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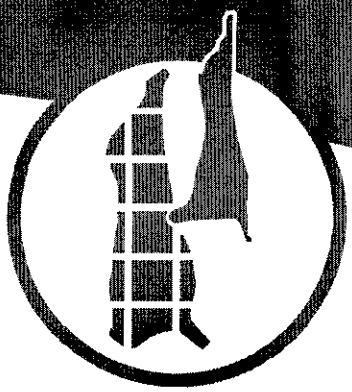
MEAT & LIVESTOCK AUSTRALIA



Sampling and test protocols MSHE.003

1997

Prepared by:
Victorian Institute of Animal Science

Published: December 1997
ISBN: 1 74036 626 2

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

MEAT & LIVESTOCK
AUSTRALIA

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Summary report

Research Summary

The project addresses key issues in the adoption of the USDA FSIS MegaRegs sampling method. In addition a Food Microbiology Information Service has been established for the Australian Meat Industry.

Introduction

In order to effectively validate and verify HACCP protocols that are implemented in meat processing establishments, sampling and testing procedures must be identified that are robust, practical, accurate and reproducible, and that can be adopted uniformly across the Australian meat industry. In particular, it is essential that sampling techniques provide an accurate representation of the level of microbial contamination on product at critical points during processing, and that testing methods accurately record bacterial numbers. This requires that sampling and testing techniques are effectively evaluated, and the findings disseminated to industry, to promote uniform analysis of microbial contamination at all levels of the industry.

Objectives

1. Investigate aspects of the MegaRegs sampling methods including site to site variation, variation in recovery rates by different microbiological methods, differences in recovery over time, differences in recovery by different operators, effect of sponge and diluent on recovery of bacteria.

2. Establish a Food Microbiology Information Service.

Major Research Findings

The major findings of this report are:

- the elimination of the rump site from sampling will result in reduced numbers of carcasses falling in the "warning" or "fail" limits of the MegaRegs plan
- sponge sampling recovers less *E coli* than excision
- Petrifilm™ method is similar to the MPN method for enumeration of *E coli* from sponge samples
- recovery of *E coli* from sponges does not vary over 24 hours
- the sponge and diluent do not influence recovery of *E coli*
- the sponge does not trap bacteria
- there can be considerable variation in recovery of *E coli* from carcasses by the sponge method from operator to operator.

The recommendations to industry are:

- sponge sampling of 3 site with Petrifilm™ enumeration of *E coli* be used
- samplers in the abatoirs be trained and audited on a regular basis
- sponges may be stored up to 24 h at 4°C prior to sampling.

Introduction

In order to effectively validate and verify HACCP protocols that are implemented in meat processing establishments, sampling and testing procedures must be identified that are robust, practical, accurate and reproducible, and that can be adopted uniformly across the Australian meat industry. In particular, it is essential that sampling techniques provide an accurate representation of the level of microbial contamination on product at critical points during processing, and that testing methods accurately record bacterial numbers. This requires that sampling and testing techniques are effectively evaluated, and the findings disseminated to industry, to promote uniform analysis of microbial contamination at all levels of the industry.

Objectives

Prior to commencement of the project the objectives were altered to meet needs of the Meat Research Corporation and the meat industry in addressing international trade regulations. The scope of the work therefore changed and the following objectives were met:

1. Investigation of recovery of organisms from beef carcasses by USDA FSIS MegaRegs method:
   a) Comparison of excision versus sponge-swabbing in recovery of *E coli*
   b) Comparison of Petrifilm™ with Most Probable Number for enumeration of *E coli*
   c) Comparison of 3 versus 2 site sampling for evaluation of microbial status of carcasses
   d) Investigation of the operator variation in carcass sampling
   e) Investigation of the variation in recovery of organisms from meat surfaces over time
   f) Investigation of the proportion of organisms recovered
   g) Investigation of the effect of the sponge/diluent on recovery of *E coli* from swabbed samples.

2. Establishment of a data base of microbiological methods for use in the meat industry and a hard copy file of relevant tests and information on equipment, including a sample costing of setting up on-site testing facilities.

3. Evaluation of data analysis systems used in the meat industry to monitor carcass contamination.

Objective 1 - Investigation of recovery of organisms from beef carcasses by MegaRegs method:

The United States Department of Agriculture Food Safety Inspection Service has introduced a pathogen reduction scheme (MegaRegs) for the US meat industry and for any potential exporters into that market. The scheme utilises a sampling regime where a number of carcasses are sampled per works for levels of *Escherichia coli* as an indication of possible contamination with food-borne pathogens of faecal origin. The proposed rule was to sample from 3 sites per carcass by a sponge sampling method. Various methods could be used for enumeration of *E coli* in the sample, including Petrifilm™ or Most Probable Number (MPN). Aspects of the sampling regime were investigated in this project so that comment might be made to USDA and to provide information to the Australian Meat Industry.

Comparison of excision versus sponge-swabbing in recovery of *E coli*

The recommended sampling method in the MegaRegs is sponge swabbing a composite of 3 x 100 cm^2 sites on beef carcasses. Previous carcass surveys in Australia and elsewhere have sampled carcasses by excision. This study was undertaken to compare the recovery rate of *E coli* by each sampling method.

Research Methodology

1. Sampling
   Beef carcasses were sampled (3x100cm^2) by both excision and sponging at a Victorian domestic abattoir . Each carcass was sampled by sponging at rump, flank and brisket, then the same area excised. Sponge samples were stored in Whirlpak bags in Butterfield's solution and excised samples were folded inwards and stored in sterile bags. Samples were returned on ice to the laboratory and tested on arrival.

2. Sample Preparation
   Both sponge and excision samples were stomached for 2 minutes and the fluid expelled from the bag, diluted in peptone water and examined for total viable counts (TVC) and *E coli.*

3. Microbiological analysis
   Samples were analysed for TVC by AS 1766.1.3 (1991) and for *E coli* by both Petrifilm™ (3M) and Most Probable Number (MPN: AS1766.2.3, 1992). In brief:
   a. For TVC, diluted aliquots were plated in pour plates (Plate Count Agar: Oxoid) and incubated at 25°C for 48h.
   b. For *E coli* counts by Petrifilm™ 1ml aliquots were plated onto Petrifilm™ and incubated at 35°C for 48h.
   c. For *E coli* by MPN, 1 ml aliquots of the original suspension and dilutions of that were added to a total of 15 lauryl tryptose broth tubes in accordance with AS 176.6.3.

Results

Excision resulted in higher recovery rates compared with sponging (Table 1). The data is presented as a frequency distribution to allow for comparison with MegaRegs 3 class sampling plan where c=3, m=5 and M=100.

Table 1. Recovery of *E coli* from excised and sponged samples.

	Petrifilm™			MPN		
E coli/cm^2	Rump (E,S)*	Flank (E,S)	Brisket (E,S)	Rump (E,S)	Flank (E,S)	Brisket (E,S)
Not detected	64, 89	53, 85	91, 94	51, 88	36, 69	65, 86
<1	9, 5	23, 13	3, 8	17, 7	30, 26	27, 15
1-5	15, 4	19, 2	6, 0	18, 3,	29, 6	9, 1
5-100	13,3	6, 1	2, 0	12, 4	4, 1	1, 0
>100	1, 1	1, 1	0, 0	4, 0	3, 0	0, 0
% positive	Petrifilm™ (E,S)	33, 13		MPN (E,S)	51, 21	

*E = excised sample, S = sponge sample

By either MPN or Petrifilm™, excised samples gave higher *E coli* counts than sponged samples. When *E coli* counts estimates were made on excised samples by MPN, samples were found outside the cutoff point of 10^2/cm^2 in the MegaRegs sampling plan. Similar sponge samples did not fall above the cut-off. More carcasses were classified as being in the range of 5-10^2/cm^2 by excision than by sponging, whether the counts were estimated by MPN or Petrifilm™.

Discussion

Sampling carcasses by sponge method rather than excision resulted in lower *E coli* counts than by excision. Thus the use of that sampling method, recommended by the USDA, would result in greater compliance with the standard than if excision had been used to collect the sample.

Comparison of Petrifilm™ with Most Probable Number for enumeration of *E coli*

There are a range of methods approved for estimation of *E coli* numbers. The MegaRegs recommends either the MPN method or a method deemed equivalent by the IOAC. The MPN method requires a large amount of media, takes a long time to perform and requires confirmation of the organism by further testing. A more simple, user-friendly alternative is to use Petrifilm™ (3M). This method utilises media which has long shelf-life, is of similar cost to alternative methods and requires no further confirmatory tests; all these factors make it an attractive test for use in on-site testing facilities. It is important to establish that the use of this test does not disadvantage the Australian meat industry.

Research Methodology

See section above

Results

See Table 1 above for data presented as a distribution. Table 2 presents the data as arithmetic counts of *E coli*/cm^2.

Table 2. Comparison of estimation of *E coli* counts by Petrifilm™ and MPN

		Petrifilm™			MPN		
		Rump	Flank	Brisket	Rump	Flank	Brisket
Sponge	mean	0.91	1.74	0.03	1.02	0.56	0.04
	sem	1.39	2.45	0.02	1.11	0.79	0.03
Excision	mean	4.32	4.21	0.26	9.64	5.46	0.39
	sem	2.80	4.16	0.20	6.06	4.85	0.20

The estimation of *E coli* by MPN resulted in higher counts on excised samples compared to sponge samples. However the standard errors for these means are large and there is no significant difference. There were no significant differences between methods for *E coli* estimates, however, as discussed in the previous section, there would be differences in compliance with the MegRegs standard.

Discussion
Sponge sampling is the method recommended in the MegRegs and, if we look at this data only, there is little difference in *E coli* numbers whether by MPN or Petrifilm™. Estimation by the Petrifilm™ method resulted in 2 carcasses above the 10^2 cut-off while none were in that range by MPN method. In the marginal range of 5-10^2/cm^2, only one carcass was positive by MPN that wasn't positive by Petrifilm™. The Petrifilm™ method would therefore result in similar compliance with the MegaRegs as the MPN method.

Comparison of 3 versus 2 site sampling for evaluation of microbial status of carcasses

In the MegaRegs, and in other carcass surveys, multiple sites on a carcass are sampled and pooled to give an overall estimate for the carcass. The MegaRegs recommends 3 sites - rump, flank and brisket - for sampling beef carcasses in chillers. There are occupational health and safety and time issues associated with sampling the rump site. Samplers must climb ladders and perform manipulations in an aseptic manner while balancing at the top of that ladder. If 2 site sampling were equivalent to 3 site sampling as a reflection of the microbiological status of the carcass, then sampling flank and brisket alone would minimise possible injury to workers and significantly reduce sampling time.

Research Methodology
See section above

Results
The mean TVC at each site for sponge samples is shown in Figure 1and excised samples in Figure 2.

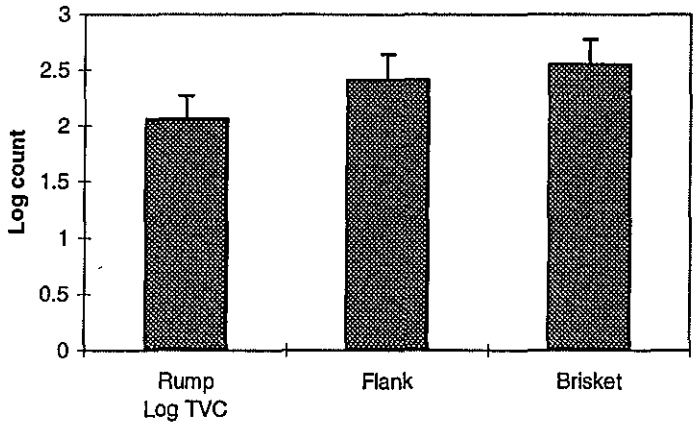


Figure 1. Bacterial numbers recovered by sponge sampling at each site.

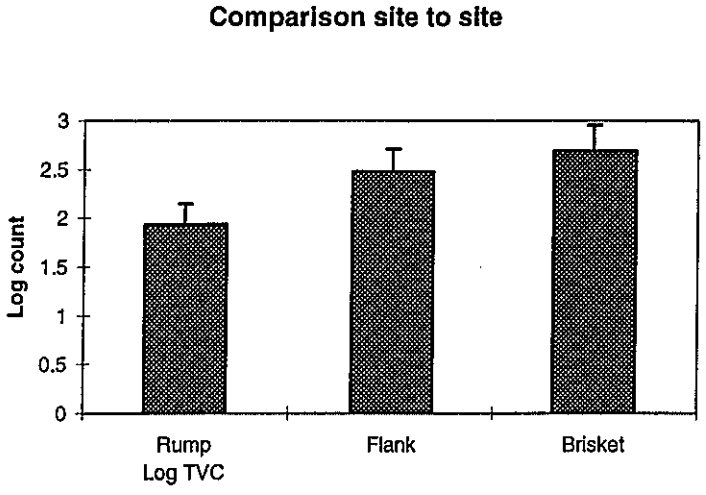


Figure 2. Bacterial numbers recovered by excision sampling at each site.

Total viable counts are similar at each site, with counts at flank and brisket marginally higher than rump. However the *E coli* data (Table 2) indicate that recovery of the indicator organism recommended in the MegaRegs is higher in rump than brisket or flank.

Discussion

Bacteria are not distributed evenly on carcasses and, while it would be an advantage to eliminate the rump site from sampling, this may result in a lower *E coli* count for the carcass as a whole than if all 3 sites were used. While the TVC data shows an opposite effect, this is not an argument to eliminate the rump site, as those bacteria are not necessarily of faecal origin and may not be significant for the safety or shelf-life of the product.

Investigation of the operator variation in carcass sampling

With the introduction of on-site sampling in a large number of meat works across Australia, many works are undertaking their own sampling, frequently using operators with little training in microbiological sampling. This may result in variation in recovery of organisms from carcasses which is due to the difference in techniques of individual operators. The method of sampling is described in the MegaRegs, however, small differences in interpretation or application may occur in practice. This experiment was undertaken to evaluate operator-operator variation in recovery of *E coli* from carcasses by the MegaRegs method.

Research Methodology

Five operators were chosen, Three (J, N, D) were experienced laboratory technicians who have had extensive experience in sponge sampling of carcasses. The other two (A, B) were QA officers at the abattoir and had some experience in testing. On each of 5 days (over a two week period), each operator sampled 10 randomly selected beef carcasses in the abattoir chiller by the MegaRegs sponge sampling method (composite 300cm^2 samples). Samples were returned to the laboratory and *E coli* and coliform counts were performed by the Petrifilm™ method. Counts are reported as cfu/cm^2.

Results

See Figures 3 and 4 for the mean recovery rates per operator. The data were not distributed normally and were analysed by One Way Analysis of Variance on Ranks. There were statistically significant differences in recovery rates for operators ($p<0.0001$).

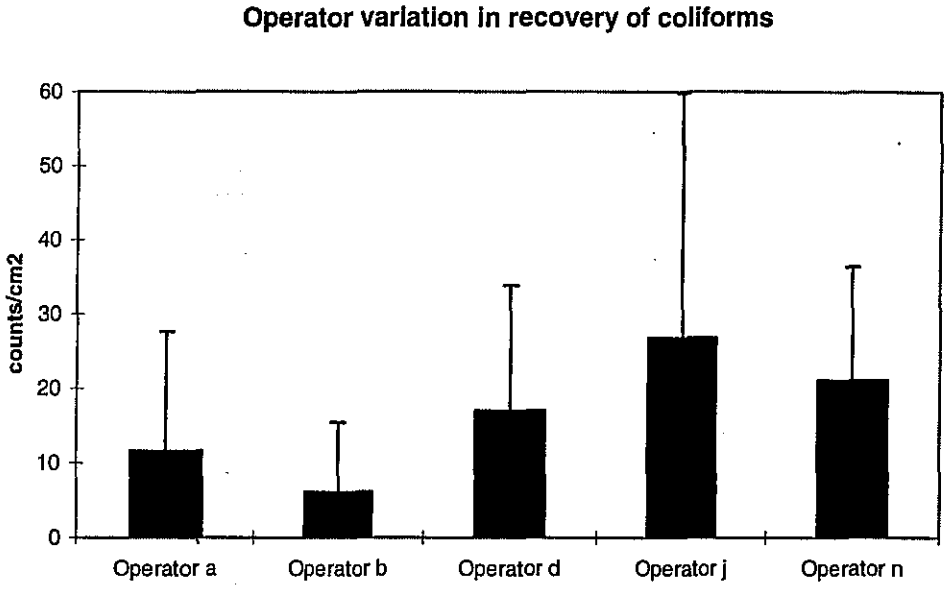


Figure 3. Recovery of coliforms by operators.

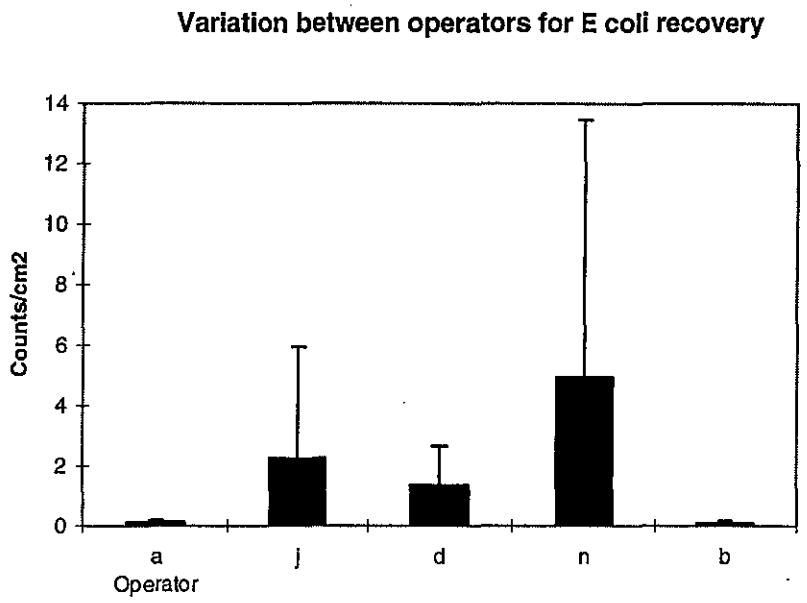


Figure 4. Recovery of *E coli* by operators.

Discussion

The rate of recovery of *E coli* varied with the operator taking the samples. All samples were taken on the same days and from the same lot of carcasses (one day's kill) and processed the same way. It is interesting to note that the skilled laboratory workers had better recovery rates than the plant QA officers. The QA officers were not re-trained in the method prior to the sampling days and were not assisted in any way. The implications for industry are that operators must be adequately trained to take the samples, with on-going auditing of their skills and that operator variation may cause false negative results. False negative results may mask an on-going problem in a works which would be revealed in validations, perhaps by external auditors.

Investigation of the variation in recovery of organisms from meat surfaces over time

The purpose of this experiment was to investigate the variation in recovery of *E. coli* over time from cattle carcasses, in an attempt to simulate the variation in time of delivery of samples to a microbiology testing facility.

Methodology

The experiment involved the collection of 200 samples from 20 randomly selected cattle carcasses from a Victorian abattoir by VIAS staff on three consecutive sampling days. As a result 3 different cattle lots were sampled. On days one and two, 7 carcasses were sampled and 70 samples were collected. On the final day, 6 carcasses were sampled and 60 samples were collected.

The samples were collected as a series of 5 paired swab samples by MegaRegs sponge swabbing methods. The samples were collected from opposite sides of the carcass midline at time 0. Samples collected from 100cm^2 areas on the left side of the carcass were the control samples. Matched samples collected from the right side of the carcass were the 5 treatment samples.

The sampled pairs were stored at 4 to 5°C to mimic travel time to a laboratory under ice, and processed for the recovery of *E. coli* at times 1, 2, 6, 12, and 24 hours. The temperature of transport and storage for the three days did not exceed a 4°C - 6°C. The difference in recovery rates of *E. coli* were estimated. Microbiological analysis was by *E. coli* and coliform Petrifilm™.

Table 3. The 10 sample sites and time until analysis for *E. coli*

Sample number	Sample site along carcass midline	Time kept in storage
1, 2, 3, 4, 5	Left (upper-lower)	0 hours
6	Right (upper)	1 hour
7	Right (upper mid.)	2 hours
8	Right (mid.)	6 hours
9	Right (lower mid.)	12 hours
10	Right (lower)	24 hours

Results

The *E. coli* results are tabulated below, and reported as a mean colony forming units/cm^2.

Table 4. *E coli* counts over time.

Treatments	mean (cfu's/cm^2)	s.d.
1 hour sample	0.4	0.8
0 hour control	1.7	6.4
2 hours sample	4.2	10.1
0 hour control	3.8	8.8
6 hours sample	2.5	4.1
0 hour control	0.8	13.7
12 hours sample	0.7	4.5
0 hour control	1.3	2.3
24 hours sample	0.5	1.9
0 hour control	0.4	1.2

The attached graph (Figure 5 attached) illustrates the effect of time on the recovery of *E. coli* compared to the average time/control mean represented as 0 cfu's/cm^2.

Discussion

The results suggest that there was no significant difference in *E. coli* counts after 1, 2, 6, 12, and 24 hours at the 0.05 level of significance. As the carcass halves were split prior to chilling, the major limitation of this experiment was the uncertainty about knowing the degree of *E. coli* contamination on both sides of the carcass along the midline. It is possible that one half of the carcass may have been more contaminated than the adjacent half of the carcass which may have caused slight variation in the first few results as seen in carcasses 1, 2 and 3. The results of this section suggest that works which must delay enumeration of *E coli* from the sponge sample (eg, send to external testing laboratory) will still get valid results up to 24 h post sampling, providing temperature of transport and storage is maintained at less than 4°C.

Investigation of the proportion of organisms recovered

Enumeration of organisms on carcass surfaces generally requires separation of the organisms from the surface. There is some variation in recovery by different sampling methods and also some concern that bacteria may become entrapped in sponges in the sponge sampling technique. In order to investigate the recovery of *E coli* from the carcass surface by sponge and the removal of those bacteria from the sponge for counting, a method was devised to measure the bacteria *in situ*. The reagent alamar Blue is a non-toxic REDOX indicator that changes colour in response to the detection of metabolic activity (see attached information).

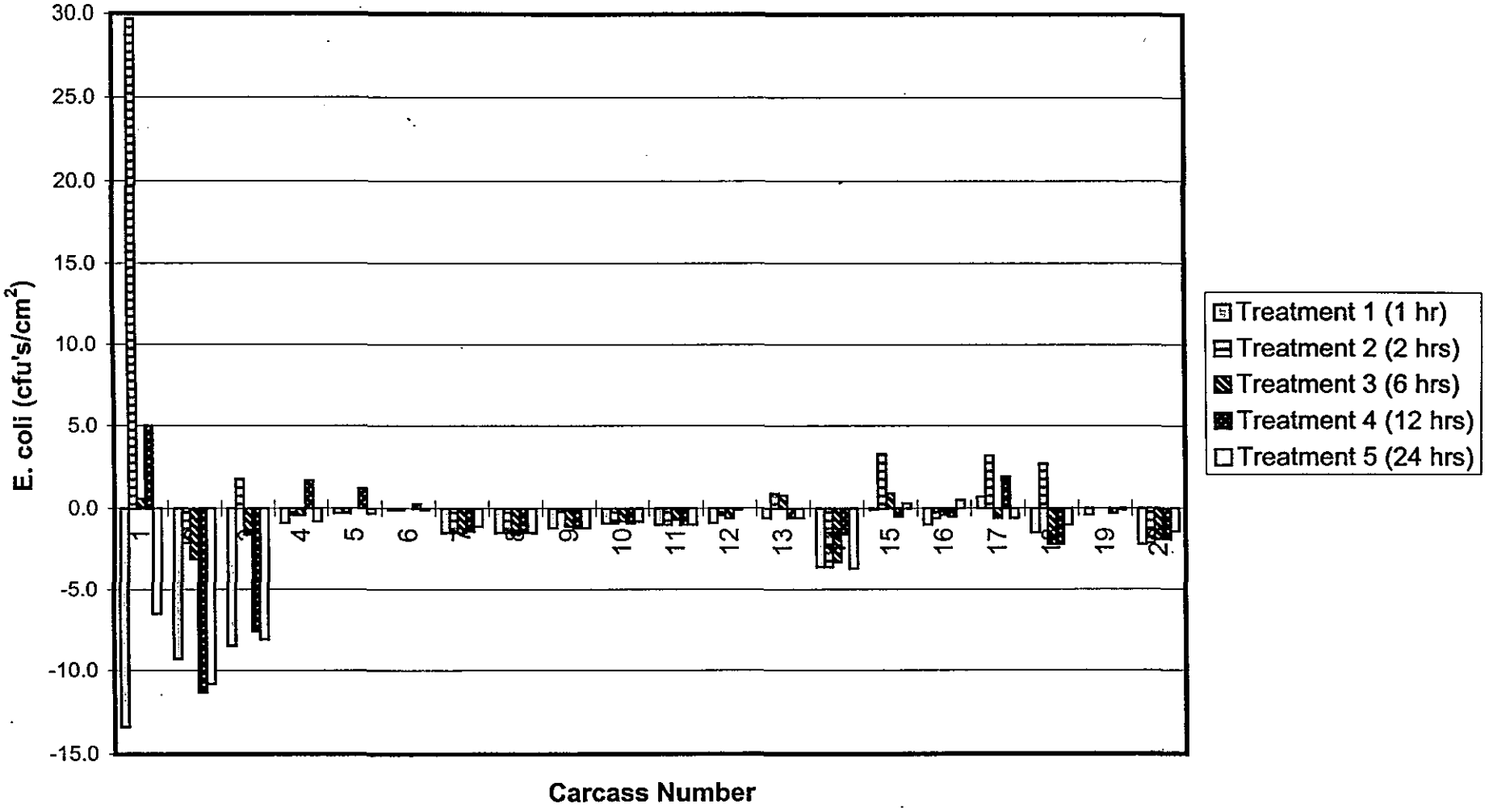
Research Methodology

Initial trials using alamar Blue indicator were carried out to firstly determine the potential of using this system to quantitate bacterial numbers directly off meat surfaces, and secondly, determine whether bacteria are becoming entrapped on the sponge during sponge sampling methods.

Materials and Methods (see flow diagram Fig 6)

Carcass flaps of lamb were cut into approximately 100cm^2 sections and surface sterilised (80°C, 10 sec). *E. coli* was grown overnight (O/N) in 20 ml nutrient broth (NB) at 37°C then diluted to 75% transmission (%T) at 610nm. Bacterial numbers were enumerated by plate counts of serial dilutions. Two pieces of sterile meat

Figure 5. Effect of time on recovery of E coli from carcasses



Note: The control (0 cfu's/cm^2) used in this graph is an average of all 5 controls measured after 1, 2, 6, 12, and 24 hrs.

were seeded with 1ml of 75%T *E. coli* and left at room temperature (RT) for 4 hours, a third piece was left sterile as a negative control. During this incubation time 5 samples were bored out of the sterile meat, using a sterile cork borer, along with 5 samples from a sterile rehydrated sponge (Nasco whirl-pak, rehydrated with 25 ml sterile 0.1% peptone water [PW]). These, along with 5 x 2ml aliquots of the PW used to rehydrate the sponge were placed in a 24 well tissue culture Costar plate and left at RT for the remainder of the incubation. After 4 hours 5 samples were bored out of one of the pieces of seeded meat (positive control), and placed in the Costar plate. A second sponge was rehydrated with 25 ml sterile 0.1% PW and used to swab 100 cm^2 from the remaining piece of meat, returned to the diluent and stomached for 2 min. After swabbing, 5 samples were bored out of both the swabbed meat and the sponge, and added to the Costar plate. Five 2ml aliquots of the diluent used in swabbing the meat were also added to the Costar plate. Once all samples were in the plate 2 ml sterile 0.1% PW was added to all wells except those already containing diluent from the sponge bags. Plates were then incubated at 37°C for 3 hours after which time 100 µl was removed from each well and added to corresponding wells of a microtitre plate and 10µl alamar Blue added to each well. The plate was then shaken at RT for 15 min and incubated at 4°C for 18 hours before reading in a Titertek at 570nm. Optical densities were averaged over the 5 samples for each group and bacterial numbers read from a standard curve of CFU vs OD_{570} for *E. coli* in NB (see Fig 7).

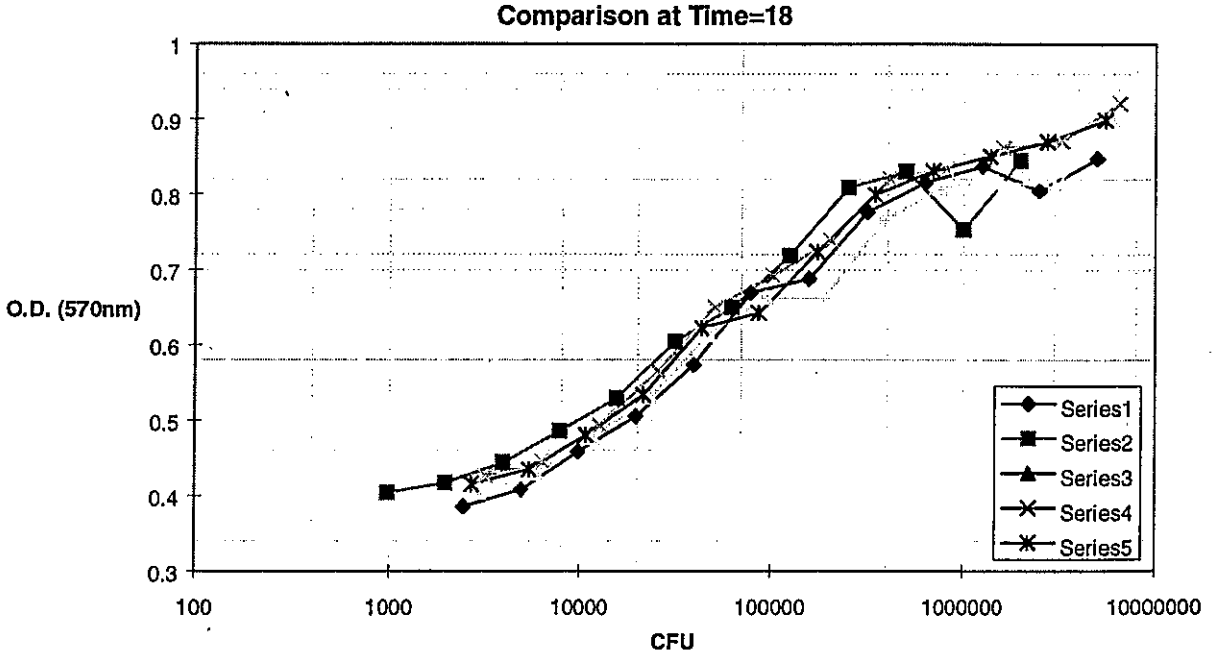


Fig 7: Standard curve for *E. coli* using alamar Blue indicator

Results

From these preliminary results it appears as though the sponge does not trap the bacteria, instead bacteria recovered from the meat via sponge sampling are released into the diluent (refer to Fig 8). Levels of bacteria, as measured by alamar Blue reaction, on swabbed meat and sponges used in swabbing are comparable with the background levels seen in the negative controls. Bacterial numbers in diluent used in sponge sampling are significantly higher than levels seen in the negative control, and similar to those on the seeded meat pre-sponging.

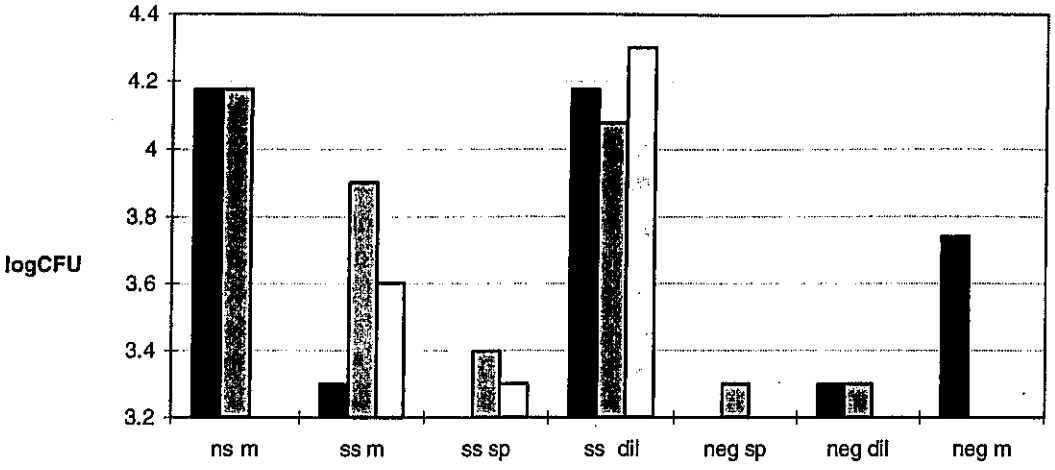


Fig 8: Bacterial numbers identified by alamar Blue indicator
(NB. each bar within a group represents a single sample averaged over the 5 sections/aliquots)

Key:		
	ns m	nonswabbed/seeded meat (positive control)
	ss m	swabbed/seeded meat
	ss sp	sponge used in swabbing
	ss dil	diluent used in swabbing
	neg sp	negative sponge (sterile)
	neg dil	negative diluent (sterile)
	neg m	negative meat (sterile)

Discussion

These results indicate that counts of *E coli*/cm^2 obtained by the MegaRegs sponge sampling method are a reasonable reflection of the actual contamination of the carcass surface. In addition, fears that bacteria are trapped in the sponge and not included in the count, would seem to be unfounded.

Investigation of the effect of the sponge/diluent on recovery of *E coli* from swabbed samples

The sponge material and diluent used in the MegaRegs may have an inhibitory effect on the organisms collected in the sampling. This experiment was designed to investigate the effect of those materials on the recovery of *E coli*.

Research Methodology

Fresh (< 1 hour post collection) bovine faecal material was collected into a stomacher bag. This was then diluted 1/10 (w/v) in Butterfields Phosphate Diluent (BPD). With a wide paintbrush, a thin layer of this faecal slurry was painted onto the skin side of five sides of lamb. This was then left to dry at room temperature for 30 min. During this time five Nasco Whirlpak sponge sampling bags were rehydrated with approximately half the volume of a 25 ml BPD sterile aliquot. After drying carcasses were sampled at 3 sites (dorsal line, flank, under front leg) with 100 cm^2 templates. New, sterile templates were used for each site, the same sponge was used for each carcass over the 3 sites before being returned to the bag and the remaining volume of BPD added to the bag. Immediately after sponging carcasses, all 5 bags were stomached by hand for 4 min and diluent squeezed from the sponge. 1 ml diluent from each bag was diluted 1:10 to 1/10,000, and 1 ml of each dilution (N to -4) was plated in duplicate on *E. coli* Petrifilm™ and incubated at 37°C for 48 hours. This sampling regime (stomaching to plating) was repeated at 30 min, 1 hour, 3 hours, 6 hours, 12 hours, 18 hours, and 24 hours.

Results
The *E coli* counts are tabulated below.

Table 5. Mean log *E coli*/cm^2 recovered from sponge over time.

	Time of Removal from Diluent					
Carcass	5 min	30 min	1 hour	3 hours	6 hours	12 hours
A	3.408617	3.418162	3.420797	3.346891	3.391102	3.334368
B	2.843987	3.16873	3.207487	3.083104	2.985406	2.897723
C	3.258268	3.304363	3.280849	3.230261	3.155612	3.27542
D	2.314194	2.361702	2.43045	2.489518	2.8677	2.851108
E	2.810003	3.021189	3.069151	2.89967	2.935713	3.070038

Due to a limited amount of diluent at 18 and 24 hours only 1 neat Petrifilm™ was plated. These plates were too confluent to count, however on visual examination the density of colonies appeared to be of the same degree as the samples taken at 12 hours.

Discussion
There was no reduction in *E coli* numbers from the sponges over time indicating that neither the material in the sponge nor the diluent had an effect on the viability or recovery of *E coli* in the MegaRegs method.

Objective 2 - Establishment of a data base of microbiological methods for use in the meat industry

Resources established

a) A database of the important spoilage bacteria and food-borne pathogens on meat has been established. This is available electronically and in hard copy. Hardcopy and electronic files of this have already been submitted and a copy is attached to this report. The database includes information on the major food-borne pathogens and spoilage organisms - organism description, growth requirements, growth rates under different conditions, standard testing methods, rapid assays and control measures.

b) A hard copy file of the major microbiological test methods has been established and a list of sources for tests and equipment.

c) An outline of requirements for setting up an on-site testing facility at a meat plant has been developed. A copy of this is attached.

d) The database is being advertised in industry via newsletter (article sent to Rosa Bertucci for Vicki Treadwell) and by fliers to industry and statutory authorities.

Objective 3 - Evaluation of data analysis systems used in the meat industry to monitor carcass contamination

A copy of this report is attached.

Implications And Recommendations

The major findings of this report are:

- the elimination of the rump site from sampling will result in reduced numbers of carcasses falling in the "warning" or "fail" limits of the MegaRegs plan
- sponge sampling recovers less *E coli* than excision
- Petrifilm™ method is similar to the MPN method for enumeration of *E coli* from sponge samples
- recovery of *E coli* from sponges does not vary over 24 hours
- the sponge and diluent do not influence recovery of *E coli*
- the sponge does not trap bacteria
- there can be considerable variation in recovery of *E coli* from carcasses by the sponge method from operator to operator.

The recommendations to industry are:

- sponge sampling of 3 site with Petrifilm™ enumeration of *E coli* be used
- samplers in the abatoirs be trained and audited on a regular basis
- sponges may be stored up to 24 h at 4°C prior to sampling.

Budget

Project Funding		**Period**	**1996/7**
	MRC	**VIAS**	**Total**
- Salaries	$56,680	30710	87390
- Travel			
- Operating	63320		
- Capital			
TOTAL FUNDING	120,000		207390

Acknowledgments

We would like to acknowledge the generous assistance and cooperation of the staff and management of the Victorian abattoir in this trial.

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FSIS Pathogen Reduction Program

The Presentation of Analytical Results for HACCP Systems in Abattoirs

Introduction

Domestic and export abattoirs must comply with regulations which require microbiological testing of carcasses. For example, USDA MegaRegs require *E. coli* in a 3 class sampling plan with limits set for each species of animal slaughtered. Australian Standards use a Total Viable Count (TVC) approach. Effective data management is the first step for monotoring test results to comply with such regulations.

Hazard Analysis Critical Control Point (HACCP) based computer programs can provide specialised data analysis with immediate and informative results. Such programs are user friendly and can be easily applied to every day quality assurance (Q.A.) systems. Some laboratories with access to computers may find it useful to use computerised HACCP programs to assist with every day quality assurance. However, such systems can be imitated manually for those without access to a compatible computer. This review looks at two HACCP computer packages readily available to the public are reviewed as well as the different methods used by such programs that can be easily performed manually for those without access to a computer. First of all it is best if we understand specific statistical terms such as the normal distribution curve, mean and standard deviation.

The Normal Distribution Curve

The Normal Distribution Curve (Fig. 1) is a statistical term used to describe the distribution of a population in which truly random values fall within an even number of deviations on either sides of the mean value (X). That is how much variation there is within a group (population).

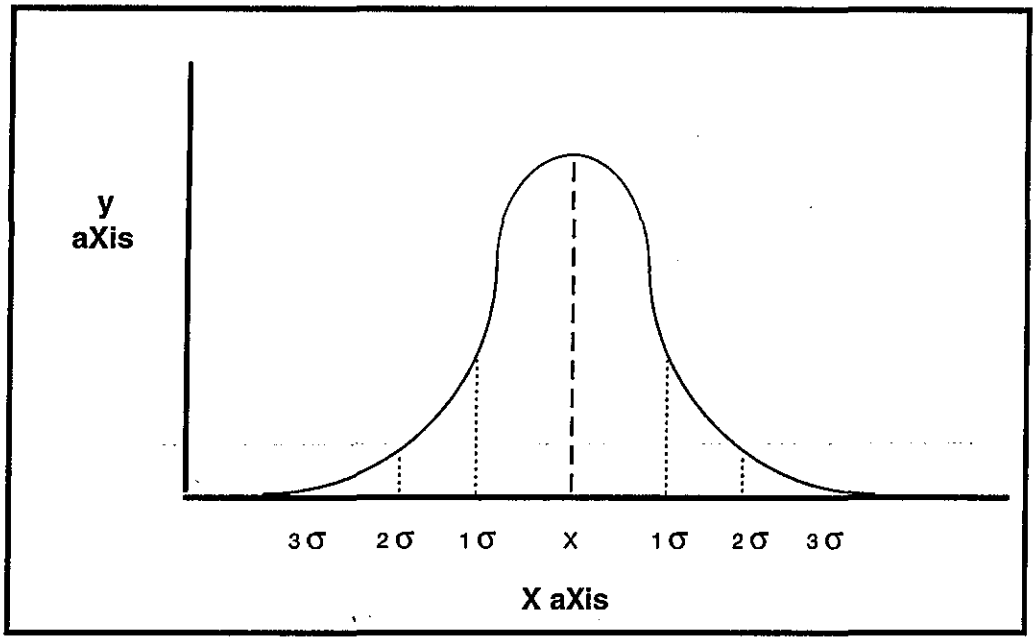

Fig. 1. Normal Distribution Curve. Note: σ = Standard Deviations.

For example, the weight range of yearling livestock carcasses may vary from 220 kg to 280 kg. If most of the carcasses weigh around 250 kg, this is known as the modal (most common) weight. Fewer cattle carcasses heavier than 260 kg and lighter than 240 kg will be in the upper and lower range.

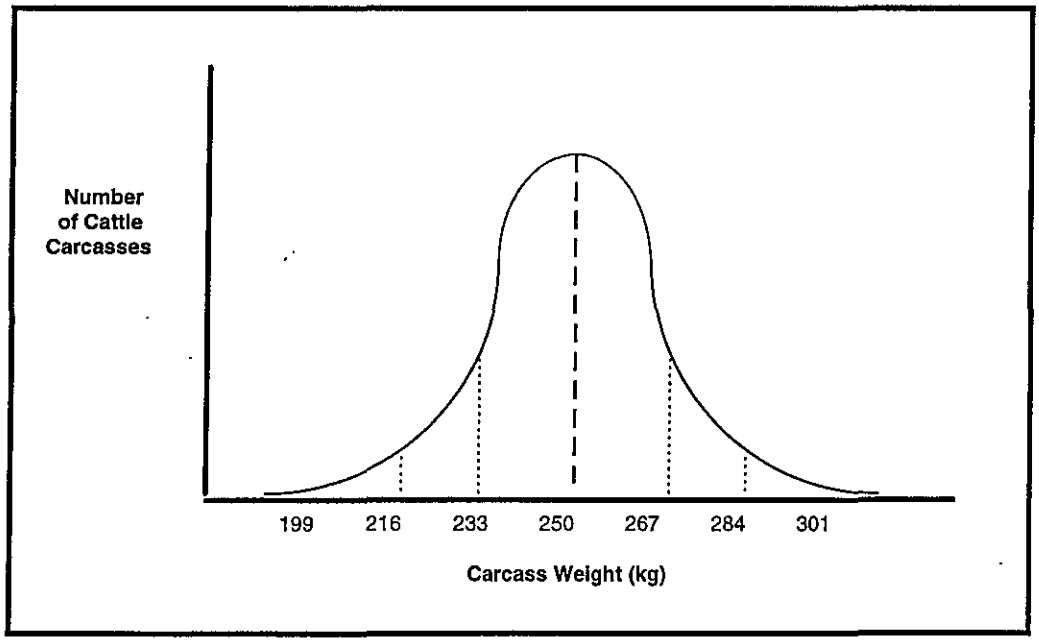

Fig. 2. Normal Distribution Curve for cattle carcasses. Where σ = 17 kg, X = 250 kg.

The Mean (X)

The Mean is a statistical term used to describe the average of truly random values and is a numerical value derived from the normal distribution curve. The mean is calculated as follows:

$$X = \frac{(\sum X)}{n}$$

Where: X = mean

n = number of values

$\sum$ = sum of

For example, if the weight of yearling livestock carcasses were as follows - 220, 220, 240, 252, 248, 260, 280, 250, 240, 252, 248, 260, 280, 250, 220, 240, 252, 248, 260, 280.

The mean (X) weight for the yearling livestock carcasses is calculated as follows:

Step 1.

$\sum X = 220 + 220 + 240 + 252 + 248 + 260 + 280 + 250 + 240 + 252 + 248 + 260 + 280 + 250 + 220 + 240 + 252 + 248 + 260 + 280$

$\sum X = 5{,}000$ kg

Step 2.

n = 20 carcasses

Step 3.

$$X = \frac{5{,}000}{20}$$

X = 250 kg

This indicates that the mean weight for the yearling cattle carcass was equal to 250 kg.

The Standard Deviation (σ)

The standard deviation (σ) is also a numerical value derived from the normal distribution curve and is a measure of the scatter of a group of related values. The formula for the standard deviation is as follows:

$$\sigma \rightarrow \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n}}$$

Where: σ = standard deviation
n = number of values
$\sum$ = sum of
X = results
$\sqrt{}$ = square root
^{2} = squared

For example, the standard deviation (σ) of carcass weight for the yearling livestock carcasses is calculated as follows:

Step 1.

$\sum X^2 = 220^2 + 220^2 + 240^2 + 252^2 + 248^2 + 260^2 + 280^2 + 250^2 + 240^2 + 252^2 + 248^2 + 260^2 + 280^2 + 250^2 + 220^2 + 240^2 + 252^2 + 248^2 + 260^2 + 280^2$

$\sum X^2 = 1{,}256{,}024$

Step 2.

$(\sum X)^2 = (220 + 220 + 240 + 252 + 248 + 260 + 280 + 250 + 240 + 252 + 248 + 260 + 280 + 250 + 220 + 240 + 252 + 248 + 260 + 280)2$

$(\sum X)^2 = 25{,}000{,}000$

Step 3.

$n = 20$

Step 4.

$$\sigma \rightarrow \sqrt{\frac{1{,}256{,}024 - \frac{25{,}000{,}000}{20}}{20}}$$

$\sigma = 17$ kg.

This indicates that the standard deviation for the yearling cattle carcass weight was equal to 17 kg.

Shewhart chart

There are a number of ways to present data and this will depend on the eXperiment and the equipment available ranging from computer software to pen and paper. A Shewhart chart (Fig. 3.) is a good graphical solution to display. The Shewhart chart shows variation around a mean and requires the calculation of a mean value and standard deviation for a sample. Subsequently, results are plotted on a chart containing ±2 standard deveiations (perimeters).

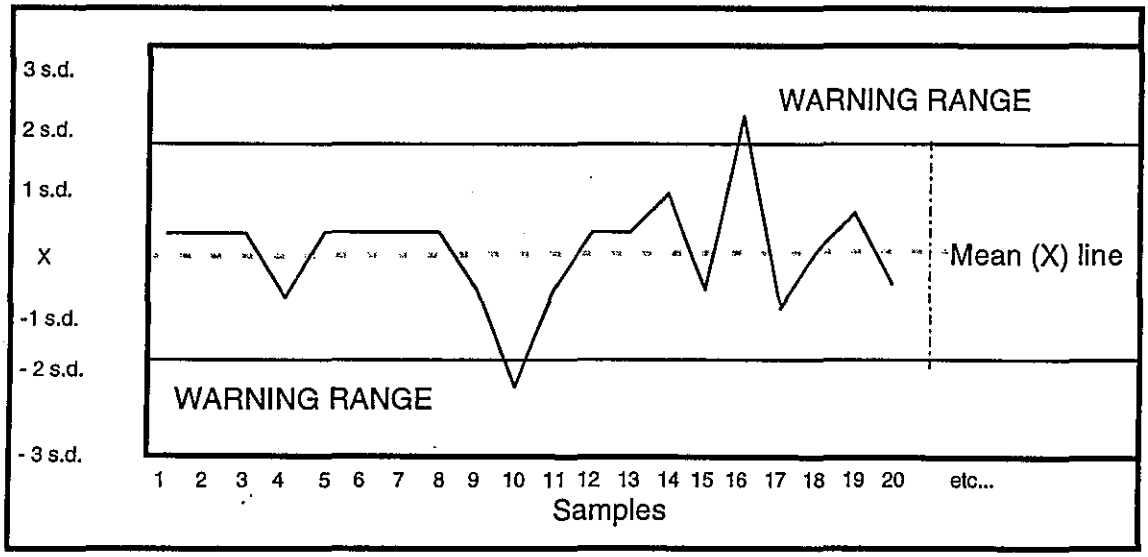

Fig. 3. A warning has been included between 2 and 3 standard deviations to highlight variation in both the precision and accuracy of sampling.

Analytical results can be written in a way that values which fall within either sides of the mean can be eXpressed as a percentage. For example; figure 3 demonstrates 18 from 20 values fall within the mean ±2 σ. Those values fall within the mean is equal to 90%. This type of analytical representation can be useful for Total Viable Counts (TVC's).

There are three important factors for setting up a Shewhart chart, and they are as follows:

a) There must be more than 20 measurements made.

b) The mean (X) and standard deviation (σ) can be calculated if the errors involved during this initial selection period are random.

c) Highly inaccurate results can distort the mean and standard deviation.

The USDA MegaRegs 3 Class Plan

The USDA MegaRegs requires a 3-class sampling plan be used for the microbiological testing of livestock carcasses for *E. coli*, which is an organism used to indicate faecal contamination on a carcass surface. The sample plan should consist of randomly selected samples. Based on USDA MegaRegs a specific number of carcasses must be sampled which differs between species (Table 1). Thirteen carcasses sampled are grouped together in a 'window' and tested against the requirements of the 3 Class Plan.

The 3 Class Plan consists of 'm' and 'M' values which are limits based on percentile levels into which the sample result falls. The 'M' values, which is set at the 98th percentile (eg. 98% of all carcasses tested fell below this level as per USDA MegaRegs), are related to the safety/quality limit (ICMSF 1986). The 'm' values, which is set at the 80th percentile (eg. 80% of all carcasses tested fell below this level as per USDA MegaRegs), are associated with good commercial and manufacturing practices by retailers and/or producers (ICMSF 1986).

Counts exceeding the 'M' value indicates unacceptable contamination. Where as the 'm' value is a critical range in which no more than 3/13 carcasses must not exceed. According to the USDA MegaRegs, permitted *E. coli* counts for different species can be seen below.

Table 1. E. coli testing frequences and cfu limits of *E. coli* for different animal carcasses

Slaughtered Species Class	Number of carcasses to be tested	'm' value cfu's/cm^2	'M' value cfu's/cm^2
Cattle	1/300	5	100
Broiler	1/22,000	100	1,000
Pigs	1/1,000	10	10,000

Note: cfu's/cm^2 - Colony Forming Units per centimetre squared. From USDA MegaRegs.

For example, if an abattoir was slaughtering 6000 head of cattle per day, then 1 in every 300 carcasses must be sampled by using methods as per USDA MegaRegs. Therefore, at the end of the day 20 cattle carcasses would have been randomly sampled.

A graph showing *E. coli* counts for each sampled carcass can be used to monitor bacterial levels under MegaRegs recommendations. Such a graph can indicate wether a problem exists within the current production chain. There are 2 perimeters in the graph - ①: the upper limit (M) - those samples which exceed unacceptable levels (eg. 100 cfu's/cm^2 for weaned cattle), and ②: a minimal limit (m) - for which no more than 3 out of 13 samples may exceed permitted levels (eg. 5 cfu's/cm^2 for weaned cattle) in the last 13 samples (see Fig. 4).

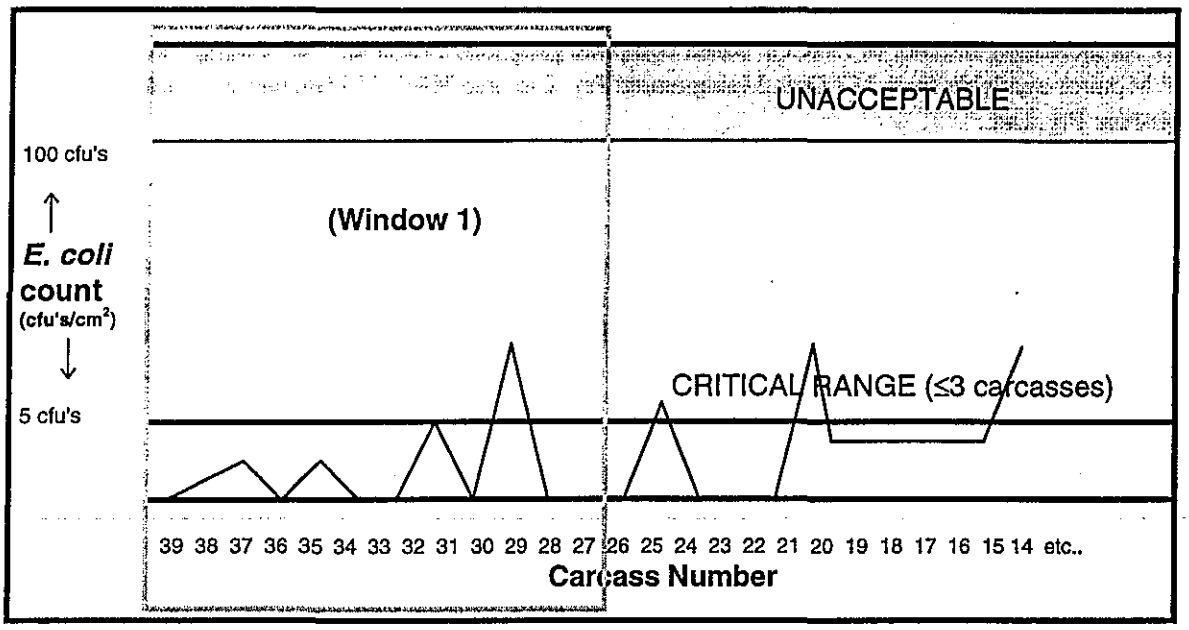

Fig. 4. A 'moving window' for cattle carcases. Note: A warning has been included above 5 cfu's and above 100 cfu's to highlight unacceptable results.

Note: To make a more user friendly graphical presentation a cardboard frame may be placed over the graph to highlight the last 13. This makes it much easier to focus on specific units (Fig. 5).

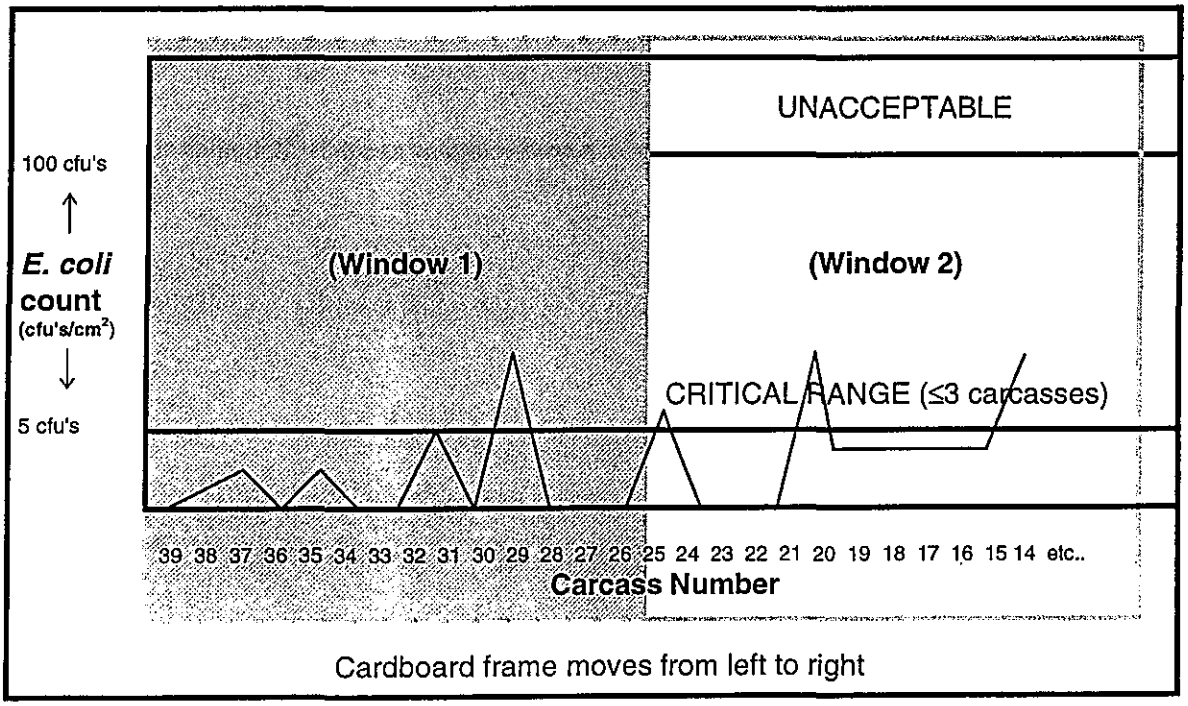

Fig. 5. A 'moving window' for cattle carcases. Note: A cardboard frame placed over the graph highlighting a particular unit of carcasses.

Available HACCP programs

There are an increasing number of HACCP based computer programs available. Two examples are HACCP Monitor available from M2 Data Management Pty. Ltd. and HACCP Manager available from Icon Software Pty. Ltd..

For consumers that require a program be customised for their needs then HACCP Manager may be appropriate. The HACCP Manager is a computer program that has been designed to monitor the performance of a HACCP plan for an abattoir and can be customised for the operator by Icon Software Pty. Ltd. Once a HACCP plan has been created the HACCP Manager is capable of producing Critical Control Point working sheets, Detailed Critical Control Point Information, Laboratory Test Result/Graphs, and Exception Reports. The program is compatible with other Icon Software packages such as LIMS and LAB Manager.

The HACCP Monitor is also a computer program that has been designed to monitor the performance of a HACCP plan for an abattoir. However, this program cannot be customised for the operator. The database program has been written in Microsoft™ 'Access'. It is capable of storing raw data collected from the abattoir floor, and can generate meaningful results in numerical and graphical presentation. To demonstrate the effectiveness of the production system the HACCP Monitor is capable of producing specific information such as:

a) *E. coli* counts over time for MegaRegs requirements

The HACCP Monitor is also capable of producing graphical data of total carcasses which have failed to comply with USDA MegaRegs requirements on a weekly basis.

b) Defect ratings (number of contaminated carcasses) for lots processed for AQIS MHA guidelines
c) Slaughter carcass assessments of total defects by severity (give Q.A. staff an idea of where on the carcass defects are most likely to occur).

References

ICMSF (1986) Micro-organisms in Food 2. Sampling for microbiological analysis: Principals and Specific Applications. Second Ed.

VICTORIA

VIAS Food Microbiology Information Services

Victorian Institute of Animal Science Food Microbiology Information Services

Victorian Institute of Animal Science Food Microbiology Information Service:

Located at the VIAS Attwood campus, the Food Microbiology Information Service provides information regarding current issues and practices in the meat industry.

- Information about establishing on-site microbiological testing facilities.
- Current news in food microbiology
- Information about up to date recommended microbiology testing

Establishing on-site microbiology testing:

If you don't have in-house research facilities the Food Microbiology Information Service can help by providing information about:

- Complying with Australian Standards
- Complying with Codes of Practices
- Requirements for materials and equipment

Current News in Meat Microbiology:

- Up to date news on current microbiology issues
- Domestic and Export meat microbiology testing requirements (eg. USDA MegaRegs)

Microbiological Testing:

- Current methods required for microbiology testing
- What materials to use
- Available training courses
- Trouble shooting
- Information about data analysis

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A project funded by the

Meat Research Corporation

Appendix 3

MATERIALS AND EQUIPMENT REQUIRED FOR USDA MEGAREGS TESTING

The following is the information required to set up a laboratory for the USDA 'Megaregs' testing. Included are the latest prices of items. Those that are marked with an asterisk are estimated costs. Many of the items can be sourced through a number of laboratory suppliers.

You will require the following materials and equipment,

ITEM	COST	UNIT	COST PER UNIT
Whirl Pak Speci-sponge bags	$140.00	100	$1.40
Sterile Gloves	$42.00	100	$0.42
Sterile Diluent (Butterfields 25ml)	$10.00	10	$1.00
0.1% Peptone water (9ml)	$10.00*	10	$1.00*
Sterile Pipettes (1ml)	$2.50	20	~$0.13
Petrifilms -*E.coli* + coliforms	$105.00	50	$2.10
Petrifilms - for total counts	$100.00	100	$1.00

Template 10cm^2

These are not yet available commercially although there is at least one company who are intending to get them made for sale. At this stage it is acceptable to simply make them yourself, this is easily acheived using wire (coathangers are ideal) and bending it into a square of the required dimensions.

Sanitiser

Can use whatever brand you are currently using for sanitising knives eg 70% Ethanol or Hypochlorite solution.

Incubator

Here is an example of two incubators which are currently available. The Boekel Incubator is recommended - although slightly more expensive it has a larger internal capacity which would be more suited to your needs. Other models of incubator are available but only two are quoted as a quick indication of cost. Should you wish to go ahead and purchase an incubator, it would be advisable to obtain a couple of extra quotes to see what other models are available and their cost (bearing in mind that if you purchase two incubators, which you may need for both Aerobic plate counts at 25°C and E.coli Petrifilms at 35°C, you will be able to negotiate a better price.
Examples of costs include:

SUPPLIER = Arrow Scientific
PRODUCT = Cultura®s Incubator 25-45°C ± 1°C
= Dimensions, internal B126 x H113 x T135 mm
COST = $460.00

SUPPLIER = Extech Equipment Pty Ltd**
PRODUCT = Boekel Incubator Model No: 132000; Max = 60°C
= Dimensions, internal D12 x W11.5 x H10.5 inches
COST = $525.00

Other

(equipment that is not mandatory but which you may wish to purchase)

Stomacher	~ $3000-$5000
Bunsen Burner	~ $60
Pipettor	~ $400

The materials and equipment that are required is dependant upon how much of the testing you intend to perform yourself and how much you will send out to an outside laboratory (this also depends on NATA accreditation). The materials quoted are the minimum that is required for screening carcasses for *E.coli* and coliforms using the Megaregs Sponge method. *Salmonella* testing may have to be performed off-site at a cost starting from about $25.00 per sample and increasing in price depending on individual laboratories and whether or not the samples test positive or negative for *Salmonellae*.