumeration of the 5 individual samples associated with the composite sample testing positive for *S.* Adelaide suggested a low concentration of the pathogen, with 3 of the individual samples having no *S.* Adelaide detected (95% Confidence Limit: 0 – 0.09 MPN/g) and two with a MPN of 0.04 MPN/g (95% Confidence Limit: 0.002-0.17).

Enumeration of the 5 individual samples associated with the composite sample of sunflower seed sprouts testing positive for *L. monocytogenes*, did not result in any detectable colonies, suggesting a bacterial concentration of $\leq$100 CFU/g (limit of detection is 100 CFU/g).

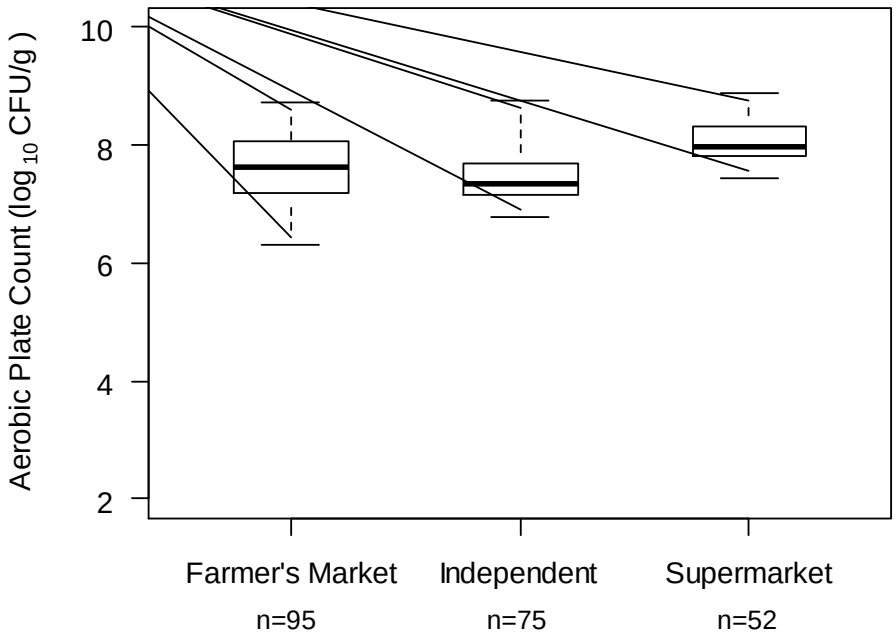
Generic *E. coli* testing of the 222 subsamples found *E. coli* in only four subsamples, from two different samples. Both of these products were also positive for *Listeria* spp. and are listed in Table 4. Generic *E. coli* was detected in 3 out of 5 of the individual samples of Brand B sunflower sprouts and 1 out of 5 of the individual Brand D soybean sprouts. Levels of *E. coli* in the other 218 subsamples were all below the detection limit of 3 MPN/g. The MPN counts obtained from the four *E. coli*-positive subsamples were between 3 and 99 and are therefore considered undesirable according to FSANZ guidelines http://www.foodstandards.govt.nz/publications/pages/guidelinesformicrobi1306.aspx (accessed February 2015). Ministry of Health guidelines propose that *E. coli* is not detected in samples of sprouted seeds (MoH 1995).

Mesophilic aerobic microflora (APC) concentrations on seed sprouts and shoots in the current survey ranged from 2.1×10^6 CFU/g, for an individual unspecified beansprout subsample, to 7.7×10^8 CFU/g for an individual mung bean sprout sample. Figure 2 compares the APCs from samples taken from different retail outlet types. The APCs are consistent across the different

retail outlets with median APCs of 7.6, 7.3 and 7.9 $\log_{10}$ CFU/g for farmer's markets, independent sellers and supermarkets respectively. The greatest variation in APCs occurred in products from farmer's markets; however this group also had the greatest number of samples. These results are in compliance with the FSANZ guidelines for microbiological examination of ready-to-eat foods http://www.foodstandards.govt.nz/publications/pages/guidelinesformicrobi1306.aspx which state that it would be expected that fresh fruits and vegetables would have an inherent high plate count because of the normal microflora present. However, guidelines for microbiological limits in ready-to-eat foods published by the Ministry of Health (MoH 1995) document the samples with APCs above 10^6 CFU/g to be unacceptable.

High APC counts were not necessarily a useful predictor of the potential presence of *Listeria* or *Salmonella* as the samples positive for *L. monocytogenes* had some of the lowest APC counts of all the samples tested. Furthermore all seed sprout samples were tested within +/- 2 days of the manufacturers stated expiry/best before date so the APC counts could be an indicator of the concentration of spoilage organisms present.

Figure 2: Distribution of aerobic plate counts (APCs) from seed sprouts and shoot samples grouped by retail type. (Bold horizontal line is the median value, bottom and top of the box 25th and 75th percentiles and the dashed vertical line the range of values)



4 DISCUSSION

The results of this survey provide information on the microbiological quality of seed sprouts and shoots available at retail outlets and through farmers markets in New Zealand and allow an assessment of compliance with the Food Standards Code (FSC 1.6.1, (http://www.comlaw.gov.au/Details/F2014C01189; ; http://www.foodsafety.govt.nz/elibrary/industry/factsheet-microbiological-limits-for-l-monocytogenes-application-of-food-standards-code.pdf). This Standard was has been recently reviewed and changes came into effect in New Zealand in September 2014. The results of this survey have been analysed based on updated microbiological limits for *L. monocytogenes*. The Food Standards Code states that for ready-to-eat foods in which the growth of *L. monocytogenes* can occur, *L. monocytogenes* must not be detected in 25g for any of the samples analysed. Furthermore, no *Salmonella* species should be detected in 25g for any samples analysed. MPI has also recently published information to assist operators and regulators determine the shelf life of food http://www.foodsafety.govt.nz/elibrary/industry/determine-shelf-life-of-food/ (accessed February 2015).

Seed sprouts and shoots are gaining popularity with consumers and, as these products (often sold as the entire germinated plant which includes the root, shoot, cotyledons and remnant seed coat) are typically eaten raw or minimally processed, pathogen contamination can represent a serious risk to human health. Microbial contamination can occur during any of the steps in the farm to consumer continuum. However, information from seed sprout associated outbreaks suggests that contamination of the original seed itself represents the most common source (although often the seed itself is not consumed). Once present on or in the seeds, pathogens are likely to survive for extended periods of time and grow rapidly as the germination and favourable conditions of the sprouting processes provide near optimal environment in terms of water activity, pH, temperature, time and nutrients for exponential bacterial growth. *L. monocytogenes* may contaminate either seeds or sprouts at various stages during the production and processing of the product. This pathogen can also grow at refrigeration temperatures on sprouts (Schoeller et al. 2002), thereby increasing the risk of contamination during the shelf life of the product, and has been isolated from commercially-produced sprouted seeds (EFSA 2012; Farber et al. 1990). Post-harvest contamination of seed sprouts (due to poor sanitation of equipment and poor personal hygiene for example) can also result in

the presence of pathogenic bacteria being detected in the final product. In the case of this survey, *L. monocytogenes* and *S.* Adelaide were detected in concentrations of < 100 CFU/g which may suggest that the contamination had occurred during or post-harvest.

In the current survey, *L. monocytogenes* and *Listeria* spp. (*L. monocytogenes*, *L. innocua* and *L. seeligeri*) were isolated from 1/50 (2%) and 7/50 (14%) of the seed sprouts and shoots respectively. However, the counts of all samples containing *Listeria* spp. were <100 CFU/g according to the criteria used for testing. Seed sprouts and shoots are a ready-to-eat product in which the growth of *L. monocytogenes* can occur (Farber et al. 1990; Schoeller et al. 2002) therefore, in order to comply with the microbiological limits contained in the Food Standards Code 1.6.1, it is not permitted to detect this pathogen in 25g of sample (minimum sample size of 5). *L. innocua* and *L. seeligeri* identified in this study indicates the potential for contamination with *L. monocytogenes* as the soil-plant environment is considered a natural niche for *Listeria* spp. (Dowe et al. 1997; Sauders et al. 2012).

There is limited information detailing the prevalence of *Listeria* spp. associated with seed sprouts and shoots (Little et al. 2004; FSANZ 2011; Abadias et al. 2008; Olaimat and Holley 2012). In the UK, an extensive survey (2096 samples) of ready-to-eat fruit salads, seed sprouts and unpasteurised fruit and vegetable drinks found 1 (0.05%) sample of unspecified seed sprouts that was positive for *L. monocytogenes* at > 10^2 CFU/g (Little et al. 2004). A survey of 300 ready-to-eat products in Spain found 2 products (0.7%) to be positive for *L. monocytogenes*, but none of the 15 soybean and alfalfa sprout samples were positive (Abadias et al. 2008) In a Malaysian survey *L. monocytogenes* was isolated from 7 of 8 unspecified seed sprout samples (Arumugaswamy et al. 1994).

STEC was not recovered from any of the samples tested in the current survey. However, 2/50 (4%) samples contained individual subsamples that were positive for *E. coli* in low numbers. These were the same samples of sunflower seed and soybean sprouts that were positive for *L. monocytogenes* and *L. innocua* respectively. As there is not enough evidence from this survey to determine the source of contamination, a review of the procedures used, by seed sprout producers and processors, would be a recommendation from these results. Historical evidence would indicate that post-processing contamination (rather than seed contamination), possibly due to poor hygiene practices, would be a likely cause of the low concentration of pathogens isolated from a minority of seed sprout samples (EFSA 2012). Within New Zealand there are no standard guidelines for seed sprout producers with respect to seed decontamination

and treatment regimes for maturing seed sprouts. A recent review has concluded that most chemical treatments disinfect incompletely with low level survival of pathogens still present on seeds after completion of the process (Dechet et al. 2014). For more effective treatments, including irradiation, cost and consumer concerns may preclude implementation. The FDA has therefore recommended that testing spent irrigation water for pathogens is crucial for verifying that pathogens are not present.

In the current study, 1/50 (2%) samples were positive for *S.* Adelaide at < 0.04 MPN/g. In cultured seeds and grains the presence of any *Salmonella* spp. is not permitted, in 25g of sample, according to the requirements of FSC 1.6.1. Therefore, the results obtained in this survey indicated that a small number of alfalfa sprouts and snow pea shoots did not comply with the microbiological limits set out in the New Zealand FSC 1.6.1 and may therefore pose a risk to human health. The ESR Enteric Reference Laboratory (ESR-ERL) serotypes isolates from almost all notified cases of salmonellosis in New Zealand. *S.* Adelaide is an infrequently-isolated serotype from clinical samples, with only six notified cases in New Zealand between 1992 and 2013 (1 each in 1992, 1993, 1996, 2000, 2001 and 2007). There were two notified cases in 2014 (one in October from a patient in the Nelson Marlborough Health District, and a second in December from the Canterbury Health District) and one case in January 2015 from the Otago Health District (ESR. 2014) (pers. comm. Muriel Dufour, ESR-ERL). All three of the recent clinical samples have the same PFGE profile as that identified from the seed sprout samples. As such, this is an association that may require further investigation. Since 1988, 33 isolates from non-human sources have been identified as *S.* Adelaide, mostly isolated from meat & bone meal samples (ESR. 2014) pers. comm. Muriel Dufour, ESR-ERL). *S.* Adelaide has occasionally been associated with Australian egg layer farms (Gole et al. 2014), and has been isolated from sheep in New Zealand (Clark 1999). There are no reports in the literature suggesting that this serotype has been found in seed sprouts or shoots. Other *Salmonella* spp., however, are frequently reported as being a contaminant associated with this food source (NACMCF 1999; Olaimat and Holley 2012; Dechet et al. 2014) with numerous different serotypes having been identified. For example, a large outbreak associated with alfalfa sprout consumption in 1996, involved more than 500 cases. Both *S.* Montevideo/Meleagridis was isolated from these cases, and sprouts and unsafe sprouting practices were cited as being the likely cause of the outbreak (NACMCF 1999).

The samples in the current survey that were positive for *L. monocytogenes* and *E. coli* had comparably low APC values suggesting that the use of this test is not necessarily a good indicator of the presence of pathogenic bacteria. The acceptable level of mesophilic aerobic microflora is not specified in FSC 1.6.1. APCs can however be an indication of hygiene at different stages of the production and manufacturing process with concentrations of alfalfa and rape seeds being reported to increase by 3.0 $\log_{10}$ cfu/g during sprouting, reaching in excess of 7.5 $\log_{10}$ cfu/g by the final stages of manufacture (Kim *et al*. 2013). While results may be useful in developing control measures for growers involved in the sprout industry, care should be taken when interpreting such results as the significance of APCs can vary markedly according to the type of food product, processing and stage of shelf life (IFST. 1997). A Spanish study of 15 sprout samples found similar APCs to those detected in this survey, with a range of 6.3-8.1 $\log_{10}$ CFU/g (Abadias *et al.* 2008).

5 CONCLUSIONS

This survey of ready-to-eat seed sprouts and shoots in New Zealand identified the bacterial pathogen *L. monocytogenes* in one sample set (5/5 subsamples) of sunflower seed sprouts and the bacterial pathogen *S.* Adelaide in one sample set (2/5 subsamples) of alfalfa seed sprouts and snow pea shoots As both of these pathogens pose a potentially serious risk to human health, the quantifiable detection of these microorganisms in ready-to-eat foods of this nature did not conform with the microbiological limits set out in the Food Standard Code 1.6.1.

Taken together, the presence of *L. monocytogenes*, *S.* Adelaide, non-pathogenic *Listeria* spp. and *E. coli* in sample sets of seed sprouts within New Zealand would suggest that a review of practices applied along the primary production and processing chain for seed sprouts would be recommended. This would include seed production on-farm, seed processing and sprout production.

Microbiological surveys of similar seed sprouts and shoots reported in the international literature do not always test for all organisms of significance. Many factors, including geographical location (and associated climatic factors) and processing methods significantly influence the presence and species of microbial contaminants. Each study, including the one described here, therefore represents only a time-bounded snapshot of some, but not all microbial risks related to ready-to-eat sprouts and seeds. While this report should be viewed in this context, the information gathered can be used to assess risk and inform decision-makers in relation to evaluating the on-going food safety issues associated with *L. monocytogenes*, STECs and *Salmonella* spp. in ready to eat seed sprouts and shoots in New Zealand.

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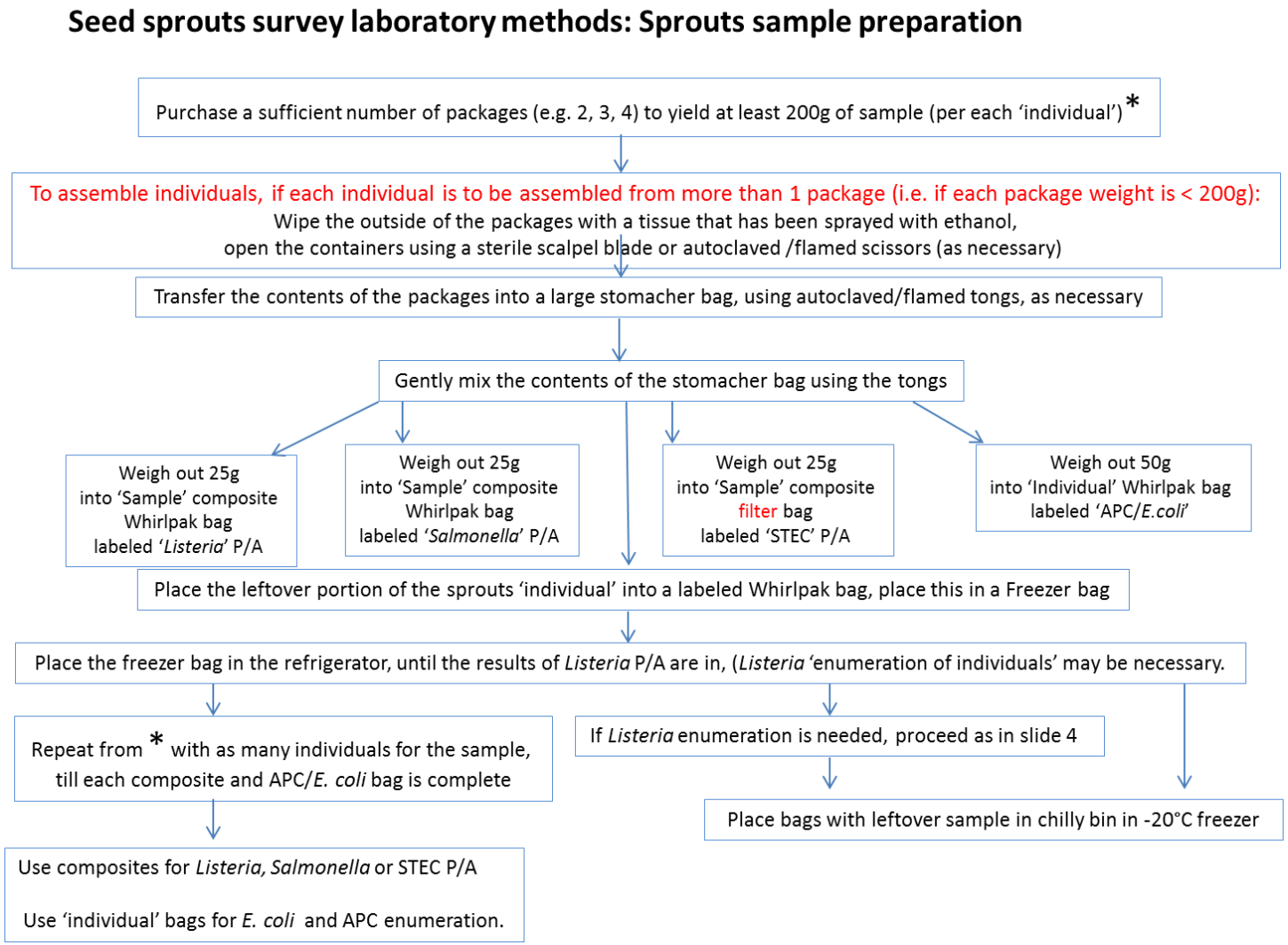
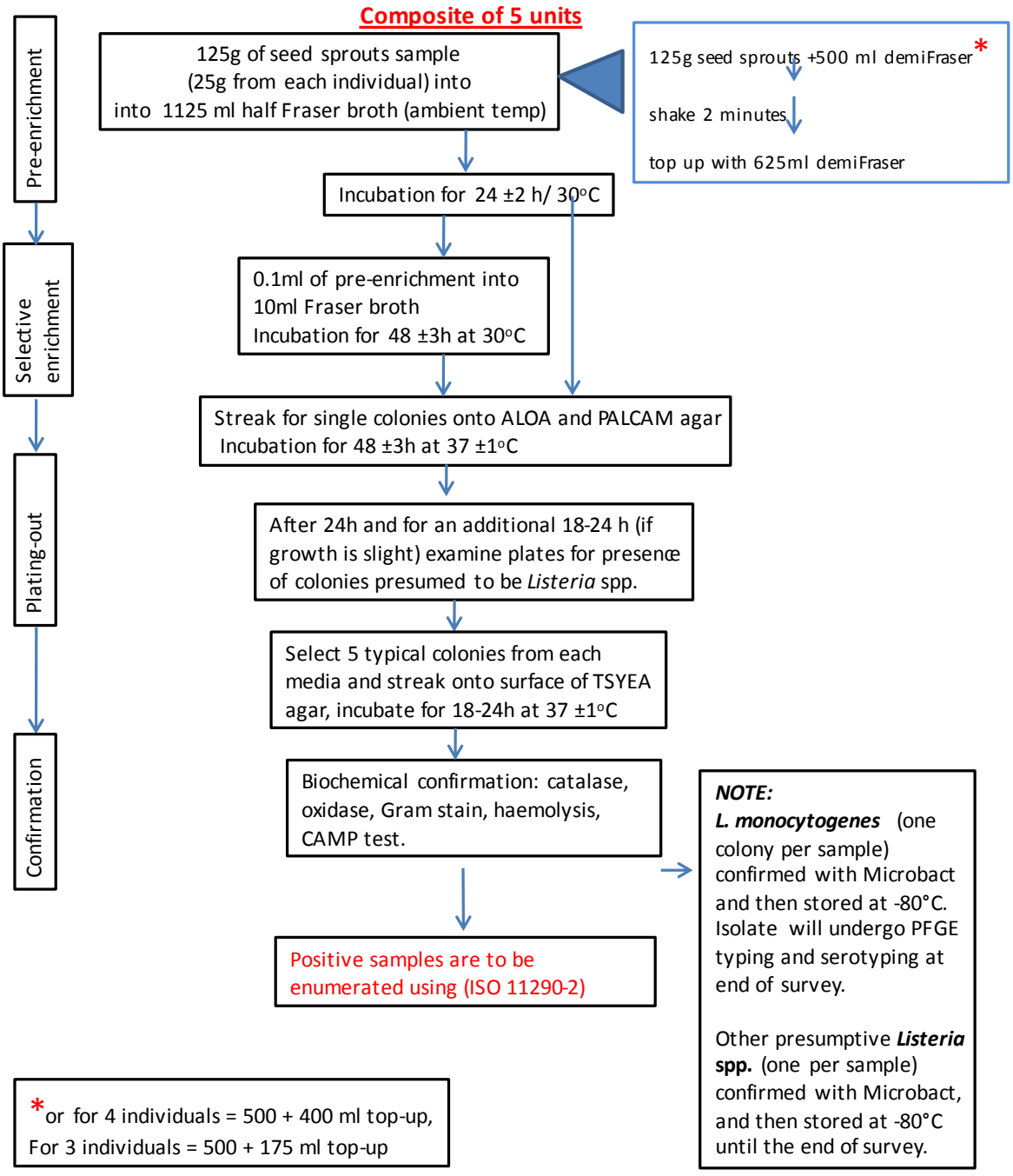
Appendix 1: Flow diagram detailing laboratory methods used for the seed sprouts survey

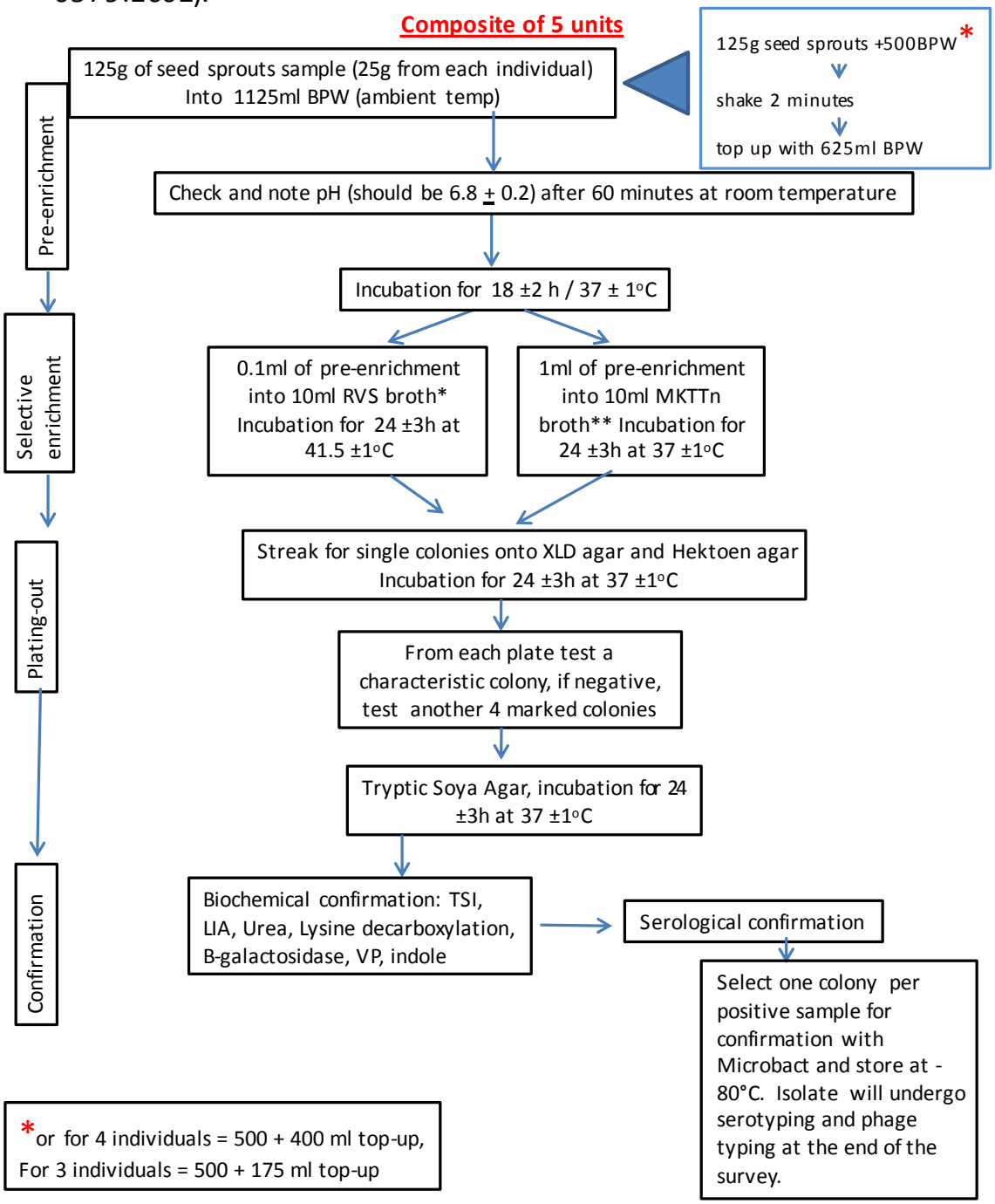


Appendix 2: Individual laboratory methods used for the seed sprouts survey:

L. monocytogenes: **P/A:** 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method" (ISO 11290-:1996/Amd.1:2004).'

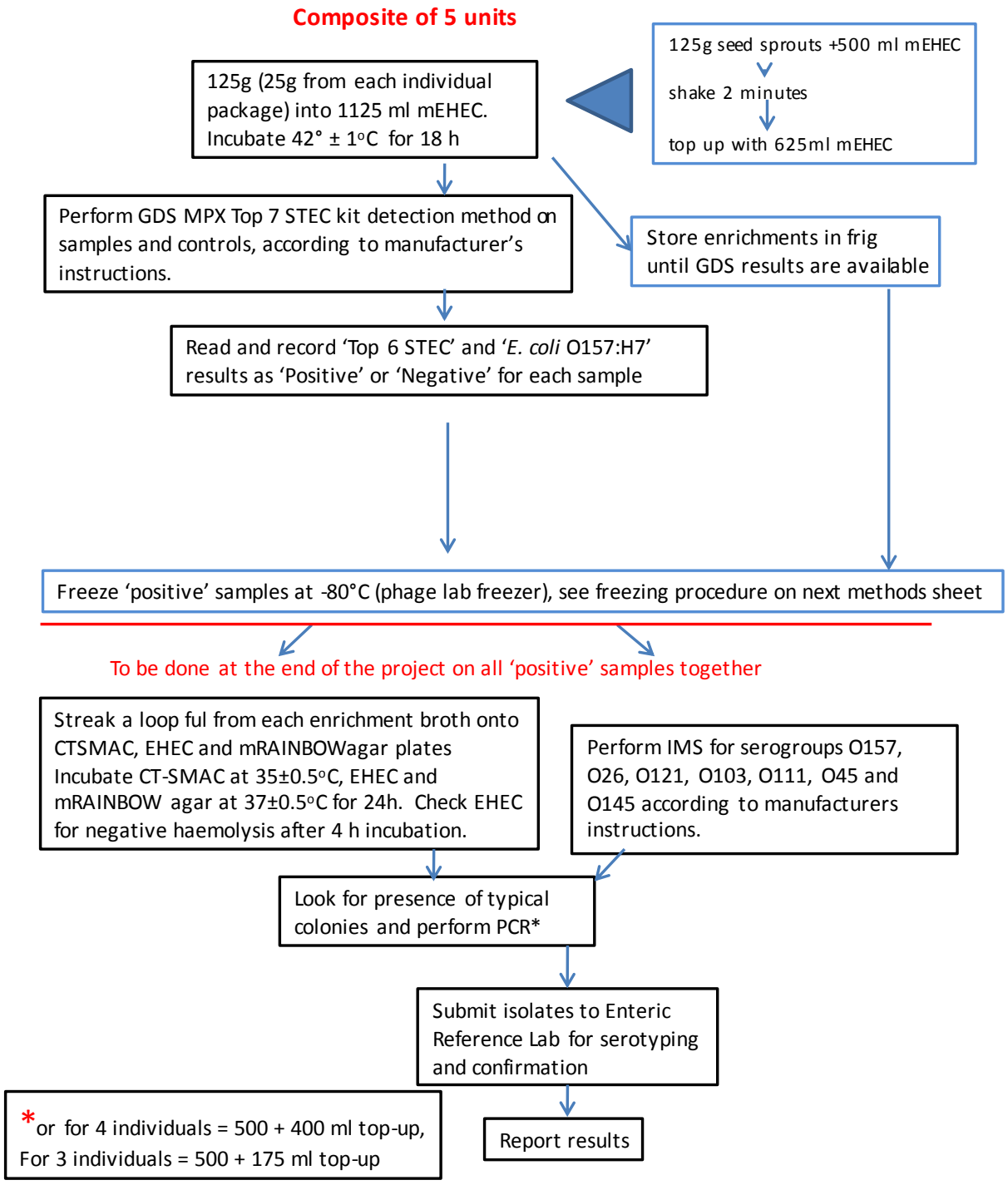


Salmonella P/A: "Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp." (ISO 6579:2002).



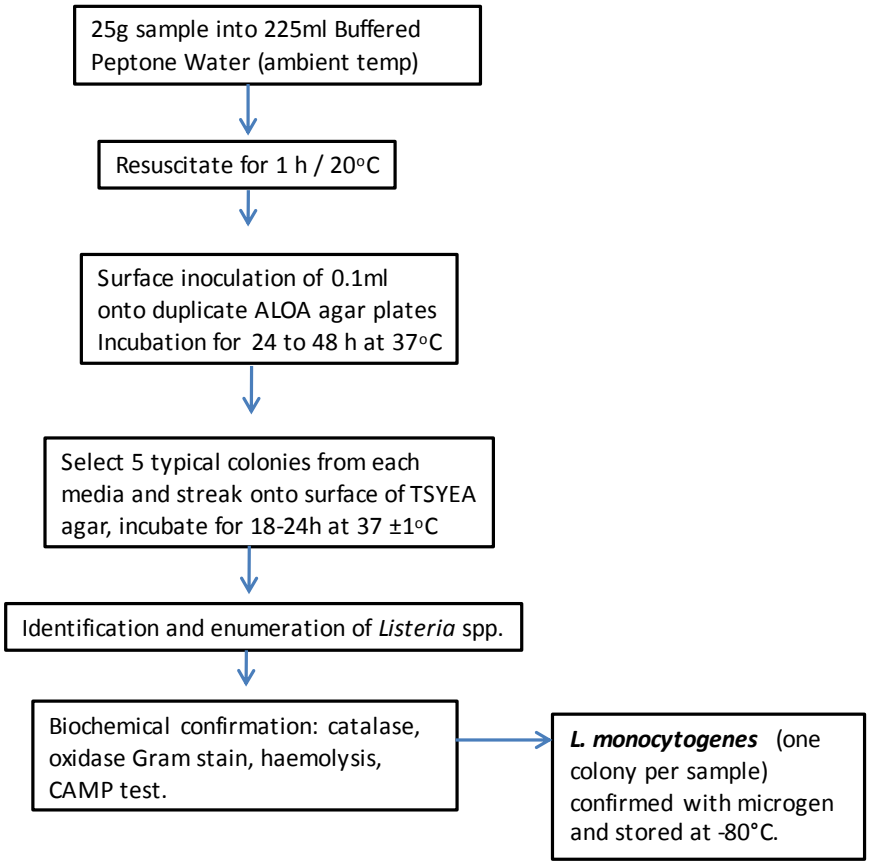
STEC P/A – Assurance GDS method

(Method using BIOCONTROL Assurance GDS machine and methodology)

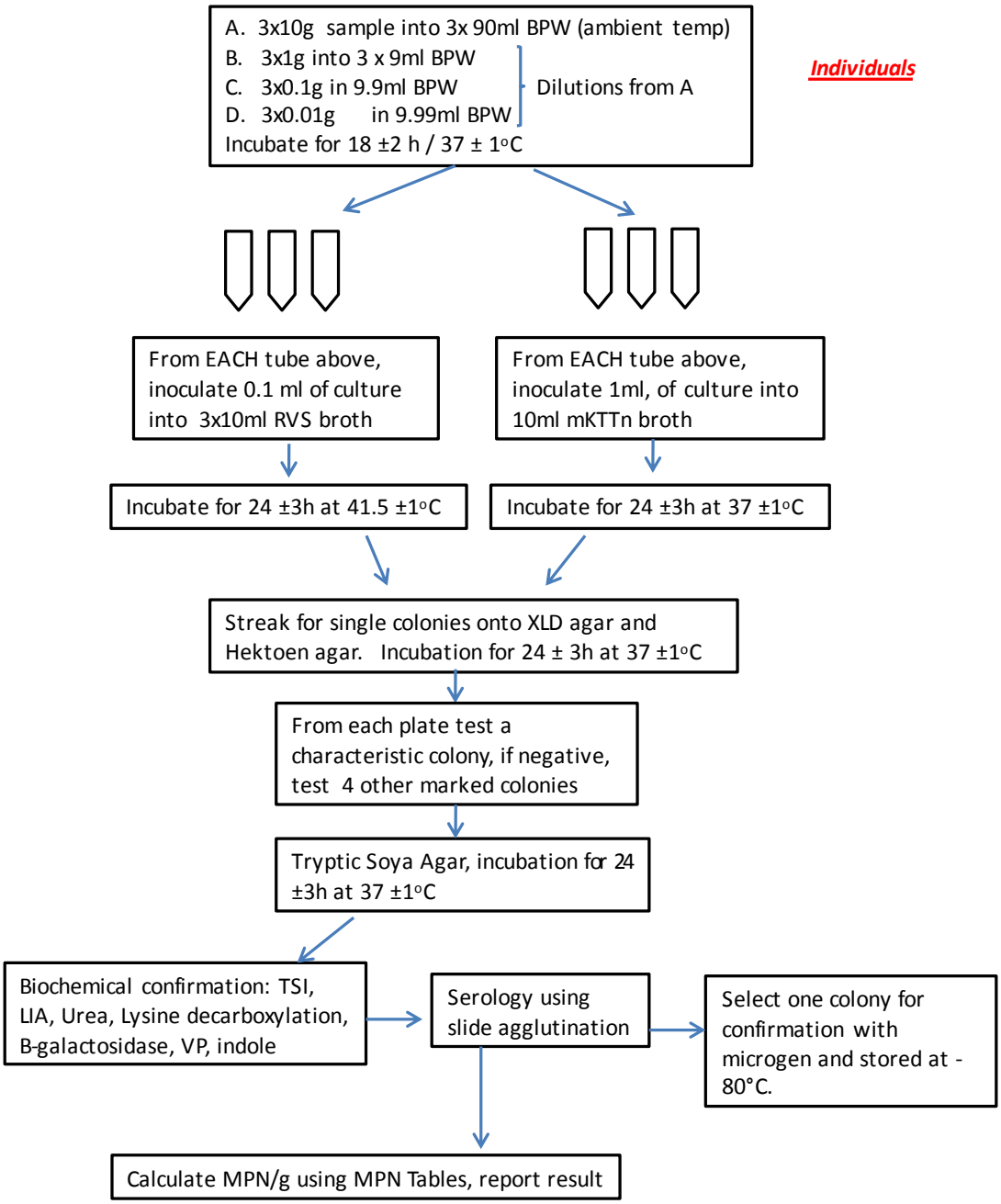


***L. monocytogenes* enumeration:** "Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method (ISO 11290-2:1998/Amd.1:2004)."

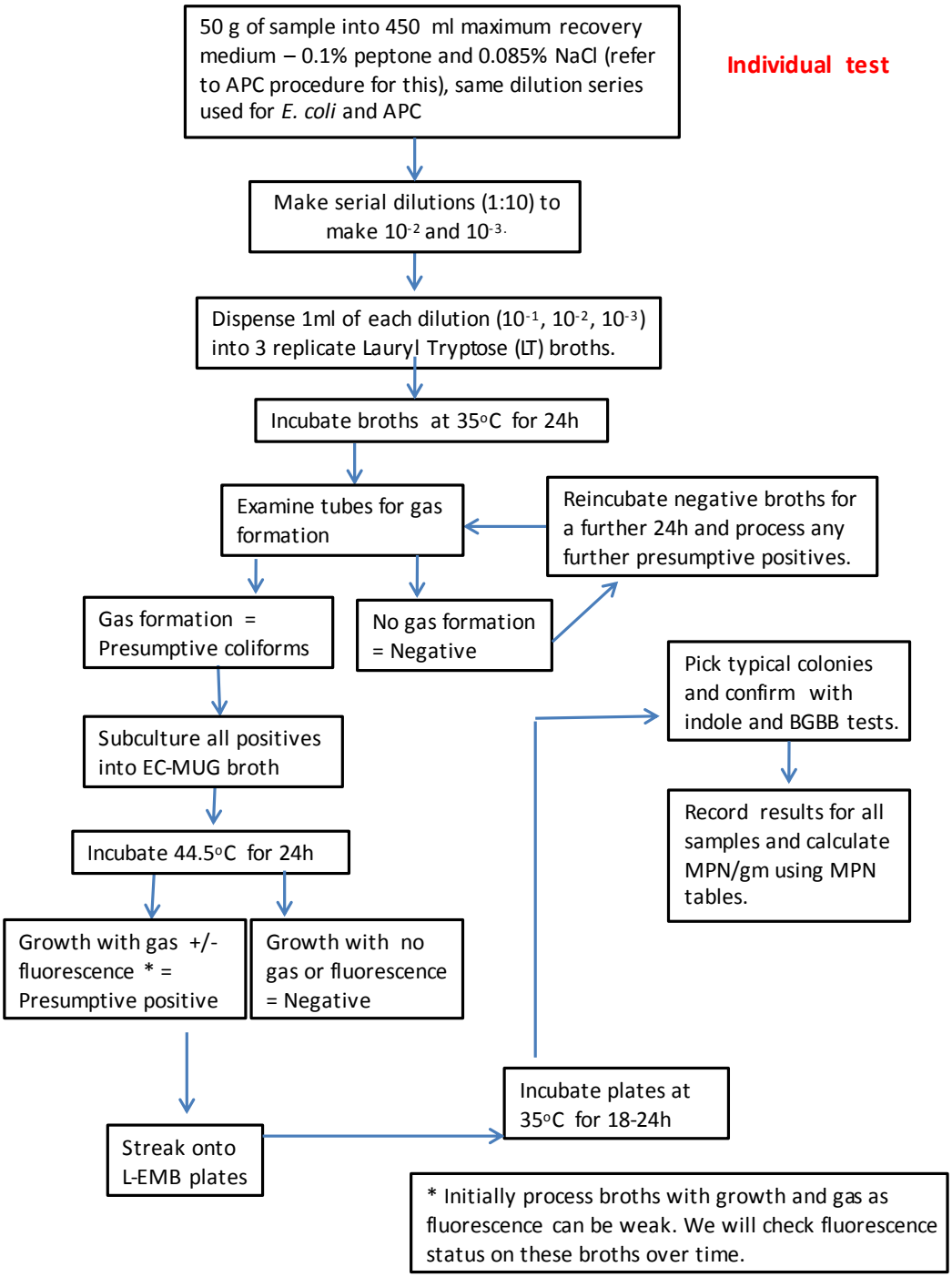
Individuals, on a per positive P/A basis



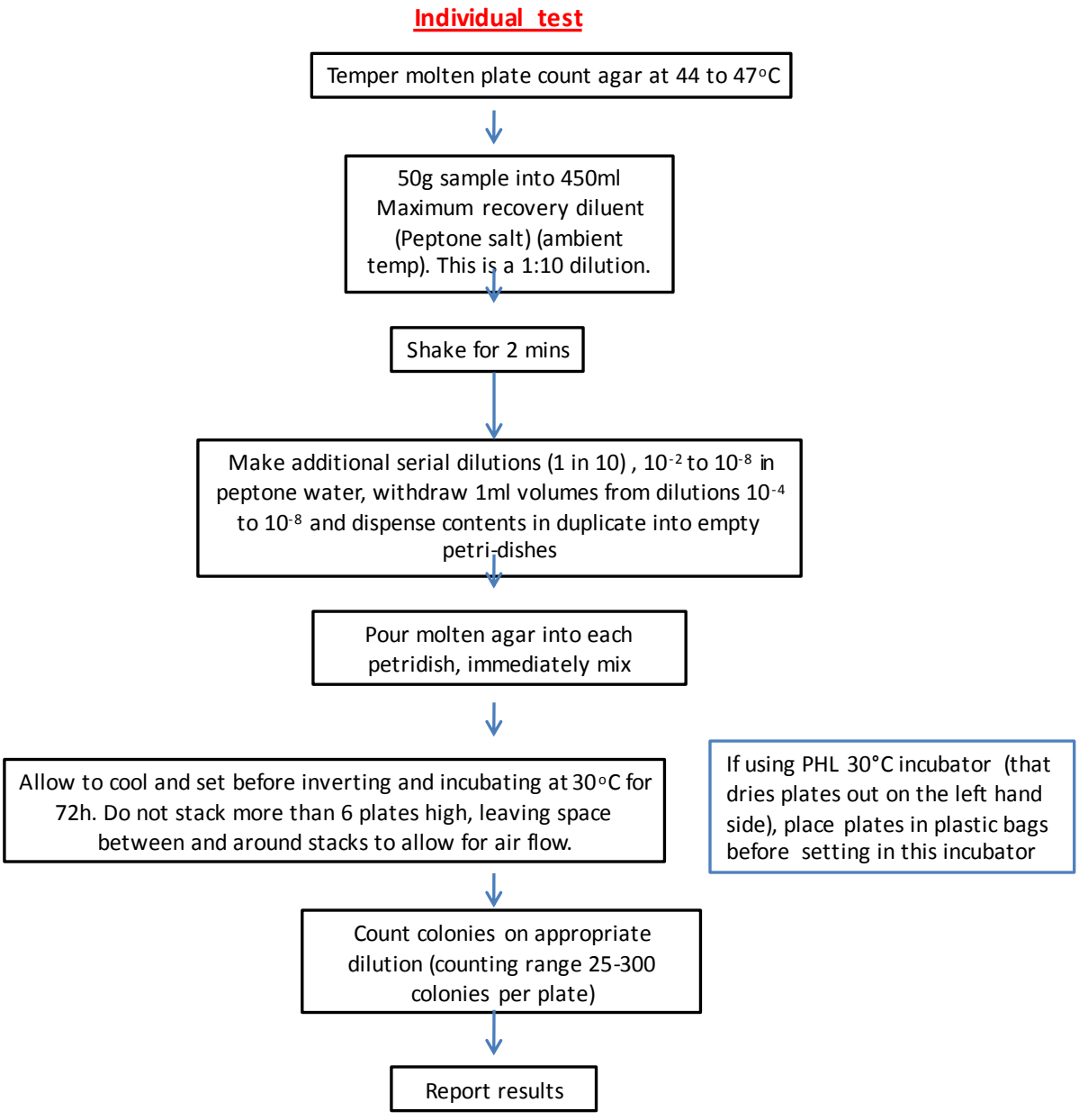
***Salmonella* Enumeration 12 Tube MPN:** 'Microbiology of food and animal feeding stuffs – general requirements and guidance for microbiological examinations (ISO 7218:2007; ISO: 6579:2002).



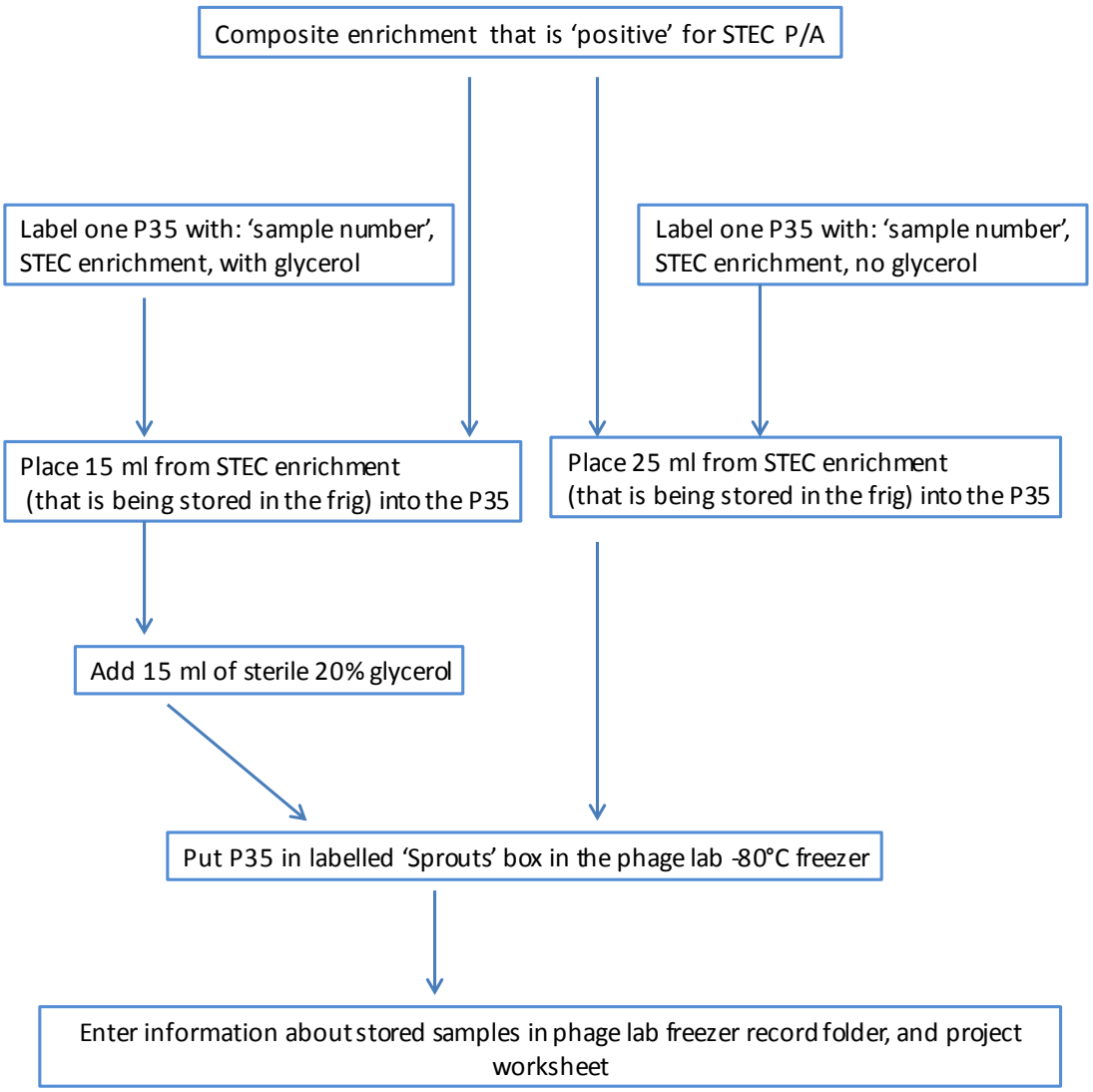
E. coli MPN method (APHA (2001) 8.7/8.8/8.9)



Aerobic Plate Count – "Microbiology of food and animal feeding stuff – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C" (ISO 4833:2003)."



Freezing method for 'positive' enrichments from STEC P/A PCR



Appendix 3: Assurance GDS Top 7 STEC manufacturer's technical data sheet

ASSURANCE GDS Genetic Detection System MPX Top 7 STEC

BIOCONTROL Results. Right now

AOAC Performace Tested Method 071301

GENERAL DESCRIPTION

Assurance GDS® MPX Top 7 STEC is an automated nucleic acid amplification system for the detection of *E. coli* O157:H7 and the "Top Six" non-O157 Shiga Toxigenic *E. coli* (STEC) in a variety of foods, including raw ground beef, raw beef trim, raw spinach, and raw mixed greens. The Top Six non-O157 STEC are defined as *E. coli* belonging to serogroups O103, O111, O121, O145, O26 or O45 that possess both the *eae* gene and at least one of the Shiga Toxin genes *stx*1 or *stx*2. Assurance GDS MPX Top 7 STEC utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to 7 specific Top STEC O serogroups (O103, O111, O121, O145, O26, O45 and O157) prior to genetic analysis for the associated pathogenicity genes.

Part No: 71015-100 (100 tests)

KIT COMPONENTS

Each Assurance GDS MPX Top 7 STEC kit contains the following:

- MPX Top STEC Amplification Tubes
- Top 7 STEC Concentration Reagent
- Resuspension Buffer Tq
- Top STEC Wash Solution

EQUIPMENT / MATERIALS REQUIRED

Other necessary materials not provided include:

- mEHEC® media
- Assurance GDS Rotor-Gene®
- PickPen® and PickPen tips
- Vortex mixer
- Adhesive film
- Sample wells and sample wells base
- Resuspension Plate
- Gel cooling block
- Stomacher / Masticator or equivalent
- 8-channel micropipette capable of accurately dispensing 30 µL
- Repeat pipette
- Adjustable micropipette
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1.0 mL)
- Incubator capable of maintaining 42 ± 1°C

SAMPLE PREPARATION

A. Test Portion Preparation & Enrichment

(a) Beef Samples – Aseptically weigh 375 g test portion into 1,500 mL pre-warmed (42 ± 1°C) mEHEC® media (for 25 g samples, use 225 mL mEHEC). Masticate or homogenize sample by hand for 2 min. Incubate for 10 – 18 h at 42 ± 1 °C.

(b) Fresh Vegetables – Aseptically weigh 25 g test portion into 225 mL pre-warmed (42 ± 1°C) mEHEC media.

Masticate or homogenize sample by hand for 2 min. Incubate for 10 – 18 h at 42 ± 1°C.

Note: Contact BioControl Systems, Inc. for recommended procedures for testing alternate sample types or sizes.

B. Sample Preparation Protocol

Change gloves prior to handling reagents.

(a) Vortex **Top 7 STEC Concentration Reagent.** Immediately transfer 20 µL to each of the required number of Assurance GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.

(b) Transfer 1.0 mL of **Top STEC Wash Solution** to each of 2 additional sample wells (2 well/sample) using a repeat pipette and 10 mL pipette tips.

(c) Transfer 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

(d) Carefully remove adhesive film from 1 strip of sample wells containing Top 7 STEC Concentration Reagent. Add 1.0 mL of incubated sample to each sample well. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip of wells. **Immediately return samples to incubator for use during confirmation if necessary.**

(e) Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10– 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

(f) Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from sample wells containing Top STEC Wash Solution.

(g) Load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen tips against the side of the sample wells to remove excess media droplets.

(h) Transfer PickPen to corresponding sample wells containing Top STEC Wash Solution and retract PickPen magnets to release particles into Top STEC Wash Solution.

(i) Discard PickPen tips and load a new set of tips onto the PickPen.

(j) Extend the PickPen magnets and insert into the strip of wells containing the Top STEC Wash Solution and particles. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.

(k) Transfer PickPen to the second set of sample wells containing fresh Top STEC Wash Solution and gently swirl for 10 s (do not release particles into solution). Tap the PickPen tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.

(l) Transfer particles to corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq.

(m) Repeat steps (f) through (l) for all samples. Cover resuspension plate with adhesive film strips.

TEST PROCEDURE

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

(a) Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15 °C) for 6 h. When frozen the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -25 to -15 °C.

(b) Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

B. Preparation of Amplification Tubes

(a) The Assurance GDS Rotor-Gene set up and data entry should be completed prior to transferring samples from the resusupension plate into the Amplification Tubes.

(b) Remove **MPX Top STEC Amplification Tubes** from foil pouch and place them in the frozen gel cooling block. Reseal pouch.

(c) Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipettor and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.

(d) Prior to placing in rotor, invert Amplification Tubes and shake with a snapping motion to thoroughly mix contents.

(e) Place Amplification Tubes into Assurance GDS Rotor-Gene in sequential order, beginning with position #1. Start Rotor-Gene cycle. Refer to Assurance GDS user manual for detailed instructions on operating the Rotor-Gene.

Note: The Assurance GDS Rotor-Gene must be started within 15 min after addition of the samples to the Amplification Tubes.

RESULTS

Upon completion of the run each sample will be identified as **Positive** or **Negative** for Top 6 STEC, and **Positive** or **Negative** for *E. coli* O157:H7 or **No Amp.** The individual gene results (*eae*, *stx*1, *stx*2) are also presented.

Top 6 STEC (*eae*/*stx*) Results:

Positive: Samples are positive for *E. coli* that belong to O serogroups O103, O111, O121, O145, O26 and O45 and contain the *eae* gene and one or both of the shiga toxin genes *stx*1 or *stx*2.

Negative: Samples are negative for *E. coli* that belong to O serogroups O103, O111, O121, O145, O26 and O45 and contain the *eae* gene and one or both of the shiga toxin genes *stx*1 or *stx*2.

No Amp: Amplification did not occur. Repeat the test beginning from step **B. Sample Preparation Protocol**. If the No Amp result is repeated contact BioControl Technical Service.

No.	Name	Top STEC Result	eae Result	stx1 result	stx2 result	Assay	Kit lot
1	Sample 1	Positive	+	+	+	Top STEC MPX	abc123
2	Sample 2	Positive	+	+	-	Top STEC MPX	abc123
3	Sample 3	Positive	+	-	+	Top STEC MPX	abc123
4	Sample 4	Negative	+	-	-	Top STEC MPX	abc123
5	Sample 5	Negative	-	+	+	Top STEC MPX	abc123
6	Sample 6	Negative	-	+	-	Top STEC MPX	abc123
7	Sample 7	Negative	-	-	+	Top STEC MPX	abc123
8	Sample 8	Negative	-	-	-	Top STEC MPX	abc123
9	Sample 9	No Amp	-	-	-	Top STEC MPX	abc123

E. coli O157:H7 Results:

Positive: Samples are positive for *E. coli* O157:H7.

Negative: Samples are negative for *E. coli* O157:H7.

No Amp: Amplification did not occur. Repeat the test beginning from step **B. Sample Preparation Protocol**. If the No Amp result is repeated contact BioControl Technical Service.

No.	Name	E. coli O157:H7 Result	Assay	Kit lot
1	Sample 1	Positive	Top STEC MPX	abc123
2	Sample 2	Positive	Top STEC MPX	abc123
3	Sample 3	Positive	Top STEC MPX	abc123
4	Sample 4	Negative	Top STEC MPX	abc123
5	Sample 5	Negative	Top STEC MPX	abc123
6	Sample 6	Negative	Top STEC MPX	abc123
7	Sample 7	Negative	Top STEC MPX	abc123
8	Sample 8	Negative	Top STEC MPX	abc123
9	Sample 9	No Amp	Top STEC MPX	abc123

CONFIRMATION

An aliquot of the mEHEC enrichment from Assurance GDS MPX Top 7 STEC positive samples may be confirmed for Top STEC via USDA-FSIS *Microbiology Laboratory Guidebook*, 5B.00 or for *E. coli* O157:H7 via USDA-FSIS *Microbiology Laboratory Guidebook 5A.01*.

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Samples may also be confirmed using the Assurance GDS IMS Panel – Top STEC kit which contains individual IMS Particles for the Top 7 STEC O serogroups (Part No. 61019-100), or Assurance GDS Poly IMS - Top STEC Kit which contains a single mixture of all the Top 7 STEC O serogroups (Part No. 61030-100.) Contact BioControl Systems, Inc. for more information.

STORAGE

Store Assurance GDS MPX Top 7 STEC kit components at 2 – 8 °C. Kit expiration is provided on the product box label.

PRECAUTIONS

This product is not intended for human or veterinary use. Assurance GDS MPX Top 7 STEC must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

Do not use test kit beyond expiration date on the product box label. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state and federal regulations.

Do not open used Amplification Tubes. Do not autoclave used Amplification Tubes. Dispose of used Amplification Tubes in a sealed container with 20% bleach or 1.2% sodium hypochlorite solution. Waste may be contaminated with *E. coli* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

PRODUCT WARRANTY

BioControl Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer, or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer's instructions.

This product contains MGB Eclipse™ probes and primers which are manufactured for BioControl Systems, Inc. by Epoch Biosciences. Certain reagents are covered by patents owned by Epoch Biosciences including, but not limited to, US Patents 6,312,894; 6,127,121; 5,801,155; 6084,102; 6,426,408; 6,492,346 and 6,485,906, and there is no implied license for any other use with respect to this product. The purchase price of this product includes a limited, nontransferable license under US patent nos. 6,030,787; 5,723,591; and 5,876,930 and corresponding foreign patents to use only the amount of MGB Eclipse detection reagents provided in the product for use only in the provided kit and solely for food and environmental testing, the express purposes noted in the instructions. This product may be used in the Polymerase Chain Reaction ("PCR") process, certain aspects of which are covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd ("Roche"). A license to use the PCR process accompanies the purchase of polymerase reagents from licensed suppliers, including those recommended by BioControl Systems, when used in conjunction with an authorized thermal cycler, or is available from Applied BioSystems.

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List of abbreviations used in appendices

ALOA (agar)	Agar *Listeria* Ottaviani and Agosti
APC	Aerobic Plate Count
APHA	American Public Health Association
BGBB	Brilliant green bile Broth
BPW	Buffered Peptone Water
C	Celcius
CTSMAC (agar)	Cefixime Potassium Tellurite MacConkey
EC-MUG (agar)	*E. coli* 4-Methylumbelliferyl-β-D-glucoronide
EHEC	Enterohaemorrhagic *E. coli*
g	gram
GDS	Genetic Detection system
H	hour
IMS	Immuno Magnetic Separation
ISO	International Organisation for Standards
L-EMB	Levine Eosin Methylene Blue
LIA	Lysine Iron Agar
ml	millilitre
mEHEC (broth)	Modified Enteroharmorrhagic *E. coli*
MKTTn (broth)	Meller-Kauffman Tetrathionate Novobiocin
MPN	Most Probable Number
NaCl	Sodium chloride
P/A	Presence/Absence
PCR	Polymerase chain reaction
PFGE	Pulse field Gel electrophoresis
RVS (broth)	Rappaport-Vassiliadis
Spp	Species
STEC	Shiga toxin-producing *E. coli*
TSI	Triple Sugar Iron
TSYEA (agar)	Tryptic Soy Yeast Extract Agar
VP	Voges Proskauer
XLD (agar)	Xylene Lysine Deoxycholate Agar