final report

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Prepared by: A. Kiermeier
Statistical Process Improvement Consulting & Training Pty Ltd

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Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Process Risk – 13/14 and 14/15

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Executive Summary

The maintenance and further development of the existing risk assessment model is important to MLA for a number of reasons. Firstly, the model can be used to predict prevalence and concentration of those pathogens which are foreseen to cause problems, but for which, little data exists. In addition, the model can be used to identify particular steps through the processing chain that present significant risk, thus providing direction as to what areas require further investigation and data collection. Models can also be used to support Australia's testing and control of pathogens such as *E. coli* O157.

As part of this project, the findings from a previously developed *E. coli* O157 risk assessment were published. This was done to gain international scientific acceptance for a novel modelling approach and to demonstrate the low risk of *E. coli* O157 infection that Australian manufacturing beef poses for American consumers, when consumed as hamburgers. Given the recent trends related to *Salmonella*, it is recommended that the *E. coli* O157 risk assessment model be adapted to allow incorporation of the results from an Australian survey of *Salmonella* in manufacturing beef, once they become available.

Work has also been undertaken to better understanding the implications of different sampling programs for Shiga Toxin producing *E.* coli (STEC). Utilising the risk assessment model for *E.* coli O157, this work showed that "increased testing beyond the current N-60 sampling plan provides marginal additional public health benefit." In addition, some Australian processors have received advice from overseas that reducing lots size increases the sensitivity of detecting STEC. However, when this advice was evaluated statistically it was shown to be incorrect. This has allowed processors make more informed decisions when deciding on a sampling and testing program.

In addition, interest by some trading partners, e.g. the United States, in process control requires assessment of proposals from the USA for their effect on Australian processors, and the need to collect and analyse additional data on processing in Australia. This project has supported MLA project G.MFS.0294 "Statistical Process Control – Hygiene and Hazards", which assisted red meat processors to better understand and control microbial hazards during slaughter and dressing.

Table of Contents

1 Background

Previously a process risk model was developed to utilise existing data from MLA projects and the wider literature, and place it into a risk context which could be used as a research tool to better understand risks and identify areas within the program (particularly the pathogen and microbial contamination area) requiring further investigation. The model allows for analysis of data in a descriptive and mathematical manner, and is useful within the pathogen program plan as a tool to ensure MLA and the industry stays "ahead of the play" rather than just "reacting/responding" to food safety concerns. Modelling has also been used to understand contamination of cartons of manufacturing beef and develop risk assessments.

The maintenance and further development of the existing risk model is important to MLA for a number of reasons. Firstly, the model can be used to predict prevalence and concentration of those pathogens which are foreseen to cause problems, but for which, little data exists. In addition, the model can be used to identify particular steps through the processing chain that present significant risk, thus providing direction as to what areas require further investigation and data collection. Models can be used to support Australia's testing and control of pathogens such as *E. coli* O157.

Current interest by some trading partners (for example, USA) in process control requires assessment of proposals from the USA for their impact on Australian processors, and the need to collect and analyse additional data on processing in Australia.

This project is a continuation of MLA Project A.MFS.0261, which was undertaken by the Principal Investigator (PI) while working for the South Australian Research & Development Institute (SARDI). In addition, due to the recent national and international focus on process control, the PI also worked closely with S. Rogers (SARDI) on MLA Project G.MFS.0294 "Statistical Process Control – Hygiene and Hazards".

2 Projective Objectives

Provision of advice through an understanding of process risk, based on the Process Risk Model will involve:

1. Documenting and explaining the model to maintain transparency and accessibility to MLA and MLA's scientific risk management panel
2. Identifying parts of the existing model which may need improvement / updating
3. Identifying areas within existing data, where there may be incomplete data, and a need for additional collection
4. Specifying the data requirements and allow for data obtained from a wide range of different projects within the program to be fed into the model for evaluation
5. Contributing to the development of experimental and survey design for projects related to the model (including responses to actions by Australia's trading partners)
6. Identifying areas within the processing chain which may be more important from a risk viewpoint, and therefore require a greater degree of investigation / knowledge

7. Assisting in the development of recommendations for complete risk assessments, and performing risk assessment, when required
8. Assisting in the development of risk management options, based on outcomes from the use of the process risk model
9. Interacting with MLA's scientific risk management panel, as required.

Furthermore, the following Additional Details were specified:

The MLA scientific risk management panel will identify and discuss significant hazards, assess the risks in scientific terms and ensure that knowledge gaps in the understanding and management of the hazard in the Australian red meat industry are filled in an appropriate manner. The process risk model will provide information and insights for the scientific risk management panel and it is expected that some face-to-face meetings with the panel will be held. It is expected the Scientific Risk management panel will meet three times per year, and it is expected that a nominated person may need to attend some or all of these meetings, depending on the agenda. Interactions between the Nominated Person/s and the scientific risk management panel will be at the direction of MLA. The specific development of the model and the use of the outputs will be directed by MLA from time to time.

3 Methodology

This project was undertaken in collaboration with Ian Jenson, MLA. Pieces of work were completed at the direction of MLA in response to ongoing and emerging issues facing the red meat industry.

4 Results

The major themes of this project were a risk assessment for *E. coli* O157 in hamburgers made from Australian manufacturing beef, testing for Shiga toxin producing *E. coli* (STEC) in Australian manufacturing beef and process control during slaughter and dressing of cattle. Activities related to these themes are described below.

In addition, the PI provided photos, figures and text to support the MLA publication "Shelf life of Australian red meat."

4.1 *E. coli* O157 Risk Assessment

As part of a previous MLA Project (A.MFS.0261), a risk assessment for *E. coli* O157 in burgers made from Australian manufacturing beef was undertaken. Consequently, the following two journal publications, and one conference poster, were produced as part of the current project.

- A. Kiermeier, J. Sumner, I. Jenson (2014) *Impact of sampling programs on the risk of E. coli O157 illness from consumption of hamburgers made from Australian manufacturing beef*, Annual Meeting of the International Association for Food Protection, Indianapolis, 4-6 August 2014
- A. Kiermeier, I. Jenson, J. Sumner (2015) Risk assessment of *Escherichia coli* O157 illness from consumption of hamburgers in the United States made from Australian manufacturing beef, Risk Analysis, 35(1), pp77-89

- A. Kiermeier, J. Sumner, I. Jenson (2014) Effect of sampling plans on the risk of *Escherichia coli* O157 illness, Journal of Food Protection, 78(7), pp1370-1374.

4.2 Testing for Shiga Toxin producing *E. coli* in manufacturing beef

On 4 June 2012, verification testing of manufacturing beef for STEC was introduced in Australia (Department of Agriculture, Meat Notice 2012/3). In early 2014, an industry delegation to the United States was presented with information that reducing lot size would increase the sensitivity of detecting *E. coli* O157 / STEC. Consequently, some establishments reduced their lot sizes from 700 cartons to 350 or 175 cartons to (a) reduce the cost of disposition in the case of a detection, and (b) increase the sensitivity of the sampling program. However, the advice about increased sensitivity was incorrect and a guidance document was prepared in September 2014 to assist the industry make informed decisions about the statistical properties of their sampling programs (Appendix 1).

Some beef processors have developed additional sampling programs to those required for *E. coli* O157 under Meat Notice 2008/9, with the aim of decreasing the risk of Port-of-Entry detections or product recalls. For example, many processors now routinely test for STEC, while a few test manufacturing beef prior to freezing, and some also test for generic *E. coli* and Total Viable Counts (TVC) in their carton product. One processor wanted to investigate whether generic *E. coli* detections/levels, coliform detections/levels or TVC levels of fresh manufacturing beef at the end of boning were related to the likelihood of STEC detections. Ten lots with and 20 lots without STEC detections were interrogated, but no relationship could be established between STEC detection and hygiene indicator levels.

By late 2014 an increase in the national *E. coli* O157 detections in manufacturing beef destined for the US had been observed, as well as an increase in *E. coli* and coliform carcase prevalence for cow/bull and steer/heifer. Consequently, an industry advisory document was developed for the Export Meat Industry Advisory Council (EMIAC) in March 2015 to alert industry of the need to be even more vigilant and proactive in addressing these trends.

On 9 and 10 March 2015, a Symposium was held at Charles Sturt University, Wagga Wagga, entitled “Human pathogenic *E. coli* in cattle: from farm to fork.” The PI was invited by MLA to present on “*E. coli* in manufacturing beef and the risks to human health: Reducing risks post processing”. Subsequently, the “Shiga toxin-producing *Escherichia coli* and beef production” symposium factsheet was developed by MLA.

4.3 Process Control

Due to the increasing national and international focus on process control MLA previously funded a project in this area (MLA Project G.MFS.0294 “Statistical Process Control – Hygiene and Hazards”). The PI worked closely with S. Rogers (SARDI) on project G.MFS.0294, including advice on experimental work undertaken by Sam Rogers. In addition, Sam Rogers (SARDI), Clive Richardson (MINTRAC) and Andreas Kiermeier visited 8 beef slaughter establishments in November 2014. These establishments were selected because of the range of *E. coli* O157/STEC detection rates (low to high), with the aim of obtaining detailed information about their slaughter, dressing and boning operations. The findings were used to revise the MLA “Incoming livestock and slaughter process assessment tool for

beef" created in 2005, and the details can be found in the final report for project G.MFS.0294.

In May 2015, Al Almanza, Deputy Under Secretary for Food Safety, of the United States Food Safety Inspection Service (FSIS) visited Australia. The PI was invited to present on the Australian red meat industry's approach to process control, including investigations throughout the supply chain and continual process improvement work undertaken in the processing sector. Unfortunately, a late change in A. Amanza's itinerary resulted in the cancellation of this presentation.

5 Discussion

The extent to which the project objectives were achieved is summarised below.

1. *Documenting and explaining the model to maintain transparency and accessibility to MLA and MLA's scientific risk management panel.*
   The risk assessment model, and the results obtained from modelling different strategies for sampling for *E. coli* O157, were published in two journal publications and a conference poster.

2. *Identifying parts of the existing model which may need improvement / updating.*
   While several assumptions were made as part of the *E. coli* O157 risk assessment, none at this stage require improvement / updating at this stage. However, there may be a need in the future to extend the model to an earlier stage in the processing chain, e.g. to include boning room operations.

3. *Identifying areas within existing data, where there may be incomplete data, and a need for additional collection.*
   Due to data gaps, assumptions were made in the risk assessment model and these were identified and discussed in the journal publications. In particular,

4. *Specifying the data requirements and allow for data obtained from a wide range of different projects within the program to be fed into the model for evaluation.*
   MLA has recently funded a survey of *Salmonella* in manufacturing beef and there have been some preliminary discussions about utilising the resulting data in the model. This would allow a risk assessment model for *Salmonella* to be constructed and evaluated quickly.

5. *Contributing to the development of experimental and survey design for projects related to the model (including responses to actions by Australia's trading partners).*
   The PI worked closely with Sam Rogers on various aspects of project G.MFS.0294, including experimental work undertaken as part of that project. In addition, a presentation to Al Amanza (FSIS) was prepared on Australia process control work, and

6. *Identifying areas within the processing chain which may be more important from a risk viewpoint, and therefore require a greater degree of investigation /*

knowledge.
The PI assisted on MLA project G.MFS.0294, including the investigation of livestock and slaughter practices that may lead to an increased risk of *E. coli* O157 /STEC contamination of manufacturing beef.

7. *Assisting in the development of recommendations for complete risk assessments, and performing risk assessment, when required.*
A risk assessment of *E. coli* O157 in burgers made from Australian manufacturing meat was previously developed. This risk assessment has now been published in the international scientific literature.

8. *Assisting in the development of risk management options, based on outcomes from the use of the process risk model.*
An advisory document was prepared for red meat processors on various aspects of sampling for *E. coli* O157 / STEC.

9. *Interacting with MLA's scientific risk management panel, as required.*
There was no requirement to meet with / present to the risk management panel.

6 Conclusions/Recommendations

The objectives of this project were carried forward from earlier projects and were focussed around a process model that had been developed previously. However, recent work has been broader than this. In particular, there was a clear need to publish the findings from the *E. coli* O157 risk assessment to gain international scientific acceptance for a novel modelling approach involving modelling of 700 carton lots and to demonstrate the low risk of *E. coli* O157 infection that Australian manufacturing beef poses for American consumers when consumed as hamburgers. Given the recent trends in the US related to *Salmonella*, it is recommended that the *E. coli* O157 risk assessment model be adapted to allow incorporation of the results from an Australian survey of *Salmonella* in manufacturing beef, once they become available.

Work has also been undertaken to better understanding the implications of different sampling programs for STEC. Utilising the risk assessment model for *E.* coli O157, this work showed that "increased testing beyond the current N-60 sampling plan provides marginal additional public health benefit." In addition, some Australian processors have received advice from overseas that reducing lots size increases the sensitivity of detecting STEC. However, when this advice was evaluated statistically it was shown to be incorrect. This has allowed processors to gain a better understanding of the statistical aspects involved in sampling and testing of manufacturing beef and hence has enabled them to make more informed decisions.

As indicated above, there is increasing emphasis on maintaining and improving process control during the slaughter and dressing of cattle (and also sheep). This project has supported MLA project G.MFS.0294, which resulted in a revision of the "Incoming livestock and slaughter process assessment tool for beef." Details for this tool can be found in the final report for project G.MFS.0294.

7 Appendix

7.1 Appendix 1: Sampling beef trim for E. coli O157 and STEC

Sampling beef trim for *E. coli* O157 and STEC

8 September 2014

In this document the sampling and testing of beef trim using an enrichment test is discussed. While we have used testing for *E. coli* O157 as the example, the approach applies equally to the big 6 STEC, or any other bacteria tested in a similar way.

In an earlier document the effect of lots size on the probability of detecting *E.* coli O157 or STEC was discussed. In this document additional information is provided with respect to "production lot testing" of fresh beef trim compared to testing containers / port marks of frozen beef trim.

1 Background

In 2007 FSIS commenced testing of beef trim destined for grinding using surface slice and "Robust N-60" sampling[1] at US processing establishments. Subsequently, Australian establishments were required to also sample and test all lots of beef trim destined for the US using an N-60 protocol (Australian Meat Notice 2007/17). The meat notice stipulated a maximum lot size of 700 cartons (a container equivalent) and that a lot would consist of product packed on a given packing line and based on Sanitation SOPs and/or determined by the establishment based on the implementation of a statistically based sampling program to distinguish between segments of production. For each lot, five 5-10g samples – surface slices or small grab samples – were to be collected from a minimum of 12 randomly selected cartons, to a total sample weight of at least 375g.

Initially, sampling was undertaken on cartons of fresh meat prior to carton sealing and freezing, though collection of frozen samples was an option. However, it wasn't until implementation of Australian Meat Notice 2008/9 that testing of frozen samples became more established throughout the industry, with many establishments testing at load-out or shortly before. This was possible because there was no longer a requirement to define lots through "Sanitation SOPs" but only through Robust N-60 sampling and testing and hence could be confined to a single container load (or less). That is, lots that were tested separately could be 'deemed independent' and a detection of *E. coli* O157 in one lot did not trigger a rejection of other lots provided they had be tested separately – even if they had been produced during the same production period.

As far as we know, this approach was also applied at Port-of-Entry (PoE) testing and further downstream in the supply chain. For example, if a lot was found to contain *E. coli* O157 at

[1] FSIS (2011) National Prevalence Estimate of Pathogens in Domestic Beef Manufacturing Trimmings (Trim): December 2005 – January 2007, http://www.fsis.usda.gov/PDF/Baseline_Data_Domestic_Beef_Trimmings_Rev.pdf

PoE and provided it had previously been tested as a lot, then all other lots from the same establishment were unaffected, that is, other lots that had been tested separately were not required to be recalled, even if they were produced from the same source materials.

2 Recent Changes by FSIS

In August 2014, FSIS announced (Doc No. 2014-19141[2]) "*that it will begin requesting an establishment to recall product if an establishment was the sole supplier of beef manufacturing trimmings source materials for ground beef product that FSIS or another Federal or State agency finds positive for E. coli O157:H7, evidence suggests that the contamination most likely occurred at the supplier establishment, and a portion of the product from the originating source lot produced by the supplier establishment was sent to other establishments.*"

The potential implications for this scenario are illustrated in Figure 1 below, where an Australian establishment produces three lots (based on customer specifications such as CL) on a single production day, using the same source animals and without "clean down" between these lots. Also assume that *E. coli* O157 is not detected when each of these three lots is tested prior to export to different US customers. If Lot 1 is the only material used by US Customer 1 and FSIS detects *E. coli* O157 in the corresponding ground product, then FSIS could require Lots 2 and 3 (shipped to other customers) to also be recalled. This is because all three lots are produced from the same "source lot", i.e. a production day.

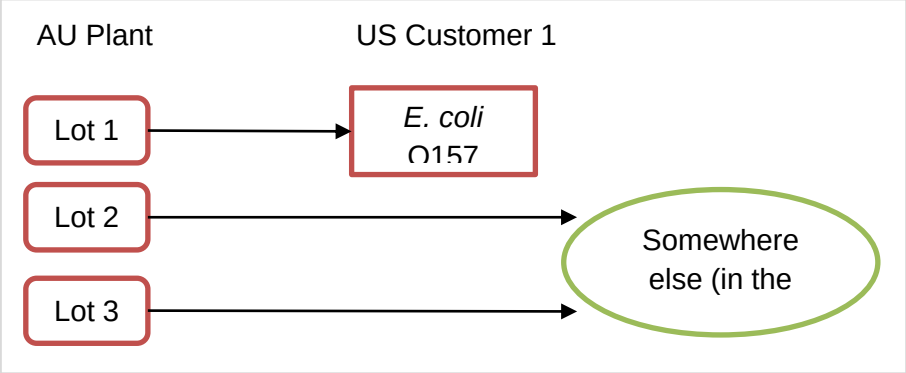

Figure 1 Potential recall scenario – Lots 2 & 3 may need to be recalled if they are produced from the same source materials as Lot 1, e.g. on the same day of production.

Some Australian establishments produce lots by collecting cartons that meet particular specifications over a number of production days. In this case, the recent FSIS Notice is even more far reaching than shown in Figure 1, as now all lots produced on those production days could be implicated and be required to be recalled.

[2] Federal Register Volume 79, Number 156 (13 August 2014), Pages 47417-47424, http://www.fsis.usda.gov/wps/wcm/connect/ad0a350c-f874-4b4a-bbe5-7e82dadfa526/2011-0009.htm?MOD=AJPERES

It should be noted that the same FSIS traceback requirement does not apply (at least not yet) when an *E. coli* O157 detection occurs in comingled product. This is because it is not possible to determine which of the source lots contained *E. coli* O157.[3]

3 Container Lot Testing versus Production Lot Testing

In response to the recent FSIS Notice, some Australian establishments have shown an interest in production lot testing, either as a replacement of or in addition to container lot testing, where we define the two testing approaches as follows:

- **Production lot testing**: Lots are defined through Sanitation SOPs, i.e. all cartons produced in a single production period that is completely separated from all other production periods by effective sanitation. Sampling involves collecting five fresh meat samples (5-10g each) from at least 12 cartons and testing them as one composite sample (total weight at least 375g).
- **Container lot testing**: Lots are defined by cartons in a container (or port mark), usually based on customer specifications. Cartons are frequently produced during multiple production periods and are not separated from other lots by Sanitation SOPs. Sampling involves collecting meat samples, usually frozen and possibly involving drill coring, from at least 12 random cartons. The total weight of 375g is tested as a single composite.

Let's now have a look at how the probability of detecting contamination in a lot might be affected by these two testing approaches and what the implications might be. Before we do, however, it is important to keep in mind the following important principle that applies to any sampling and testing scheme applied in a food safety setting.

> *Detecting a pathogen in a food product is evidence of the presence of the pathogen. However, not detecting the pathogen does not provide evidence that the pathogen is not present in the food product (i.e. of no contamination).*

This is because sampling and testing is very much a "game of chance" rather than a "game of skill" and hence the chance of detecting a pathogen depends on how much contamination there is in the lot. As such, gross contamination (high levels and wide spread) is likely to be detected (though not guaranteed!), while low levels of contamination are very unlikely to be detected (not unlike six correct numbers in lotto).

3.1 External carcase surface area sampled

Sampling fresh trimmings during production lot testing allows the collection of meat slices from the external carcase surface, which is the area most likely to be contaminated during dressing (after hide removal). This type of sample collection was used in the FSIS baseline[4] to maximise the chances of detecting *E. coli* O157 contamination. While surface slices are the "ideal" sample type, research indicates that similar results could be achieved with small

[3] This could change in the future if genetic fingerprinting methods can be developed to uniquely link *E. coli* O157 isolates with establishments.

[4] FSIS (2011) "National Prevalence Estimate of Pathogens in Domestic Beef Manufacturing Trimmings (Trim)" http://www.fsis.usda.gov/wps/wcm/connect/f07f5e1d-63f2-4ec8-a83a-e1661307b2c3/Baseline_Data_Domestic_Beef_Trimmings_Rev.pdf?MOD=AJPERES

grab samples of fresh beef trimmings[5] and this type of sampling was allowed as part of Australian Meat Notice 2007/17.

In contrast, sampling frozen cartons of beef trimmings requires the collection of frozen meat chipped of the edges of the carton, or through core sampling. Neither of these approaches is likely to yield similar levels of external carcase surface. In fact, the authors of an MLA funded study[6] of drill core sampling concluded that "the use of drill coring to obtain the same surface area would require a sample weight of about six times the amount stipulated by FSIS." That is, instead of testing 375g you would need test over 2.25kg to represent the same external carcase surface area.

Consequently, sampling and testing fresh beef trimmings, through the collection of surface slices or small grab samples, will increase the chances of detecting *E. coli* O157 (if present).

3.2 Effect of freezing on *E. coli* concentrations

Research has shown that freezing can result in a reduction of *E. coli* O157 between 0.5 and 2 $\log_{10}$ cfu/g (32-99% reduction).[7] However, the author also noted that "the inability to eliminate *E. coli* O157 and the presence of sub-lethally injured strains which are still infectious but undetectable on selective media make freezing an unreliable method to assure the safety of beef trimmings."

Consequently, sampling and testing fresh beef trimming presents a "worst case scenario" in terms of levels of *E. coli* O157 and hence increases the chances of detecting contamination when present. In contrast, sampling and testing frozen beef trimmings reduces the chances of detecting *E.* coli O157 when there is some contamination. While this applies at both export testing (in Australia) and PoE testing in the USA, establishments should not rely on the effect of freezing to eliminate *E. coli* O157 and mitigate their risk.

3.3 Traceability implication

When cartons from multiple production days are combined into a single lot, the potential implication of an *E. coli* O157 detection can be broad and affect multiple lots (hence the recent FSIS notice).

For example, consider a lot that consists of cartons produced during four production periods. We sample 12 cartons from this lot and a total of 375g of meat is collected and tested for *E. coli* O157 (as per Australian Meat Notice 2008/9). However, if the presence of *E. coli* O157 is confirmed, then it is impossible to know during which of the four production periods the *E.* coli O157 contamination occurred, or in fact if it was confined to just one – it could be as many as all four. Consequently, the "safe" action would be to withdraw *all* meat from all four production periods from commerce – even if other lots containing cartons from these periods did not have *E.* coli O157 detected when tested (remember, you may have just been "lucky" not to detect the contamination in those lots).

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[5] A. Kiermeier, G. Holds, M. Lorimer, I. Jenson, J. Sumner (2007) "Sampling cartons of beef trim for microbiological analysis: Comparison of portions versus surface slices" Food Protection Trends, 27(11), p899-902

[6] A. Small, N. McPhail, A. Kiermeier (2010) "The potential use of Carton Coring as a sampling method for *E. coli* O157 testing"

[7] G. A. Dykes (2001) "The effect of freezing on the survival of *Escherichia coli* O157:H7 on beef trimmings" Food Research International, 33, p 387-392

In contrast, if lots are defined based production periods that are clearly separated by “Sanitation SOPs” then lots produced during different periods can be considered independent.

4 Conclusion

Recent changes by FSIS with respect to traceability of product from sole suppliers has prompted some Australian processors to consider testing of fresh beef trimmings at the end of production, i.e. production lot testing, to reduce the risk of sending contaminated product to the USA.

Such production lot testing has two potential benefits, namely:

1. Increased likelihood of detecting contamination because more external carcase surface can be sampled and because the ability to detect *E. coli* O157 has not be reduced by freezing. This results in more contaminated lots being removed from commerce and therefore results in a reduction of risk of a PoE detection.
2. Independence of lots produced in production periods that are separated by “Sanitation SOPs” and hence a detection in one lot will not trigger a recall of microbiologically independent lots.

As a consequence of 1), establishments can expect to detect *E. coli* O157 more frequently. However, how much more frequently is unknown as this depends on how frequently the establishment’s product is contaminated and to what extent this contamination occurs. But because current testing methods utilise enrichment of a composite 375g sample there is no establishment-specific information about the extent of contamination (number of cartons and concentration of *E. coli* O157) which could be used to obtain better estimates.