final report

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Prepared by: Andreas Kiermeier

South Australian Research and Development Institute

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

Process Risk Models – Continued Development 13/14 and 14/15

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Contents

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 predictive tool to ensure MLA and the industry stays “ahead of the play” rather than just “reacting/responding” to food safety concerns. Modelling was also used to understand contamination of cartons of manufacturing beef.

The maintenance and further development of the existing risk model is important to MLA for a number of reasons. Firstly, using data collected for a pathogen known to currently pose a food safety problem, 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 throughout the processing chain that present significant risk, thus providing direction as to what areas require further investigation and data collection. Carton beef models can be used to support Australia’s testing and control of pathogens such as *E. coli* O157

Project Objectives

The maintenance of 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
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.

Results and Discussion

The following is a summary of the work undertaken as part of this project between the period of 1 July 2013 and 30 September 2013.

Risk assessment of *E. coli* O157 in burgers made from Australian beef trim

A risk assessment for *E. coli* O157 in burgers made from Australian beef trim, which was initiated in project A.MFS.0222, has been further developed and documented. This risk assessment uses data on the contamination of cartons of Australian beef trim[1] and subsequently models the *E. coli* O157 concentration in beef patties through retail storage, transport to the home, home storage, cooking and consumption. A manuscript reporting the risk assessment and results is still being prepared for scientific publication in *Risk Analysis*.

The key results from this risk assessment have been presented at the Annual Conference of the International Association for Food Protection in Charlotte, North Carolina from 28-31 July 2013 (Attachment 1).

Abattoir visits with Sam Rogers

As part of MLA project G.MFS.0294 "Statistical Process Control – Hygiene and Hazards" Dr Kiermeier visited five abattoirs as part of Sam Rogers' industry familiarisation. Abattoirs included the Northern Co-operative Meat Company, Oakey Abattoir, Greenmountain Meats and JBS Australia (Dinmore) on 29 & 30 August 2013, and Thomas Foods International on 27 September 2013.

MINTRAC QA Manager's Network meeting

Dr Kiermeier delivered an MLA presentation entitled "*Does your performance measure up?*" at the Adelaide MINTRAC QA Manager's Network meeting on 21 August 2013 (Attachment 2).

MINTRAC Conference

Dr Kiermeier contributed to the development and in the delivery of an MLA presentation entitled "*Increasing your certainty of meeting market requirements*" at the MINTRAC 2013 Meat Inspection and Quality Assurance Conference in Melbourne on 11 & 12 September 2013 (Attachment 3).

Discussion document on aspects related to sampling beef trim for *E. coli* O157

Exporters of manufacturing beef undertake *E. coli* O157 testing of each lot using the 'Robust N-60' sampling plan. An argument which is frequently made in the USA is that testing smaller lots results in higher probabilities of detecting contamination. Subsequently, there has been pressure on establishments to reduce the lot size from a maximum of 700 cartons to 350 or even 175 cartons. A document to address some of the issues related to *E. coli* O157 testing has previously been produced and this document has been revised following discussions with Dr Mansour Samapour at the Annual Conference of the International Association for Food Protection in Charlotte in July 2013. The most recent version of this document is included with this report (Attachment 4).

[1] Kiermeier, A., Mellor, G., Barlow, R. & Jenson, I. (2011). Assumptions of Acceptance Sampling and the Implications for Lot Contamination: *Escherichia coli* O157 in Lots of Australian Manufacturing Beef. *Journal of Food Protection* 74(4): 539-544.

Conclusions and Recommendations

This project has provided MLA with a flexible mechanism to address a variety of statistical and modelling issues. As of 4 October 2013, Dr Andreas Kiermeier will no longer be working for SARDI. Because of this loss of key scientific capability to deliver this research project, it is recommended that this project is terminated.

A. Kiermeier (SARDI)
J. Sumner (MLA)
I. Jenson (MLA)

Risk Assessment of *E. coli* O157 in Hamburgers Made from Australian Beef Trim

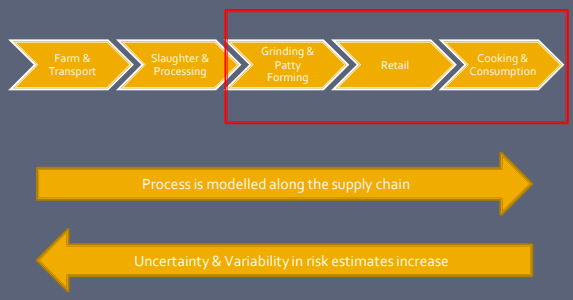
Background

- Australia exports large amounts of lean beef destined for grinding to the US
- All lots are tested for *E. coli* O157 using N-60: Detections are infrequent
- Microbial status of Australian beef has been well documented
  - 4 baseline studies

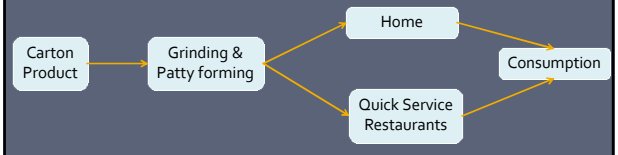
Aim

To assess the risk posed by *E. coli* O157 from the consumption of burgers made only from Australian beef trim and to compare the effects of sampling and cooking interventions on illness estimates.

Supply Chain



Model Components



Simulation

- Based on export sampling *E.coli* O157 not found frequently
  - Estimate that only about 0.5% of AU lots are contaminated
- Simulate only contaminated lots (10,000)
  - 700 cartons per lot – each carton 27.2kg
  - 190,400 hamburger patties (100g) per lot

Carton Contamination

- Five contaminated lots (A-E)
- Identified through standard N-60 sampling
- The 12 original cartons were sampled again
- 75 surface slice per carton – 900 samples per lot – tested individually for *E. coli* O157

Carton Contamination

- Lots A, B, C: could not isolate *E. coli* O157
  - Assume one carton was contaminated
  - Estimate <0.0013 org/cm²
- Lot D: 1 piece of meat from 1 carton
  - Estimate 0.0014 org/cm²
- Lot E: 27 pieces of meat from 2 cartons
  - Estimate 0.019 and 0.093 org/cm²

Grinding and Patty Forming

- Individual cartons
- No cross contamination between cartons
- Organisms in a carton end up in patties

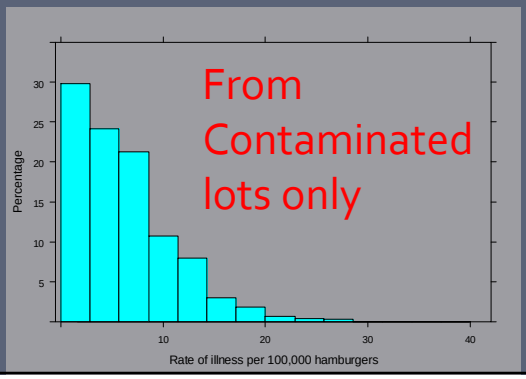
Storage & transport

- Quick service restaurants
  - Stored frozen ⇒ No growth
- Retail, transport to home and home storage
  - Based on FSIS 2001 RA approach
  - Using updated EcoSure (2008) data

Cooking & Consumption

- Quick Service Restaurant
  - Cooked to internal temperature of constant 68˚C (154.4 ˚F)
- Home
  - Variable internal endpoint temperature based EcoSure (2008) data
- Cassin's Dose-Response model

Results



Results – All AU lots

- Baseline – no testing undertaken (!)
- Take non-contaminated lots into account
- Through the home – variable cooking: 3.0 illnesses per 10,000,000 hamburgers
- Through QSR – cooking to 68˚C (154.4 ˚F) 7.3 illnesses per 100,000,000,000 hamburgers

Results – All Australian lots

Scenario	Rate of illness per burgers consumed
Home cooking (variable temp.)	3.0 per 10,000,000
Sampling N60 + home cooking	1.5 per 10,000,000
Sampling N120 + home cooking	1.1 per 10,000,000

Results

Location	Patties consumed	Illnesses estimated
Home	232,500,000	34.10
QSR	2,085,750,000	0.07
Total	**2,425,000,000**	**34.17**

Conclusions

- Sampling removes the most contaminated lots
- Less contaminated lots are harder to detect, but they also cause less illness
- Law of diminishing returns

Conclusions

- Cooking is (still) the best way to reduce risk

Conclusion

Australia's contribution

- About 10% of raw materials for hamburger production in the US
- About 0.2% of illnesses

Thank You!

Does your performance measure up?

Process control – what are you trying to achieve?

Your customers (and DAFF, as their agents) require you to control your process so that:

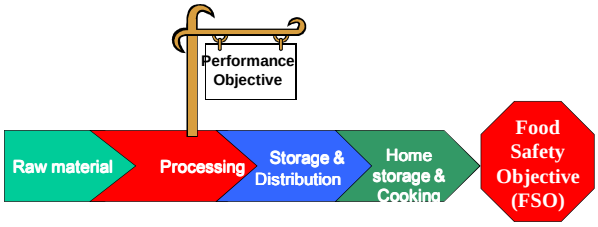
- Trim for grinding in USA has no STECs detected in the sampling program (currently 375g from 60 surface slices)
- No visible faeces on pieces of meat
- ESAM windows are OK

These are called PERFORMANCE OBJECTIVES

E. coli beef carcases Australian establishments (Jan 2010-July 2012)

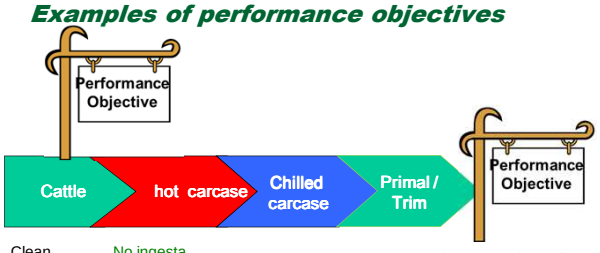
Establishment	*E. coli* prevalence (%)
1	0.0
2	0.3
3	1.0
4	1.1
5	1.6
6	2.1
7	2.6
8	2.6
9	2.7
10	3.0
11	3.5
12	3.7
13	4.0
14	4.6
15	4.9
16	5.1
17	5.2
18	5.5
19	5.8
20	5.9
21	6.7
22	7.8
23	8.9
24	10.5
25	16.4

Your place in the chain



The PO is linked to the FSO – the quantity of a pathogen your customer will accept to meet their public health needs

Examples of performance objectives



Clean

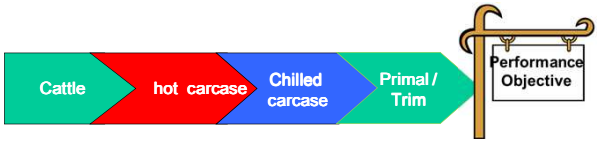
No ingesta visible

Carton meat assessment is OK

The FSO only applies at the time of consumption

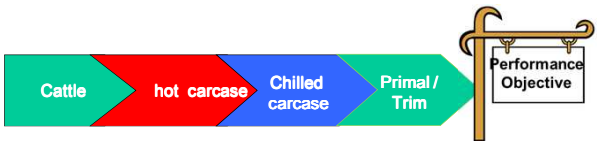
Processing – we need to think about all of this from scratch

Contamination on the animal varies – we have to meet the PO with the worst case



HACCP: Prevent, eliminate, reduce the hazard to an acceptable level

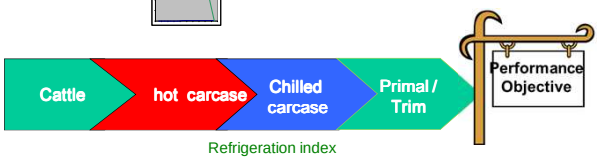
Contamination on the animal varies – we have to meet the PO with the worst case



A lot of different ways to meet the performance objective
A number of measurements to know you are on track

Performance criteria

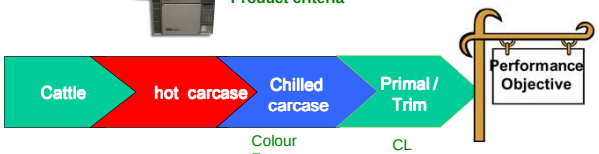
Performance criteria



Refrigeration index

Product criteria

Product criteria

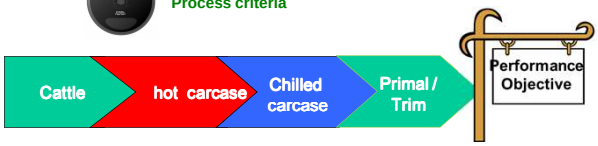


Colour
Fat score
Ultimate pH

CL

Process criteria

Process criteria

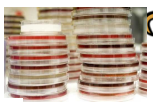


MHA process score less than ?

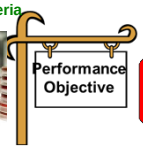
How the terms work together

Performance criteria

Microbiological criteria



Process criteria



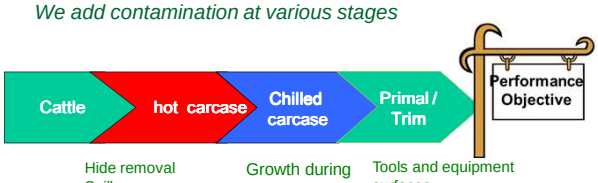
Food Safety Objective (FSO)

Product criteria

We need all these things working together!!

We start with a sterile carcase under the hide

We add contamination at various stages

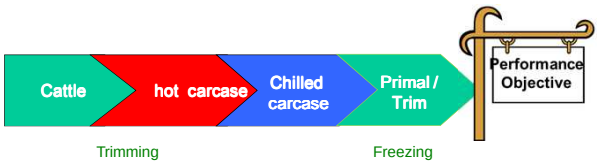


Hide removal
Spillage
Tools and equipment
Hands

Growth during chilling

Tools and equipment surfaces

We not only add contamination, we may be able to remove it



We start with a sterile carcase under the hide + Sum of contamination from various places (increases) - Sum of treatment to remove contamination (removal) ≤ The performance objective that contributes to meeting the Food Safety Objective

Sigma = the sum of

Less than or equal to

$$H_0 + \sum I - \sum R \leq PO$$

Do you know your process?

Do you know where you put contamination on?
And how much you're putting on?

Do you know if you're reducing contamination during processing – and by how much?

If you don't know, you're process is random – sometimes it works and sometimes it doesn't – and we don't know why.

When things go wrong and DAFF says "review your HACCP" – what information do you have?

Some companies are investigating their processes

Establishments 1, 2 and 3

Example of some increases - contamination from hide to carcase immediately after hide removal – sponging a large area

	Mean Log TVC (cfu/sample) [~ cfu/cm$_2$]	Prevalence E. coli
Est 1	4.20 [1.6]	82% positive
Est 2	3.75 [1.15]	40% positive
Est 3	4.39 [1.79]	27% positive

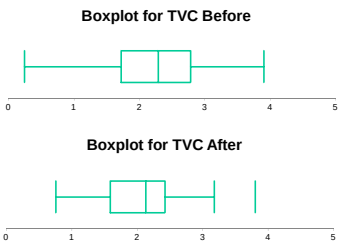
Establishment 1, 2 and 3

Knife trimming reduces contamination by the end of the slaughter floor

	Mean log TVC decrease	Prevalence E. coli decrease
Est 1	0.98	82 → 30%
Est 2	0.43	40 → 22%
Est 3	0.66	27 → 11%

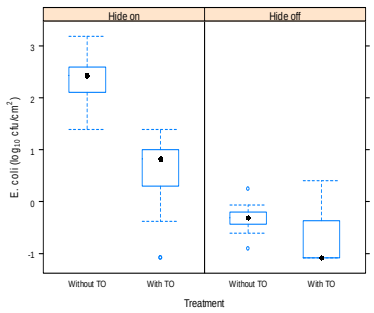
Establishment 4

Steam vac of brisket – not much reduction



Establishment 5

Twin Oxide, hide and carcase – some reduction



We start with a sterile carcase under the hide	+	Sum of Contamination from various places (increases)	-	Sum of treatment to remove contamination (removal)	≤	The performance objective that contributes to meeting the Food Safety Objective
H_0	+	$\sum I$	-	$\sum R$	≤	PO (FSO)
0 sterile	+	1.6 carcase 0.2 RI	-	0.5 trim	≤	2.0
0	**+**	**1.8**	**-**	**0.5**	**≤**	**2.0**

Chilling as an intervention

How did we get here?

Known for many years that chilling leads to a lowering of bacterial count on the carcase

Counts always “higher” on hot-boned carcases compared with chilled carcases

This led to hopes that chilling could be used as an intervention

Attractive because it would be a no-cost intervention

But if it were that easy everyone would be doing it

Need to know how bacteria behave during chilling

UTAS work on chilling and E. coli

- Australian meat industry/MLA since 1990’s
- Predictive microbiology/risk assessment, also expert panels
- Given us tools which are routinely used
  - Refrigeration Index
  - EHECs in fermented meats
  - Shelf life/spoilage of VP meats

UTAS methodology

Have equipment which allow them to model, in test tubes, what happens on meat

They can change the temperature and water activity to mimic what happens to the carcase surface during chilling

They can also do modern microbiology which tells them about how bacteria behave at the genetic level

Which genes switch on and off during chilling, as conditions change through the chilling cycle

They’ve done this for E. coli.

Food Control 27 (2012) 385–397

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

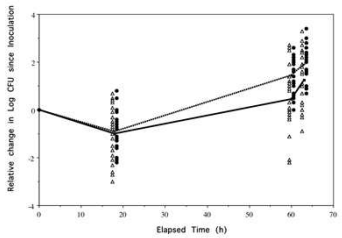
The efficacy of interventions applied during primary processing on contamination of beef carcasses with *Escherichia coli*: A systematic review-meta-analysis of the published research

J.D. Greig [a,*], L. Waddell [a,b], B. Wilhelm [a,b], W. Wilkins [c], O. Bucher [a,b], S. Parker [d], A. Rajić [a,b,d]

[a] Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada N1G 5B2.
[b] Department of Population Medicine, Ontario Veterinary College University of Guelph, Guelph, Ontario, Canada N1G 2W1
[c] Saskatchewan Ministry of Agriculture, Livestock Branch, 3085 Alberta Street, Regina, Saskatchewan, Canada S4S 0B1
[d] Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5B4

"Existing research indicates that … dry chilling is beneficial for reducing the contamination of beef carcasses with generic *E. coli* and potentially pathogenic strains.
The predicted risk difference in carcass contamination with E. coli was 35 per 100 carcasses after 24 h dry chill"

Greig *et al*., *Food Control*, (2012)



Relative change in coliform numbers over time for weekend chilling. Numbers drop within 24 hours but then come back EVEN THOUGH GROWTH IS NOT POSSIBLE (Upper line: brisket; Lower line: striploin).

Implications for testing

If we test for E. coli after 24h chilling we will underestimate the count

They are able to "come back from the dead"

Hot boners have a more accurate picture of contamination levels on their carcases

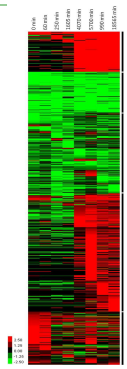
So what's happening?

Bacteria are shocked and inactivated during active chilling:

- Cold shock by rapid temperature fall
- Osmotic shock as water evaporates from the surface

UTas are working to *explain* (scientifically) what happens **And** to exploit and to design improved approaches/technologies as interventions

Gene regulation changes



There are 1600 genes in E. coli

They respond to stress by up-regulating (red) or down-regulating (green)

Changes occurring during chilling are illustrated in the diagram

Genes are responding to oxidative stress during chilling

E. coli reacts by producing a slime, which is protective

POSSIBLE applications

Still a long way to go……

Spray chilling could be enhanced if the initial cycle was in air, then spraying commenced after 10h when the rise in water activity is occurring (osmotic shock #2)

Spraying the carcase with an antimicrobial which works by oxidising (Twin oxide or Acidified sodium chlorite) may enhance the oxidising damage

In the mean time…….

Many companies are investigating their processes

MLA want to pair with establishments to help identify where contamination is added and removed

It's the future.

Increasing your certainty of meeting market requirements

Andreas Kiermeier,	SARDI
Casey Smith,	E C Throsby
Ian Jenson,	MLA
John Sumner,	MLA
Mandy Smart,	Midfield International
Sam Rogers,	SARDI
Teresa Hore,	Southern Meats

Market access

Meeting expectations

- Residues
- Physical contamination
- Suitability parameters
- Microbiological contamination
- Animal welfare
- Environmental management
- Child labour
- …

Trends

- Longer list of requirements
- More testing
- More documentation
- More audits
- More checks

Need to deal with these requirements in a consistent way

- No control over requirements
- We have to meet them
- We have to manage our systems
- We need to be confident that our product meets requirements
- Best if we can use a common approach to thinking about how we meet requirements

Outline

- Traditional process control
- New approaches to meeting requirements
- Investigating your operations

TRADITIONAL PROCESS CONTROL

HACCP and GHP

- Seven HACCP principles
- Focus on Critical Control Points …

  "*A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level*"

- … setting critical limits, monitoring and corrective action.

What if no CCP exists?

- Codex: "*Redesign of the operation should be considered if a hazard which must be controlled is identified but no CCPs are found*"
- Not usually possible to 'create' a CCP
- Changes in individual tasks / process to make them more effective may be possible

Monitoring and Verification

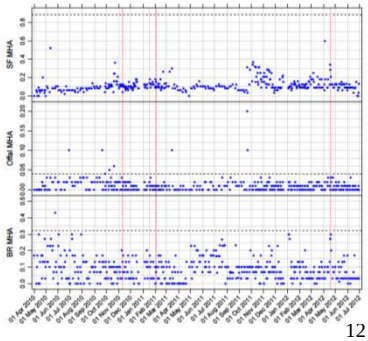
- Monitoring: visual aspects – MHA
  - Processes
  - Product – Carcases
  - Product – Cartons
- Verification: microbiological aspects
  - ESAM
  - Carton micro
  - O157 & STEC testing

⇒ All feeds into the Product Hygiene Index

But …

- Data analysis indicates that pathogens can occur even though our existing systems indicate that things are 'in control'

Plant A: MHA history



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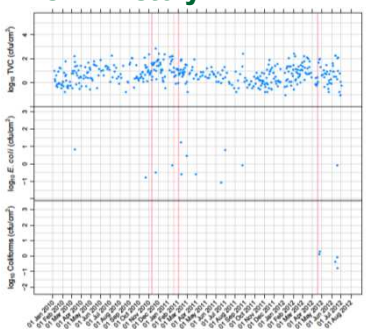
Plant A: ESAM history



So what's going on?

- ESAM designed to gain / maintain market access
- Micro contamination is hard to see
- Australian processing is generally slower & thorough
  - Less of a problem to start with compared to the US
- Less contamination ⇒ need more data to verify control!

More data where?

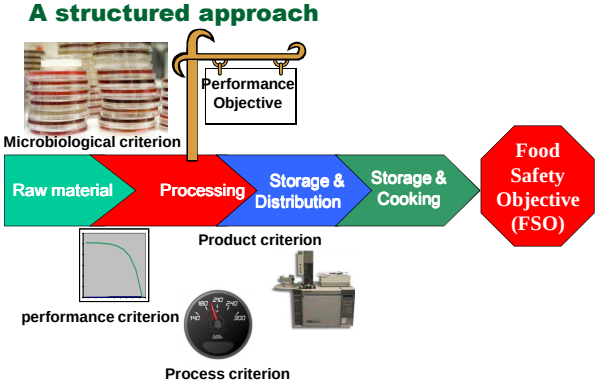
- No CCP ⇒ series of hurdles
- Need data on these hurdles throughout the process
- Ian will talk to you more about this and then we'll see some examples.

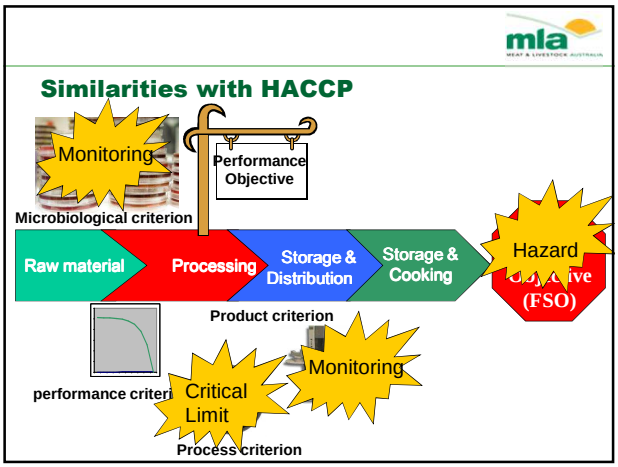
THE PERFORMANCE OBJECTIVE APPROACH

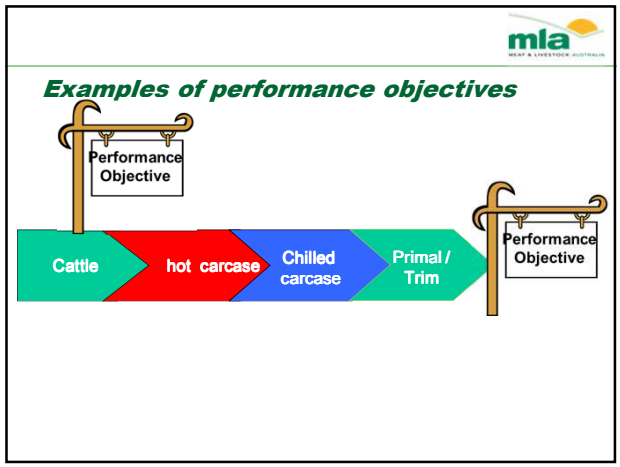
Your place in the chain

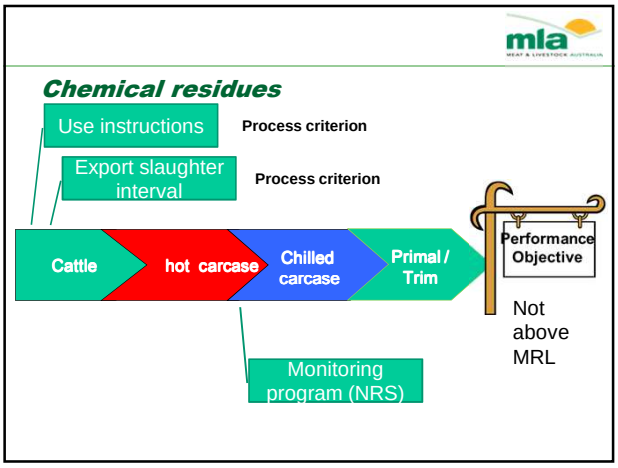
The PO is linked to the FSO – the quantity of a hazard your 'customer' will accept to meet their public health needs

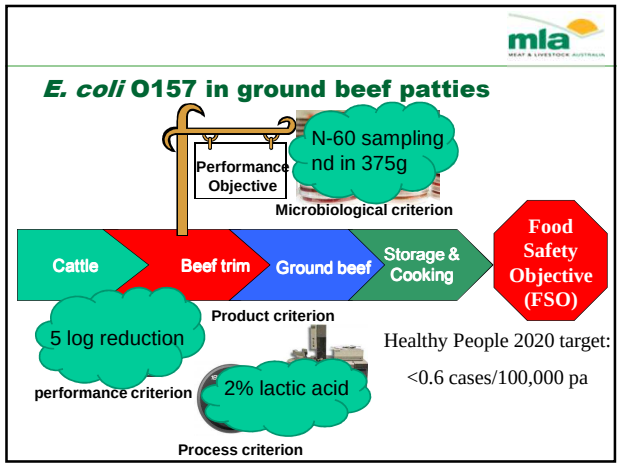
A structured approach

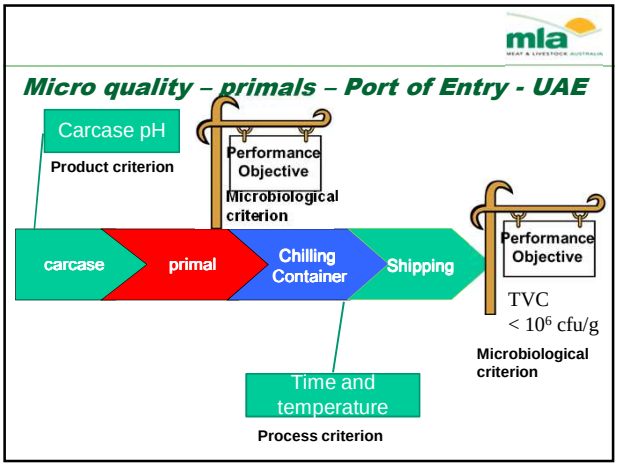



Similarities with HACCP
Monitoring
Performance Objective
Microbiological criterion
Raw material
Processing
Storage & Distribution
Storage & Cooking
Hazard
(FSO)
Product criterion
performance criteri
Critical Limit
Monitoring
Process criterion


Examples of performance objectives
Performance Objective
Cattle
hot carcase
Chilled carcase
Primal / Trim
Performance Objective


Chemical residues
Use instructions
Process criterion
Export slaughter interval
Process criterion
Cattle
hot carcase
Chilled carcase
Primal / Trim
Performance Objective
Not above MRL
Monitoring program (NRS)


E. coli O157 in ground beef patties
Performance Objective
N-60 sampling
nd in 375g
Microbiological criterion
Cattle
Beef trim
Ground beef
Storage & Cooking
Food Safety Objective (FSO)
Product criterion
5 log reduction
performance criterion
2% lactic acid
Process criterion
Healthy People 2020 target:
<0.6 cases/100,000 pa


Micro quality – primals – Port of Entry - UAE
Carcase pH
Product criterion
Performance Objective
Microbiological criterion
carcase
primal
Chilling Container
Shipping
Performance Objective
TVC
< 10⁶ cfu/g
Microbiological criterion
Time and temperature
Process criterion


INVESTIGATING UNIT OPERATIONS

How well do you know your process?

Do you know where you put contamination on?
And how much you're putting on?

Do you know if you're reducing contamination during processing – and, if so, by how much?

When things go wrong and DAFF says "review your HACCP" – what information do you have?

Some companies are investigating their processes

Staff from 29 plants attended MLA workshops on how to set up, carry out and analyse the data

Typical investigations

- Difference in micro quality of cartoned meat between shifts
- Performance of a neck sanitising robot
- Effect of hot water pasteuriser
- Carcase hygiene of dorper versus crossbred sheep
- Hand hygiene
- Cutting board hygiene
- Tail removal
- Twin oxide decontamination

How they did the work

- Identified the problem and the key sites
- Thought through how they could be sampled on the factory floor
- Did the sponging and the micro (Petrifilm TVC and E. coli)
- Did the counts and dropped them into the tools designed by AK

TVC of dorpers versus crossbreds

Summary	Dorper (log)	Cross (log)
Mean	4.02	2.83
St. Dev.	0.23	0.19
n	25	25
Conf level	95%	
CI Lower	3.92	2.75
CI Upper	4.11	2.91
Significance	Highly significant	

Three examples

1. Tail flick at hide pulling – Casey Smith, EC Throsby
2. Dropping socks on smallstock line – Teresa Hore, Southern Meats
3. Cleaning chillers using ozone – Mandy Smart, Midfield International

Effect of tail cleanliness

Objective

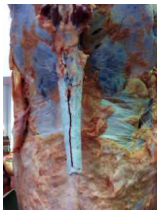
Assess whether our current procedure to remove the bushy part of the tail is effective in preventing E. coli contamination due to tail flick

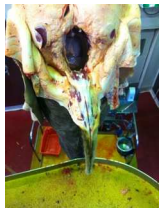
Assessment of tail brush: Rating 1-5

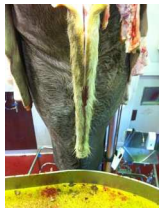
1. Clean
2. Slightly dirty
3. Dags on brush
4. Dags on brush and tail
5. Dags everywhere

Experimental

- We have a downward hide puller and we can get contamination when the tail flicks at loin
- We sponged 50 loins, and we noted the grade of contamination (clean, slightly dirty etc)
- We used TVC and E. coli Petrifilm and incubated at 35°C for 48 hours
- When we looked at the results we found ratings 1+2 looked very different from rating 3, 4 and 5.
- So we grouped the data into two categories

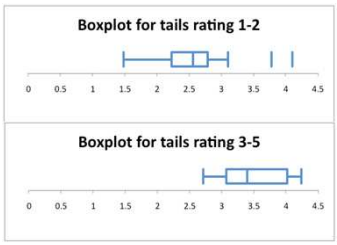






Contamination levels on tails

TVCs different – boxes don't overlap



Contamination levels on tails

Sponged loin area (400 cm^2)
Rating 1+2 very different contamination levels on Ratings 3-5

TVC

Summary	Rating 1-2 (log)	Rating 3-5 (log)
Mean	2.59	3.49
St. Dev.	0.58	0.51
n	35	15
Conf level	95%	
CI Lower	2.39	3.21
CI Upper	2.79	3.77
Significance	Highly significant	

E. coli

Summary	Rating 1-2	Rating 3-5
Detect	2	14
n	35	15
Prev	5.7%	93.3%
Conf level	95%	
CI Lower	0.7%	67.8%
CI Upper	19.8%	100.0%
Significance	Highly significant	

What's the outcome

Our current procedure requires the removal of the bushy part of the tail to eliminate the chance of cross-contamination and E. coli spreading to the loin area of the carcase

The investigation confirms that:

- Tails rating 1-2 have a significantly less chance of contaminating the loin
- Current procedures for tail bush removal reduce the likelihood but do not eliminate E. coli from the loin area

Procedures for dropping socks

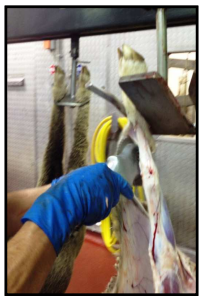
Objective

If we drop socks with an airknife how will contamination compare with current procedure using a conventional knife?

If it does we can save an operator.

Current procedure

Proposed procedure



Experimental

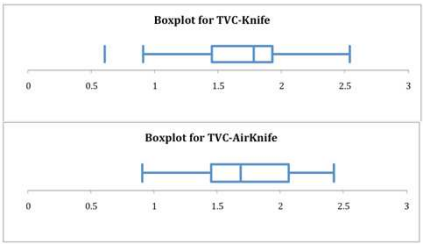
We sponged 50 foreshanks – 25 after using the current procedure and 25 after using the proposed procedure

We plated on TVC and E. coli Petrifilm

After 48 hours incubation we counted the plates and entered the data into the tools Andreas had given us

Results

Box plots overlap



TVC

Summary	Knife (log)	Air knife (log)
Mean	1.70	1.72
St. Dev.	0.45	0.43
n	25	25
Conf level	95%	
CI Lower	1.51	1.54
CI Upper	1.89	1.90
Significance	Not significant	

E. coli

Summary	EC (Knife)	EC (Air Knife)
Detect	3	2
n	25	25
Prev	12.0%	8.0%
Conf level	95%	
CI Lower	3.5%	1.2%
CI Upper	31.0%	26.3%
Significance	Not significant	

Conclusion

We think we have good evidence to change to the new procedure.

There's no difference in micro between the procedures.

And we're able to save an operator.

Using ozone to clean chiller walls

Objective

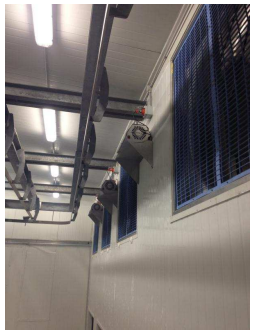
Determine if ozonation of a chiller over a 2h period will result in lower levels of TVC on the wall

Theory

Ozone is a powerful oxidizing agent which by the action of UV light and atmospheric electrical discharge has inhibitory microbiological effects

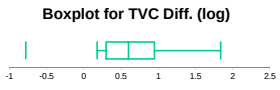
Experimental

A grid pattern was taped to the chiller wall. Twenty-five sites were sampled using a press plate. After ozonation, sites adjacent to the original 25 were sampled.



What's the result?

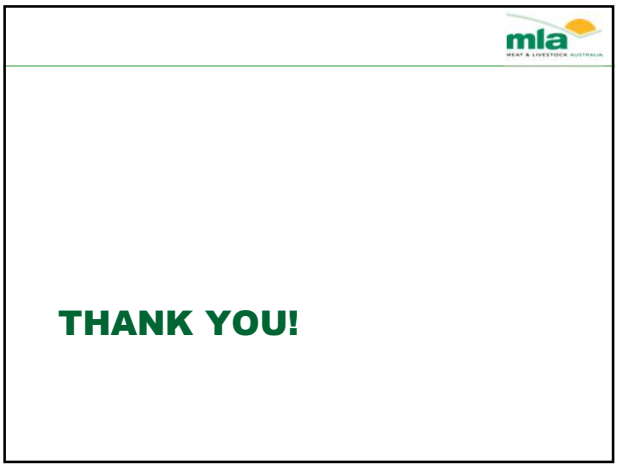
Summary	Before Ozone	After Ozone
Detect	19	5
n	21	21
Prevalence	90.5%	23.8%
Conf level	95%	
CI Lower	69.6%	10.4%
CI Upper	98.4%	45.6%
Significance	Highly significant	



- TVCs were lower after ozone treatment (just over 0.5 log)
- Great variation in effect (up to almost 2 log reduction in one case)
- But only just under ½ had detectable TVCs after treatment – average effect is bigger than 0.5 log.

Resources to help you

- Sam Rogers
- The Spreadsheet tools


mla
MEAT & LIVESTOCK AUSTRALIA
THANK YOU!

STEC Sampling

This document discusses sampling and testing using an enrichment test. 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.

Background

In 2007, FSIS commenced testing of beef trim destined for grinding using surface slice and "Robust N-60" sampling[1] at USA processing establishments. Subsequently, Australian establishments were required to also sample and test all lots of beef trim destined for the USA 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 been tested separately – even if they had been produced during the same production period.

In comparison to the USA, Australian lot sizes were large – up to 20t (700 cartons or a container load) compared with 5 combo bin lots (total of 5t). The trend in the USA has been toward smaller lots and 1 combo bin lots (2000lb or 1t) are now common. In contrast, Australian lot sizes remained largely unchanged, until recently. Some establishments have now reduced lot size and decided to have two port marks (sometimes even four) per container and have tested each of these port marks as separate lots. Reducing lot size has become more popular since the introduction of the requirement for DAFF verification testing for six additional Shiga toxigenic *E. coli* (STECs), in addition to *E. coli* O157, in June 2012 (Australian Meat Notice 2012/01). In addition, information obtained from the USA has prompted some establishments to believe that testing an increased number of port

[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

marks/containers will result in a greater chance of detecting contamination in Australia and a reduced likelihood that a lot will test positive at Port of Entry.

Unfortunately, some of the advice provided from the USA is not appropriate for the Australian situation and this document has been prepared to provide information on how lot size and testing frequency impact on sampling and likely sampling results.

The situation in the USA

Let's make an assumption that an establishment typically produces lots in which 1% of meat pieces are contaminated with *E. coli* O157. In this case, randomly collecting 60 samples, i.e. robust N-60 sampling, would result in a probability of detecting *E. coli* O157 of 45.3% (see Appendix).[2] That is, on average about half of all lots contaminated at this level (1%) would have O157 detected, while in the other half the contamination would not be detected and these lots would progress through to commerce.

Information from the USA indicates that processors create lots by combining several combo bins, possibly from different days of production. These lots are then tested by selecting an equal number of samples from each combo bin, while maintaining the overall N-60 sampling plan (Table 1). For example, if a lot contains four combo bins, then each bin would be sampled by selecting 60/4 = 15 pieces of meat. Based on an average 1% contamination rate (and assumptions detailed in the Appendix), the probability of each one of these combo bins yielding a detection is 14.0%. Because all 60 meat samples are pooled into one analytical sample, which is enriched, the whole lot of four combo bins is rejected when O157 is detected (even if the contamination originates from just from one bin). As it turns out, testing N-60 drawn from all four combo bins is equivalent, in terms of probabilities, to testing each combo bin separately using n=15 and rejecting the whole lot when only one of them results in an O157 detection. The fact that all four combo bins are rejected is important as it results in an overall probability of 45.3%[3] rejecting for the whole lot – the same probability we calculated above using a pooled sample and n=60.

Table 1: Summary of Australian and USA sampling schemes for *E. coli* O157.

	AU sampling	US sampling
Lot units	Carton (27.2 kg)	Combo Bin (1t)
Predominant lot size	175/350/700 cartons	1-5 combo bins
Number of samples	N-60	N-60
Samples are collected how?	5 samples (5-10g) from each of 12 randomly selected cartons	Equal number from each combo bin to give N-60, e.g. 15 samples (5-10g) from each of 4 combo bins in lot

As indicated above, it is possible that the four combo bins originate from different days of production. Let's assume that one of these days was a "High Event Period", that is, a day

[2] Throughout this document we use the approach and statistical assumptions detailed in the Appendix for the calculation of probabilities.

[3] $1-(1-0.14)^4 = 0.453 = 45.3\%$

with worse than average O157 contamination, and that the combo bin from that day has 4% of pieces contaminated. Let's also assume that the other three combo bins have no contamination (0%) as this gives us an average contamination rate of 1% (same as above). The probability of rejecting this lot remains at 45.3%, despite three combo bins not being contaminated (they have a 0% probability of rejection). However, the highly contaminated combo bin has a 45.3% probability of detection, although it is only sampled n=15. And this is where the concern in the USA comes from, namely that (a) lots are created from combo bins from unrelated days of production, and that (b) because of this, and the lower intensity of sampling (n=15), a highly contaminated combo bin has 'only' got a 45.3% probability of rejection. In contrast, if each combo bin was tested as a separate lot using n=60, then the heavily contaminated combo bin would have a 91.4% probability of detection while the remaining three uncontaminated combos would be cleared for commerce.

The important part to note here is that the change in probabilities – from 45.3% to 91.4% – is not because the lots size was reduced. Instead, it is a result of the things noted above, namely that

a) lots are created by combining unrelated days of production;
b) the contamination rate is higher; and
c) the more contaminated lots is tested using n=60 rather than n=15.

The situation in Australia

In Australia we have lots of up to 700 cartons, although some processors are moving to smaller lots sizes, e.g. 350 or even 175 cartons. Just like in the USA we use N-60 sampling to test lots for *E. coli* O157 (Table 1). However, in contrast to the USA, we randomly select the cartons from which we collect samples, which is due to the fact that we have many more cartons than we need samples.

So, if a lot is created by taking 175 cartons from each of 4 days of production, then we don't test each day using n=15. Instead the 12 cartons are randomly selected from the 700, and hence any particular day may have any number of cartons between 0 and 12 selected for testing. This is quite different to the USA where each combo bin is tested proportionally.

While the way we collect samples is different the USA argument is frequently *interpreted* or *portrayed* incorrectly. The case seems to be made that halving a lot's size, and hence testing both halves (or port marks) using N-60, will increase the probability of detecting a contaminated lot to $1-(0.45)^2 = 0.80 = 80\%$. Furthermore, if the initial lot is divided into four port marks, then the probability of detection will increase to $1-(0.45)^4 = 0.96 = 96\%$. However, this interpretation about the probability of detecting O157 is incorrect. That's because it implies the probability of detecting O157 in every port mark, while actually it is the *probability of detecting E. coli O157 in <u>at least one</u> of the four port marks*. But each port mark is accepted or rejected independently of other port marks. This is in fact no different to the situation in the USA – if the lot size is reduced from four combo bins to just one, then a detection in one does not trigger rejection of all four.

As described above, each individual port mark still has the same probability of detection as the original lot, namely 45.3% (just like the combo bins in the USA). That's because we are using the same N-60 sampling plan and are collecting a small amount of meat (375g) from a very large lot (700 cartons = 19,040 kg and 175 cartons = 4760 kg). If we used N-240 sampling (for a port mark or a container) then the probability of detecting O157 becomes 0.91=91% when 1% of pieces are contaminated. Again, the important thing is that the probability has increased because the number of samples tested has increased, and not because the size of the lot has changed. However, this N-240 should not be confused with testing four port marks with N-60 – that's because each port mark is accepted / rejected independently.

So, what effect does this have on your sampling and testing program? To answer this question, let's also assume that 1% of pieces are contaminated in the original 700 cartons lots. Based on the above calculations, we would expect that for every two lots tested, we would reject one of these (on average), i.e. a total of 700 cartons.

Now let's divide these two lots into four port marks each, that is, a total of eight port marks are being tested. If the contamination is distributed randomly throughout each lot, then each of these eight port marks will still have 1% of its pieces contaminated. Consequently, we again expect half of these, that is, four port marks, to result in an O157 detection. Therefore, the total amount of product rejected is equal to 4 × 175 = 700 cartons.

On the other hand, it could be that all the contaminated pieces end up in a single port mark – this might be the case if a few highly contaminated carcases are boned and packed and the contamination is not spread widely. So in our two containers with a total of eight port marks, two will be contaminated at 4% (because all contaminated pieces are together) and the remaining six port marks will be free of contamination. Using N-60 sampling, the two contaminated port marks have a probability of detection of 91% while the remaining port marks have 0% probability of O157 detection (because they contain no contamination). In this case you would reject a total of only 350 cartons.

But which of these two extremes (or anywhere in between) we are dealing with, is anyone's guess. However, if the port marks originate on different days of production, then given the above information, it may be beneficial to test them separately and independently. It is also important to remember that not detecting the contamination is not the same as "there is no contamination" and that the above scenarios assume true independence between lots and port marks.

Conclusions

There is no statistical justification for just making lots smaller – the probability of detecting O157, and similarly STECs, does not increase simply because the lots are smaller (assuming the same prevalence or level of contamination). Practically, smaller lots may be attractive as a detection will result in less product being condemned. However, all other things being equal, smaller lots require you to test two or four times as frequently and therefore we

would expect to detect STECs two or four times as frequently, but the overall amount of meat rejected is left largely unchanged.

However, combining unrelated cartons, from different days of production, into a single port mark or lot carries some risk, because the whole lot is rejected and not just the independent portions making up the lot.

Finally, don't assume that failing to detect O157 (or STECs) is the same as O157 (or STECs) not being present in the lot.

Mathematical Appendix

You may want to discuss this topic with your customers so this appendix contains some further information for calculating lot acceptance and rejection probabilities.

To calculate the probability of lot acceptance (or rejection) FSIS utilised the commonly used binomial distribution. This approach is usually utilised when the total amount of material that can be sampled is much larger than the actual amount sampled. Under the binomial distribution, there are a number of assumptions made:

1. **The total number of meat samples collected is fixed in advance**. This is clearly the case as we take 60 samples from the lot (irrespective of lot size).
2. **Each individual meat sample either contains an STEC or does not contain an STEC** – the number of STECs are not important, only the presence / absence. This is also a reasonable assumption.
   It is also assumed implicitly that any STECs present on a meat sample will be detected (i.e. no false negative results) and that compositing the 60 samples followed by enrichment is a suitable way of detect at least one STEC (and hence just as good as testing each individual piece of meat separately).
3. **The STEC prevalence and hence the chance of a single piece being contaminated is constant throughout the lot**. This assumption is harder to assess and means that there aren't known factors that increase or decrease the prevalence. For example, if the first 350 cartons in a 700 carton lot were irradiated (and hence all STECs were eliminated) then these would not yield STECs if sampled. So there is a known systematic effect that needs to be taken into account and hence these two sets of 350 cartons should be treated as separate lots (and sampled separately). However, in practice such knowledge usually doesn't exist.
4. **Individual meat samples are independent from each other**. This is not the case when samples are obtained from pieces of meat that originate from a single carcase. If parts of the carcase are contaminated with STECs then it may also be more likely to have other parts also contaminated. In many cases, meat from multiple carcases is collected in a single carton and it is not possible to discern which carcase they come from.

Under these assumptions, the probability of detecting STEC can be calculated from the binomial distribution for any given prevalence (p) and number of samples (n) as

$$P(\text{detect}) = P(1 \text{ or more STECs in sample})$$

$$= 1 - P(\text{no STECs in sample})$$

$$= 1 - (1 - p)^n$$

For *p*=0.01 (=1%) and *n*=60 we get

$$P(\text{detect}) = 1 - (1 - p)^n = 1 - (1 - 0.01)^{60}$$

$$= 1 - 0.99^{60} = 1 - 0.547 = 0.453$$

The prevalence depends on the size of the sample unit collected. In the case of N-60 sampling the sample unit is 5-10g (Australian Meat Notice 2007/17), or an average of 6.25g (to give a total sample weight of 375g). Increasing the size of sample unit has the possible effect of increasing the prevalence purely by virtue that sampling more meat is more likely to result in detecting contamination (when it is present on the piece of meat or in a carton).

It should be noted that the binomial approach does not incorporate the lot size. That is because for the binomial approach to be applicable, the lot size has to be large in relation to the amount of meat sampled. Provided the total amount sampled is less than about 10% of the amount of meat in the lot, there is no impact on the probability calculations irrespective of how big the lot is (provided the assumptions above are met). Consequently, there is no impact on the probability of detection when sampling 375g from a lot weighing 5t (about 175 cartons) or 20t (about 700 cartons).