Final Report

AUSTRALIAN MEAT PROCESSOR CORPORATION

Meat Industry Services

Effect of testing regimes on *E.coli* O157 isolation

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Prepared by:	Dr. Robert Barlow Food and Nutritional Sciences CSIRO
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Project leader:	**Dr. Robert Barlow**
MLA project manager/coordinator:	**Ian Jenson**
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Enquiries should be addressed to:

Robert Barlow
Microbiologist | Food Microbiology and Safety Group
Food and Nutritional Sciences
CSIRO

Phone: +61 7 3214 2035 | Fax: +61 7 3214 2150
Robert.Barlow@csiro.au | www.csiro.au
Address: 39 Kessels Road, Coopers Plains, QLD 4108

Distribution list

Ian Jenson	1

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Executive summary

Meat destined for export to the US and other countries must be tested and deemed free of *E. coli* O157 prior to being released into commerce. The current approach for testing for *E. coli* O157 in meat relies on first performing a screening test on an enrichment broth which contains a mixture of bacteria. Samples which test positive for the appropriate targets are typically sent to one of two commercial laboratories for confirmation. DAFF currently approves a number of methods for *E. coli* O157 testing and there is increasing concern that testing regimes may be impacting on the likelihood of isolating the target organism if it is present in the test sample. More specifically, it is hypothesised that the type of enrichment broth used, time between screening and the commencement of confirmation, and storage temperatures of broths prior to confirmation may affect the ability to isolate the target organism. All laboratories currently approved by DAFF to conduct testing for *E. coli* O157 were asked to provide details of their testing regimes. Key parameters relating to the testing of Australian manufacturing beef for *E. coli* O157 such as type of enrichment broth, length and temperature of incubation, time between testing and confirmation of a potential positive, and the temperature at which potential positives are transported or stored prior to confirmation were identified. Trials were conducted to determine if key parameters could negatively affect the likelihood of isolating *E. coli* O157 from potential positive broths.

Pure culture, low background (representative of manufacturing beef) and high background (ground beef) samples were inoculated with *E. coli* O157 and enriched in three commonly used enrichment broths for two different times and then subsequently stored at either 4°C, 10°C or 20°C for seven days. The concentration of *E. coli* O157 in each sample and the ability to isolate *E. coli* O157 from low background and high background enrichments were assessed daily. Counts of *E. coli* O157 following enrichment ranged from 3.52 to 8.46 $\log_{10}$CFU/mL with counts highest in pure culture samples followed by low background samples and then high background samples. *E. coli* O157 could be isolated from all samples immediately following enrichment. The type of sample used had minimal effect on the counts or isolation of *E. coli* O157 when stored at 4°C or 10°C with maximum reductions of 0.59 $\log_{10}$CFU/mL over the seven day storage period. Reductions in counts of *E. coli* O157 were greater (up to 2.70 $\log_{10}$CFU/mL) in samples stored at 20°C. A decrease in the ability to isolate *E. coli* O157 was also noted in these samples, particularly high background samples. The type of enrichment broth used appears to have minimal effect on the counts or the likelihood of isolating of *E. coli* O157. The average variation of *E. coli* O157 counts ranged from 0.13 to 1.06 $\log_{10}$CFU/mL regardless of sample type, incubation time or storage temperature. Reductions of >2.0 $\log_{10}$CFU/mL was observed in just seven samples with five of these being pure culture samples.

Attempts to isolate *E. coli* O157 using IMS followed by plating on rainbow agar and CT-SMAC were successful from both agars for all except two occasions for samples stored at 4°C (sample 12a – day 7) or 10°C (sample 31b – day 6). On each of these occasions *E. coli* O157 could not be isolated using rainbow agar but was recovered from CT-SMAC. However, as commercial testing laboratories currently use both agars during confirmation, samples 12a and 31b would have been confirmed positive for *E. coli* O157 using standard confirmation procedures. Consequently, isolation of *E. coli* O157 was possible from all samples stored at 4°C or 10°C regardless of enrichment broth, length of

incubation or sample type. Isolation of *E. coli* O157 from samples stored at 20°C was problematic with 10 of 24 samples having at least one occasion where *E. coli* O157 could not be isolated from either rainbow agar or CT-SMAC. High background samples were most likely to fail to yield isolates with only one low background sample unable to have *E. coli* O157 recovered from it.

The results of the study indicate that the isolation of *E. coli* O157 from a potential positive enrichment broth stored at 4°C or 10°C is not significantly affected by:

- The type of enrichment broth used
- The concentration of background microflora in the sample
- The time for which the sample is enriched
- Storage of the enrichment broth for up to seven days

The key parameters currently utilised during testing for *E. coli* O157 by Australian beef producers therefore appear to have minimal negative effect on the likelihood of isolating *E. coli* O157 from potential positive enrichment broths. It is more probable that the failure to isolate *E. coli* O157 from potential positive enrichment broths is due to factors such as the sensitivity and specificity of test systems, competition and inhibition of microflora during the isolation phase, and/or combinations of genetic targets within enrichment broths that incorrectly suggest the presence of *E. coli* O157. Increasing our understanding of these factors will assist in identifying ways of increasing the conversion of potential positives to confirmed positives.

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Introduction

Meat destined for export to the US and other countries must be tested and deemed free of *E. coli* O157 prior to being released into commerce. DAFF coordinates a testing program within Australia aimed at limiting the release of contaminated meat into export markets. Despite this, there has been a recent increase in the rejection rate of Australian beef from the US as a result of POE testing. As the US is a major beef trading partner of Australia, the increased rejection rate will reduce the confidence in the Australian beef supply and may lead to a contraction of market share in the US. A greater understanding of how individual components of the various testing regimes used in Australia affect the ability to isolate *E. coli* O157 will enable the identification of factors that result in a low conversion rate of potential positives to presumptive positives.

The current approach for testing for *E. coli* O157 in meat relies on first performing a screening test on an enrichment broth which contains a mixture of bacteria. Samples which test positive for the appropriate targets are typically sent to one of two commercial laboratories for confirmation. DAFF currently approves a number of methods for *E. coli* O157 testing and there is increasing concern that testing regimes may be impacting on the likelihood of isolating the target organism if it is present in the test sample. More specifically, it is hypothesised that the type of enrichment broth used, time between screening and the commencement of confirmation, and storage temperatures of broths prior to confirmation may affect the ability to isolate the target organism. With Australia having just two laboratories approved for confirmation testing of *E. coli* O157 distances between onsite laboratories conducting screening tests and the confirmation labs can be substantial (>1000 km). It's plausible to suggest that the transport of potential positives from screening laboratories to confirming laboratories may be a critical factor affecting the isolation of *E. coli* O157.

A greater understanding of the issues associated with failure to isolate *E. coli* O157 from positive screening tests will provide processors with information that can be used to:

- Determine if transport (time and temperature) impacts the recovery of *E. coli* O157
- Provide guidance on the most appropriate and efficient testing and transport strategy for potential positive samples to confirmation laboratories
- Continue to have a favourable trading position in international markets.

Materials and Methods

Bacterial strains

Three *E. coli* O157 strains (EC2422, EC581 and EC 2823a) from the CSIRO culture collection were used in the study. Each strain was recovered from frozen storage and sequentially passaged on Luria-Bertani (LB) agar (Oxoid) containing rifampicin (Sigma) until growth at a concentration of 100 µg/mL was achieved. Each strain was maintained on LB agar with rifampicin (100 µg/mL) at 4°C.

Inoculum preparation

A cocktail preparation consisting of the three *E. coli* O157 strains was prepared by growing each of the strains in 50 mL of LB broth containing rifampicin (100 µg/mL) at 37°C for 18-24 h. Serial dilutions of each enrichment were prepared using LB broth and then combined to form the high (100-1000 CFU/mL) or low (10-100 CFU/mL) inoculum. One mL of the appropriate inoculum was added to each test sample prior to enrichment.

Survey design

All laboratories that are approved by DAFF for routine testing of *E. coli* O157 were asked to complete a questionnaire about their O157 testing regime. A total of 15 abattoirs completed and returned the questionnaire and the information provided was collated in order to identify the key time and temperature parameters associated with *E. coli* O157 testing and confirmation. The questionnaire, identification of key parameters and survey design were tabled in the Milestone 2 report for A.MIS.1005. A copy of the report is presented in Appendix 1.

Sample preparation and storage

As per the survey design, three enrichment broths, three sample types and two incubation times were used in this study. Enrichment broths included modified tryptone soya broth plus novobiocin (8 mg/L), mEHEC (BioControl) and *E. coli* O157:H7 MP (Oxoid). Sample types were pure culture (enrichment broth only), low background (beef trim) or high background (ground beef). All samples were incubated at 42±1°C for 12 or 24 h. Samples were prepared as per the FSIS laboratory guidebook MLG 5.06 (http://www.fsis.usda.gov/PDF/MLG_5_06.pdf) with 325 ± 32.5 g product with 975 ± 19.5 mL enrichment broth used for each test sample. Following enrichment, three 50 mL aliquots of each enrichment broth were transferred to sterile 120 mL jars. One jar from each enrichment were then stored at 4, 10 or 20°C for seven days.

Sample testing

Immediately following enrichment and each day during storage, all samples were tested for the presence and quantity of *E. coli* O157 in each broth using immunomagnetic separation (as per MLG 5.06) and by direct plating onto Rainbow agar (RBA; Biolog), sorbitol MacConkey agar supplemented with cefixime and tellurite (CT-SMAC; Oxoid) and LB agar supplemented with rifampicin (100 µg/mL). All agar plates were incubated at 37°C for 20-24 h after which they were assessed for the presence

of *E. coli* O157 using an *E. coli* O157 latex agglutination kit (Oxoid). Samples were deemed positive for *E. coli* O157 if a positive agglutination result was observed.

Enrichment temperature monitoring

The rate at which enrichment broths return to 42±1°C following the addition of a beef trim sample was measured using a Gemini data logger with stab probe. The stab probes were placed into beef trim immediately prior to incubation and the temperature recorded at two minute intervals.

Results

The counts of *E. coli* O157 in high background, low background and pure culture samples grown in three enrichment broths and stored for seven days at 4, 10 and 20°C are shown in Appendix 2. The storage trial was conducted twice with samples 1-18 (a-c) representing Trial 1 samples and samples 19-36 (a-c) representing Trial 2. Inoculation levels were 42 (low background and pure culture samples) and 421 (high background) CFU/mL for Trial 1 samples and 27 (low background and pure culture samples) and 294 (high background) CFU/mL for Trial 2 samples. Background microflora counts for high background samples were 6.69 and 6.76 $\log_{10}$CFU/mL for Trial 1 and Trial 2 samples respectively. The background microflora counts for low background samples were 3.04 and 4.35 $\log_{10}$CFU/mL for Trial 1 and Trial 2 samples respectively. Following enrichment, counts of *E. coli* O157 were highest in pure culture samples with an average of 7.70 $\log_{10}$CFU/mL. Low background samples were the next highest with an average *E. coli* O157 count of 6.31 $\log_{10}$CFU/mL followed by high background samples with an average of 4.55 $\log_{10}$CFU/mL. Counts of *E. coli* O157 following enrichment ranged from 3.52 to 8.46 $\log_{10}$CFU/mL and all samples were positive for *E. coli* O157 at Day 0 regardless of enrichment media, incubation time or IMS plating media used.

Effect of sample type

Three samples types were evaluated in this study to determine if background microflora counts affect the survival or isolation of *E. coli* O157. Figure 1 shows the counts of *E. coli* O157 in high background, low background and pure culture samples stored at 4, 10 and 20°C. There was minimal variation (maximum 0.59 $\log_{10}$CFU/mL) in the counts of *E. coli* O157 in high background samples stored at 4 and 10°C. Reductions in counts of *E.coli* O157 were greatest in high background samples stored at 20°C with reductions of up to 2.70 $\log_{10}$CFU/mL observed. However, the average reduction for high background samples stored at 20°C was 1.03 $\log_{10}$CFU/mL. Counts of *E. coli* O157 in low background samples were highly stable regardless of storage temperature or enrichment broth with an average reduction of 0.30 $\log_{10}$CFU/mL observed across all low background samples. Counts of *E. coli* O157 were most variable in pure culture samples with reductions of up to 4.66 $\log_{10}$CFU/mL observed. The type of enrichment broth used appeared to effect the survival of *E. coli* O157 in pure culture enrichments with average reductions of 2.63, 2.03 and 0.46 $\log_{10}$CFU/mL observed in mTSB+n, mEHEC and O157:H7 MP broths respectively when stored at 4°C. Survival of *E. coli* O157 (pure culture samples) was significantly greater in O157:H7 MP than in mTSB+n or mEHEC broths when stored at 4°C.

Effect of enrichment broth

Three types of enrichment broth were assessed to determine if they effect the survival of *E. coli* O157. Figure 2 shows the counts of *E. coli* O157 stored for 7 days after growth in O157:H7 MP, mEHEC or mTSB+n broth. The average variation of *E. coli* O157 counts in any of the broths ranged from 0.13 to 1.06 $\log_{10}$CFU/mL regardless of sample type, incubation time or storage temperature. Reductions in *E. coli* O157 counts of >2.0 $\log_{10}$CFU/mL were observed on seven (6.5%) occasions. Five of these occasions were observed in pure culture samples with the remaining two observed in high background samples. Eighty-eight (81.5%) samples had decreases in *E. coli* O157 count of <1.0 $\log_{10}$CFU/mL. Survival of *E. coli* O157 was greater in O157:H7 MP than the other two enrichment broths although the difference was not significant across all sample types.

Isolation of *E. coli* O157

IMS was used to try and isolate *E. coli* O157 from all high and low background samples. A list of samples from which *E. coli* O157 was not isolated on both rainbow agar and CT-SMAC is presented in Table 1. Failure to isolate *E. coli* O157 on both agars occurred on just one occasion for each set of samples stored at 4°C or 10°C (samples 12a and 31b respectively). However, *E. coli* O157 was isolated using CT-SMAC from samples 12a and 31b but could not be isolated using rainbow agar. As commercial laboratories within Australia currently use both rainbow agar and CT-SMAC *E. coli* O157 would have been isolated from all samples stored at 4°C and 10°C regardless of storage time. In contrast to samples stored at 4°C or 10°C, isolation of *E. coli* O157 from samples stored at 20°C was problematic. Ten out of 24 samples stored at 20°C had at least one occasion where *E. coli* O157 could not be isolated on both agars. Five of the 10 samples had at least one occasion where *E. coli* O157 was not isolated using either agar. High background samples were most likely to fail to yield an isolate (4 out of 5) with only one low background sample unable to have *E. coli* O157 recovered from it.

Enrichment temperature monitoring

The rate at which enrichment broths return to the prescribed 42±1°C enrichment temperature following the addition of chilled samples is shown in Figure 3. The average time taken for the four enrichments to reach 42±1°C was 306 minutes with a range of 282 to 320 minutes. Although this exceeds the currently accepted four hour warm up timeframe, it should be noted that all enrichments were at 40.2°C or 40.6°C after four hours.

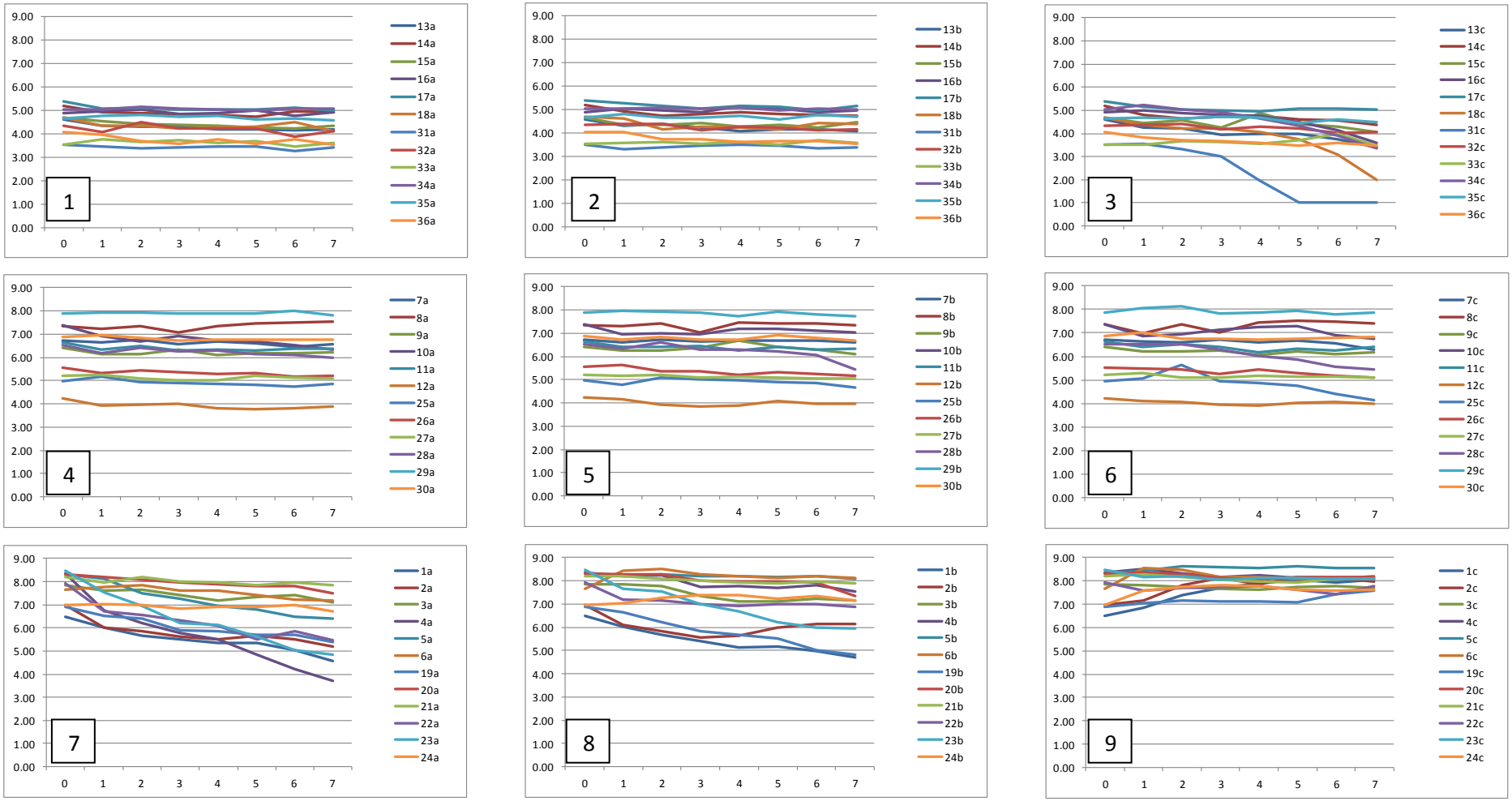

Figure 1. Counts of *E. coli* O157 in high background, low background or pure culture at 4, 10 and 20°C. All counts are $\log_{10}$CFU/mL (y axis) and are displayed for 7 days of storage (x axis). Charts 1-3 are high background samples stored at 4, 10 and 20°C respectively. Charts 4-6 are low background samples stored at 4, 10 and 20°C respectively. Charts 7-9 are pure culture samples stored at 4, 10 and 20°C respectively.

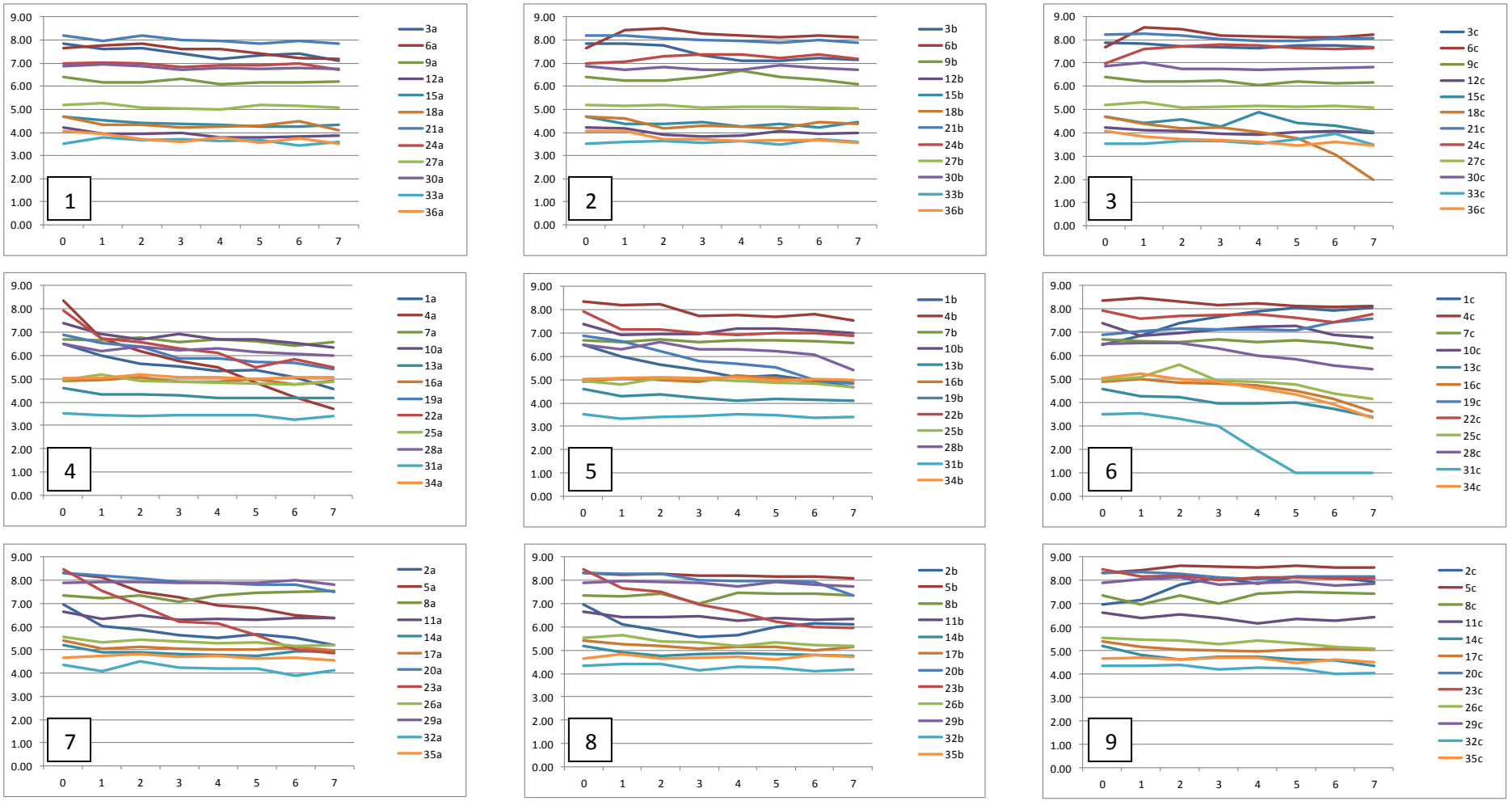

Figure 2. Counts of *E. coli* O157 in O157:H7 MP (Bax), mTSB+n (FSIS) and mEHEC (BioControl) at 4, 10 and 20°C. All counts are $\log_{10}$CFU/mL (y axis) and are displayed for 7 days of storage (x axis). Charts 1-3 are O157:H7 MP samples stored at 4, 10 and 20°C respectively. Charts 4-6 are mTSB+n samples stored at 4, 10 and 20°C respectively. Charts 7-9 are mEHEC samples stored at 4, 10 and 20°C respectively.

Table 1. List of samples from which *E. coli* O157 was not isolated on both rainbow agar and CT-SMAC agar

IMS Failures	Storage temp (°C)	Day	Count*	Detected on
12a	4	7	3.87	CT-SMAC
12c	20	1	4.12	CT-SMAC
12c	20	6	4.07	None
13c	20	2	4.23	None
13c	20	3	3.95	None
13c	20	4	3.97	None
13c	20	5	4.00	CT-SMAC
13c	20	6	3.73	None
14c	20	7	4.36	CT-SMAC
15c	20	6	4.31	CT-SMAC
15c	20	7	4.05	CT-SMAC
16c	20	5	4.49	CT-SMAC
16c	20	6	4.15	CT-SMAC
16c	20	7	3.61	None
17c	20	7	5.05	None
18c	20	1	4.39	CT-SMAC
18c	20	2	4.20	None
18c	20	3	4.23	None
18c	20	4	4.04	CT-SMAC
18c	20	6	3.08	None
18c	20	7	2.00	None
31b	10	6	3.36	CT-SMAC
31c	20	3	3.00	CT-SMAC
31c	20	4	1.95	CT-SMAC
31c	20	5	<1.00	None
31c	20	6	<1.00	None
31c	20	7	<1.00	None
33c	20	7	3.48	CT-SMAC
34c	20	4	4.63	CT-SMAC
34c	20	5	4.34	CT-SMAC
34c	20	6	3.92	CT-SMAC
34c	20	7	3.34	CT-SMAC

* all counts are $\log_{10}$CFU/mL

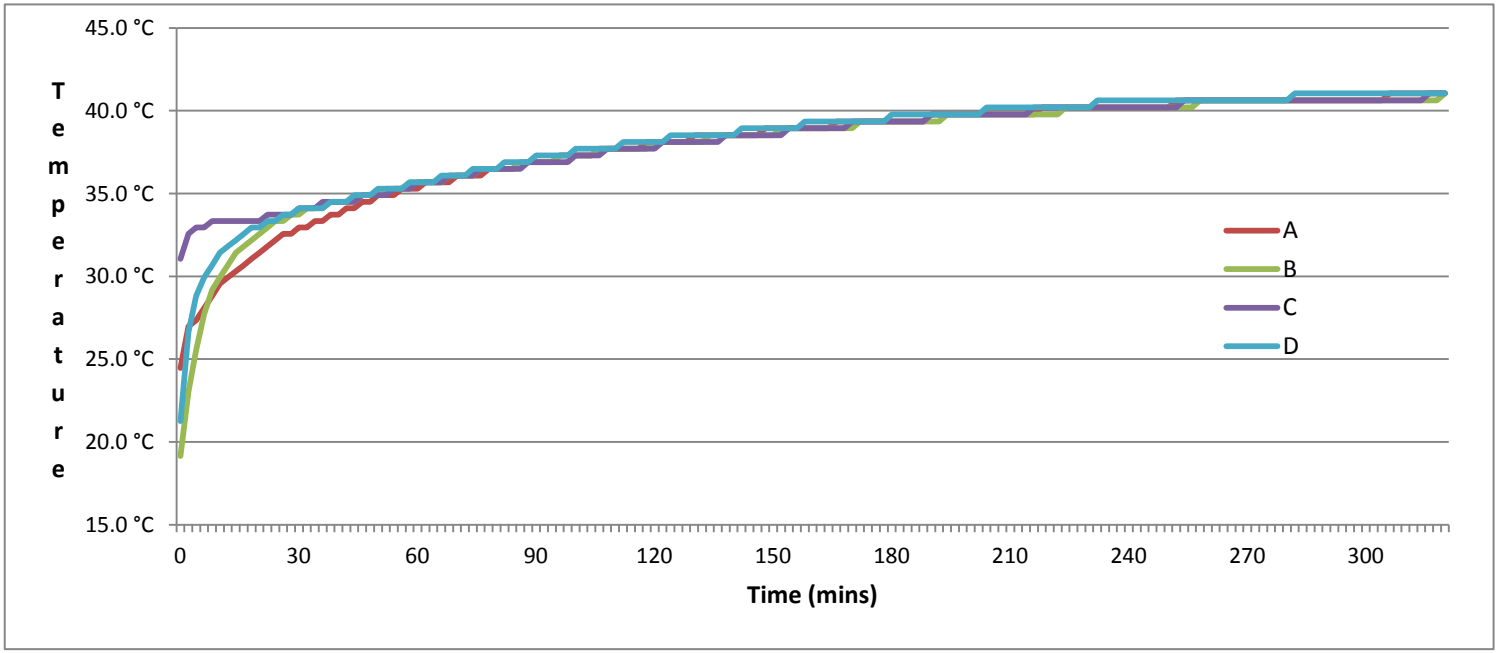

Figure 3. Rate of return of enrichment broth to the prescribed 42°C enrichment temperature

Conclusions

This study was designed to determine if key parameters associated with the testing of *E. coli* O157 are detrimental to the likelihood of isolating *E. coli* O157 from potentially positive samples. The findings of this study indicate that sample type, enrichment broth type and length of incubation are unlikely to negatively affect the likelihood of isolation. Similarly, storage of the enrichment broths for up to seven days at either 4°C or 10°C will have minimal impact on the likelihood of isolating *E. coli* O157. Storage of enrichment broths at 20°C will reduce the likelihood of isolating *E. coli* O157. However, the effect is more pronounced in enriched samples that start with high background microflora counts as opposed to low background samples. It is plausible to suggest that in samples with low background microflora counts such as manufacturing beef samples, the overall effect of elevated storage temperatures across a seven day period is minimal.

The results of the study indicate that the isolation of *E. coli* O157 from a potential positive enrichment broth stored at 4°C or 10°C is not significantly affected by:

- The type of enrichment broth used
- The concentration of background microflora in the sample
- The time for which the sample is enriched
- Storage of the enrichment broth for up to seven days

The key parameters currently utilised during testing for *E. coli* O157 by Australian beef producers therefore appear to have minimal negative effect on the likelihood of isolating *E. coli* O157 from potential positive enrichment broths. It is more probable that the failure to isolate *E. coli* O157 from potential positive enrichment broths is due to factors such as the sensitivity and specificity of test systems, competition and inhibition of microflora during the isolation phase, and/or combinations of genetic targets within enrichment broths that incorrectly suggest the presence of *E. coli* O157. Increasing our understanding of these factors will assist in identifying ways of increasing the conversion of potential positives to confirmed positives.

Appendix 1: Milestone 2 – Report to MLA – Identify key parameters of current *E. coli* O157 testing regimes. Select key criteria for use in growth and survival studies through consultation with DAFF and MLA

Introduction

Current testing for *E. coli* O157 in the meat industry requires a stepwise approach which involves initial screening of an enrichment broth followed by a process of confirmation for isolating *E. coli* O157. Many abattoir-based laboratories use commercially available testing systems to conduct their own screening tests for *E. coli* O157 and if a positive test is detected (potential positive) the sample is sent to a DAFF registered laboratory for confirmation. The potential positive tests therefore need to be transported to the DAFF registered laboratory and it is not clear if the conditions under which these samples are transported are having an effect on the ability to isolate and confirm *E. coli* O157. It is possible that the failure to confirm an isolate may lead to contaminated meat leaving Australia for export markets and could result in a positive point of entry (POE) detection in the USA. Several POE detections have already occurred and the current Australian red meat industry process of testing and confirming *E. coli* O157 has been questioned by the US Food Safety and Inspection Service who operate the POE testing program. It is therefore important to determine if the types of enrichment broths used, the time between screening and confirmation, and the temperatures that enriched broths are exposed to prior to confirmation are affecting isolation of *E. coli* O157 from potential positive samples.

Milestone 2 requires the identification of the key parameters associated with *E. coli* O157 testing and the design of growth and survival studies to measure their impact. The key parameters and experimental design are described below.

Identification of key parameters

All laboratories that are approved by DAFF for routine testing of *E. coli* O157 were asked to complete a questionnaire (Appendix 1) about their O157 testing regime. A total of 15 abattoirs completed and returned the questionnaire. Below are the key findings:

Enrichment broths

All respondents use one of three enrichments broths: modified tryptone soya broth with novobiocin (47%), mEHEC (33%) or *E. coli* O157:H7 MP medium (20%).

Pre-warming

Twelve of the 15 respondents pre-warm the media prior to use. The remaining three respondents use media at ambient temperature. Pre-warming times range from 30 minutes to 12 hours with most respondents claiming to have verified their approach.

Enrichment times and temperature

Irrespective of enrichment media or temperature (36°C or 42°C) used, broths are generally enriched for >15 hours. Laboratories using 36°C as an enrichment temperature (40%) tend to enrich for 18-24 hours as opposed to laboratories using 42°C where enrichment time is generally restricted to 15 hours.

Time prior to testing

The majority of laboratories test their enrichment broths for *E. coli* O157 within one hour of the completion of enrichment. Two laboratories reported that the maximum time between enrichment and testing can be 48 hours. In these situations the broths are stored at refrigeration temperatures until testing.

Test system

BioControl VIP is the most commonly used test systems. Bax, Rapidcheck and Reveal are also used.

Transport

All respondents said they packed and transported potential positive samples to the confirming laboratory in sealed insulated containers with ice in order to ensure that the samples arrives with a temperature of <7°C. Data from the confirming laboratory confirms that 95% of broths meet the <7°C specification with 86% arriving at temperatures below 4°C.

Twelve of 15 respondents can receive notification from the confirming lab within 24 hours of screening that their sample has been received. The remaining 3 plants can receive notification within 48 hours. Seven of 15 plants will have confirmation commenced within 24h of screening. Four plants can have >3 days between screening and the commencement of confirmation and one plant reported that in certain circumstances there may be up to 6 days between screening and the commencement of confirmation.

Confirmed samples

Only four of the 15 respondents had any of their last 10 potential positives samples confirmed as actually containing an *E. coli* O157 isolate. In total, just nine out of the previous 141 potential positives sent for confirmation had an *E. coli* O157 isolate recovered.

Selection of key criteria & experimental design

The results of the questionnaire were used to identify key criteria that are to be used in growth and survival experiments relating to *E. coli* O157 confirmation. The selected criteria and experimental methodology are described below. The criteria and methodology have been reviewed by representatives of MLA and DAFF.

Enrichments broths

Modified tryptone soya broth plus novobiocin (8 mg/L), mEHEC (BioControl) and *E. coli* O157:H7 MP (Oxoid) will be used.

Bacterial strains

A cocktail of three rifampicin resistant O157 strains will be used to inoculate all samples. The use of rifampicin resistant strains will allow detection of O157 strains at concentrations below the limit of detection of IMS. Growth rates of the wild-type O157 and the rifampicin resistant O157 will be measured to ensure that similar growth patterns occur in each of the broths to be used in the trial.

Sample types

- **Pure** - Pure culture – enrichment broth and O157 strains only
- **LB** - Low background + inoculum (rif resistant O157)– beef trim with O157 inoculated at 10-100 CFU/enrichment
- **HB** - High background + inoculum (rif resistant O157) – ground beef with O157 inoculated at 100-1000 CFU/enrichment
- Appropriate uninoculated controls will be included

Incubation times and temperatures

All broths will be pre-warmed to 42°C prior to the addition of sample or inoculum. Samples will be incubated for either 12 or 24 hours at 42°C. Temperatures of the enrichment broths will be monitored post sample addition until the completion of the scheduled enrichment time.

Storage and testing approach

Following enrichment each sample will be tested for the presence and quantity of *E. coli* O157 in each broth using immunomagnetic separation (as per MLG 5.06) and by direct plating onto selective agar containing rifampicin. Aliquots of each sample will then be stored in sealed plastic containers at 4, 10 or 20°C for seven days. Each aliquot will be tested for presence and quantity of *E. coli* O157 daily during the storage period.

Samples and experimental approach

Table 1 outlines the sample parameters and combinations that will be evaluated. The experiment will conducted in duplicate with the first set of experiments to be conducted 19th to 27th June and the second set of experiments to be conducted 10-18th July. Minor modifications to the experimental design may be made prior to the second set of experiments following a review of the initial results by CSIRO, MLA and DAFF.

Table 1. Samples required for O157 testing regime experiments

Sample	Sub sample	Broth	Sample type	Incubation	Storage temp
1	a	FSIS	Pure	42°C for 24h	4
1	b	FSIS	Pure	42°C for 24h	10
1	c	FSIS	Pure	42°C for 24h	20
2	a	mEHEc	Pure	42°C for 24h	4
2	b	mEHEc	Pure	42°C for 24h	10
2	c	mEHEc	Pure	42°C for 24h	20
3	a	Bax	Pure	42°C for 24h	4
3	b	Bax	Pure	42°C for 24h	10
3	c	Bax	Pure	42°C for 24h	20
4	a	FSIS	Pure	42°C for 12h	4
4	b	FSIS	Pure	42°C for 12h	10
4	c	FSIS	Pure	42°C for 12h	20
5	a	mEHEc	Pure	42°C for 12h	4
5	b	mEHEc	Pure	42°C for 12h	10
5	c	mEHEc	Pure	42°C for 12h	20
6	a	Bax	Pure	42°C for 12h	4
6	b	Bax	Pure	42°C for 12h	10
6	c	Bax	Pure	42°C for 12h	20
7	a	FSIS	LB	42°C for 24h	4
7	b	FSIS	LB	42°C for 24h	10
7	c	FSIS	LB	42°C for 24h	20
8	a	mEHEc	LB	42°C for 24h	4
8	b	mEHEc	LB	42°C for 24h	10
8	c	mEHEc	LB	42°C for 24h	20
9	a	Bax	LB	42°C for 24h	4
9	b	Bax	LB	42°C for 24h	10
9	c	Bax	LB	42°C for 24h	20
10	a	FSIS	LB	42°C for 12h	4
10	b	FSIS	LB	42°C for 12h	10
10	c	FSIS	LB	42°C for 12h	20
11	a	mEHEc	LB	42°C for 12h	4
11	b	mEHEc	LB	42°C for 12h	10
11	c	mEHEc	LB	42°C for 12h	20
12	a	Bax	LB	42°C for 12h	4
12	b	Bax	LB	42°C for 12h	10
12	c	Bax	LB	42°C for 12h	20
13	a	FSIS	HB	42°C for 24h	4
13	b	FSIS	HB	42°C for 24h	10
13	c	FSIS	HB	42°C for 24h	20
14	a	mEHEc	HB	42°C for 24h	4
14	b	mEHEc	HB	42°C for 24h	10
14	c	mEHEc	HB	42°C for 24h	20

15	a	Bax	HB	42°C for 24h	4
15	b	Bax	HB	42°C for 24h	10
15	c	Bax	HB	42°C for 24h	20
16	a	FSIS	HB	42°C for 12h	4
16	b	FSIS	HB	42°C for 12h	10
16	c	FSIS	HB	42°C for 12h	20
17	a	mEHEc	HB	42°C for 12h	4
17	b	mEHEc	HB	42°C for 12h	10
17	c	mEHEc	HB	42°C for 12h	20
18	a	Bax	HB	42°C for 12h	4
18	b	Bax	HB	42°C for 12h	10
18	c	Bax	HB	42°C for 12h	20

E. coli O157 testing regime questionnaire

Questionnaire – O157 testing regimes

Company name:.....

Contact person:.....

Contact details:.....

Email:.....

Phone:.....

Enrichment

1. What is the name of the enrichment broth you use for O157 testing?
   .....
2. Who is the manufacturer or supplier of the enrichment broth?
   .....
3. Do you pre-warm your enrichment broth prior to the addition of the meat sample?
   a. No ☐
   b. Yes ☐
      i. If so, what temperature do you pre-warm to?.....
      ii. How long do you pre-warm for?.....
      iii. How do you confirm the broth is at the required temperature?.....
      .....
4. What are the enrichment times and temperatures used for O157 testing in your laboratory?.....
   .....
5. Following enrichment, what is the maximum amount of time that elapses prior to a screening test being conducted?
   .....
   .....
6. If the enrichment is not tested immediately after incubation, how do you store (i.e temperature) the enrichment broth prior to screening?
   .....
   .....

Screening test

7. Which *E. coli* O157 screening test system do you use?.....

Confirmation

8. When sending a potential positive for confirmation, what is?
    a. The minimum time between the screening test and collection by the courier or transport company? .....
    b. The maximum time between the screening test and collection by the courier or transport company?.....

9. When sending a potential positive for confirmation, what is?
    a. The minimum time between the screening result and notification from the confirming lab that your sample has been received?.....
    b. The maximum time between the screening result and notification from the confirming lab that your sample has been received?.....
    c.
10. Please detail how you pack and transport your samples to the confirmation lab?
    .....
    .....
    .....

Confirmation success

11. Of the last 10 potential positive samples sent for confirmation how many were confirmed as positive for *E. coli* O157:H7? .....

Additional notes and comments: Please feel free to add any additional information that you feel might impact on your *E. coli* O157 testing regime.

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Appendix 2. Counts of *E. coli* O157 in high background, low background and pure culture samples grown in three enrichment broths and stored for 7 days at 4, 10 and 20°C

Table 2. Counts of *E. coli* O157 in high background, low background and pure culture samples grown in three enrichment broths and stored for 7 days at 4, 10 and 20°C

					Storage time (days)							
Sample	Broth	Sample type	Incubation	Storage temp	0	1	2	3	4	5	6	7
1a	FSIS	Pure	42°C for 24h	4	6.49	6.01	5.64	5.52	5.34	5.36	5.05	4.57
1b	FSIS	Pure	42°C for 24h	10	6.49	6.01	5.66	5.40	5.11	5.18	4.96	4.69
1c	FSIS	Pure	42°C for 24h	20	6.49	6.86	7.38	7.68	7.88	8.05	7.93	8.04
2a	mEHEc	Pure	42°C for 24h	4	6.97	6.01	5.85	5.63	5.52	5.66	5.52	5.20
2b	mEHEc	Pure	42°C for 24h	10	6.97	6.11	5.84	5.56	5.64	5.99	6.15	6.13
2c	mEHEc	Pure	42°C for 24h	20	6.97	7.15	7.81	8.11	7.87	8.18	8.12	7.95
3a	Bax	Pure	42°C for 24h	4	7.86	7.61	7.64	7.43	7.17	7.33	7.40	7.12
3b	Bax	Pure	42°C for 24h	10	7.86	7.85	7.75	7.34	7.10	7.11	7.22	7.14
3c	Bax	Pure	42°C for 24h	20	7.86	7.83	7.72	7.66	7.62	7.74	7.76	7.69
4a	FSIS	Pure	42°C for 12h	4	8.36	6.75	6.20	5.76	5.49	4.83	4.20	3.70
4b	FSIS	Pure	42°C for 12h	10	8.36	8.21	8.24	7.72	7.76	7.68	7.80	7.53
4c	FSIS	Pure	42°C for 12h	20	8.36	8.49	8.30	8.17	8.23	8.13	8.10	8.13
5a	mEHEc	Pure	42°C for 12h	4	8.30	8.12	7.49	7.26	6.93	6.80	6.49	6.39
5b	mEHEc	Pure	42°C for 12h	10	8.30	8.24	8.26	8.21	8.19	8.15	8.18	8.07
5c	mEHEc	Pure	42°C for 12h	20	8.30	8.45	8.62	8.60	8.56	8.62	8.55	8.55
6a	Bax	Pure	42°C for 12h	4	7.66	7.76	7.83	7.59	7.62	7.43	7.21	7.16
6b	Bax	Pure	42°C for 12h	10	7.66	8.43	8.51	8.28	8.19	8.10	8.19	8.10
6c	Bax	Pure	42°C for 12h	20	7.66	8.54	8.47	8.18	8.16	8.09	8.10	8.21
7a	FSIS	LB	42°C for 24h	4	6.71	6.64	6.76	6.56	6.70	6.60	6.44	6.57
7b	FSIS	LB	42°C for 24h	10	6.71	6.61	6.72	6.63	6.68	6.70	6.67	6.59

7c	FSIS	LB	42°C for 24h	20	6.71	6.62	6.59	6.72	6.58	6.66	6.54	6.30
8a	mEHEc	LB	42°C for 24h	4	7.36	7.22	7.35	7.08	7.33	7.45	7.51	7.52
8b	mEHEc	LB	42°C for 24h	10	7.36	7.30	7.41	7.02	7.45	7.43	7.42	7.33
8c	mEHEc	LB	42°C for 24h	20	7.36	6.98	7.37	7.02	7.43	7.52	7.49	7.42
9a	Bax	LB	42°C for 24h	4	6.40	6.15	6.15	6.34	6.09	6.16	6.18	6.21
9b	Bax	LB	42°C for 24h	10	6.40	6.26	6.26	6.39	6.68	6.40	6.28	6.09
9c	Bax	LB	42°C for 24h	20	6.40	6.20	6.21	6.26	6.06	6.21	6.11	6.17
10a	FSIS	LB	42°C for 12h	4	7.38	6.92	6.68	6.92	6.71	6.69	6.53	6.35
10b	FSIS	LB	42°C for 12h	10	7.38	6.94	6.98	6.95	7.19	7.18	7.11	7.02
10c	FSIS	LB	42°C for 12h	20	7.38	6.87	6.96	7.13	7.25	7.27	6.90	6.76
11a	mEHEc	LB	42°C for 12h	4	6.64	6.32	6.49	6.30	6.33	6.31	6.39	6.39
11b	mEHEc	LB	42°C for 12h	10	6.64	6.41	6.41	6.46	6.27	6.40	6.31	6.34
11c	mEHEc	LB	42°C for 12h	20	6.64	6.41	6.53	6.39	6.16	6.34	6.27	6.42
12a	Bax	LB	42°C for 12h	4	4.23	3.93	3.96	4.00	3.79	3.77	3.81	3.87
12b	Bax	LB	42°C for 12h	10	4.23	4.16	3.91	3.83	3.88	4.07	3.95	3.97
12c	Bax	LB	42°C for 12h	20	4.23	4.12	4.08	3.94	3.92	4.02	4.07	3.99
13a	FSIS	HB	42°C for 24h	4	4.60	4.34	4.32	4.30	4.19	4.18	4.16	4.19
13b	FSIS	HB	42°C for 24h	10	4.60	4.30	4.38	4.23	4.10	4.16	4.15	4.10
13c	FSIS	HB	42°C for 24h	20	4.60	4.26	4.23	3.95	3.97	4.00	3.73	3.38
14a	mEHEc	HB	42°C for 24h	4	5.20	4.91	4.89	4.83	4.79	4.74	4.94	4.93
14b	mEHEc	HB	42°C for 24h	10	5.20	4.92	4.75	4.82	4.89	4.83	4.79	4.74
14c	mEHEc	HB	42°C for 24h	20	5.20	4.81	4.64	4.72	4.75	4.62	4.57	4.36
15a	Bax	HB	42°C for 24h	4	4.68	4.53	4.41	4.38	4.33	4.26	4.24	4.34
15b	Bax	HB	42°C for 24h	10	4.68	4.36	4.36	4.43	4.27	4.36	4.22	4.45
15c	Bax	HB	42°C for 24h	20	4.68	4.43	4.57	4.26	4.87	4.41	4.31	4.05
16a	FSIS	HB	42°C for 12h	4	4.90	4.96	5.05	4.86	4.88	4.98	4.75	4.93
16b	FSIS	HB	42°C for 12h	10	4.90	5.05	4.98	4.91	5.17	5.05	4.90	4.97
16c	FSIS	HB	42°C for 12h	20	4.90	5.01	4.87	4.81	4.75	4.49	4.15	3.61
17a	mEHEc	HB	42°C for 12h	4	5.40	5.06	5.13	5.05	5.03	5.03	5.12	4.99
17b	mEHEc	HB	42°C for 12h	10	5.40	5.27	5.17	5.06	5.16	5.13	4.98	5.15

17c	mEHEc	HB	42°C for 12h	20	5.40	5.15	5.03	5.01	4.96	5.06	5.09	5.05
18a	Bax	HB	42°C for 12h	4	4.70	4.35	4.34	4.23	4.25	4.30	4.48	4.11
18b	Bax	HB	42°C for 12h	10	4.70	4.61	4.18	4.28	4.25	4.17	4.43	4.39
18c	Bax	HB	42°C for 12h	20	4.70	4.39	4.20	4.23	4.04	3.76	3.08	2.00
19a	FSIS	Pure	42°C for 24h	4	6.89	6.52	6.38	5.88	5.87	5.71	5.69	5.40
19b	FSIS	Pure	42°C for 24h	10	6.89	6.66	6.23	5.81	5.67	5.53	5.01	4.83
19c	FSIS	Pure	42°C for 24h	20	6.89	7.04	7.15	7.12	7.11	7.09	7.43	7.57
20a	mEHEc	Pure	42°C for 24h	4	8.32	8.19	8.07	7.94	7.88	7.79	7.79	7.48
20b	mEHEc	Pure	42°C for 24h	10	8.32	8.26	8.26	7.99	7.98	7.95	7.93	7.34
20c	mEHEc	Pure	42°C for 24h	20	8.32	8.34	8.27	8.13	8.05	8.18	8.18	8.18
21a	Bax	Pure	42°C for 24h	4	8.20	7.97	8.21	7.98	7.96	7.86	7.94	7.86
21b	Bax	Pure	42°C for 24h	10	8.20	8.21	8.08	8.01	7.94	7.89	7.98	7.90
21c	Bax	Pure	42°C for 24h	20	8.20	8.27	8.17	8.03	7.95	7.94	8.08	8.08
22a	FSIS	Pure	42°C for 12h	4	7.93	6.72	6.56	6.31	6.10	5.49	5.86	5.48
22b	FSIS	Pure	42°C for 12h	10	7.93	7.17	7.14	6.99	6.92	6.99	7.00	6.87
22c	FSIS	Pure	42°C for 12h	20	7.93	7.59	7.69	7.74	7.79	7.62	7.44	7.77
23a	mEHEc	Pure	42°C for 12h	4	8.46	7.52	6.93	6.20	6.14	5.62	5.03	4.85
23b	mEHEc	Pure	42°C for 12h	10	8.46	7.65	7.52	6.98	6.67	6.23	5.98	5.94
23c	mEHEc	Pure	42°C for 12h	20	8.46	8.18	8.19	8.03	8.11	8.12	8.10	8.08
24a	Bax	Pure	42°C for 12h	4	6.97	7.04	6.98	6.82	6.89	6.92	6.97	6.72
24b	Bax	Pure	42°C for 12h	10	6.97	7.05	7.28	7.38	7.38	7.22	7.36	7.16
24c	Bax	Pure	42°C for 12h	20	6.97	7.58	7.72	7.81	7.76	7.62	7.58	7.62
25a	FSIS	LB	42°C for 24h	4	4.96	5.18	4.93	4.89	4.85	4.81	4.76	4.86
25b	FSIS	LB	42°C for 24h	10	4.96	4.78	5.08	5.02	4.96	4.88	4.84	4.67
25c	FSIS	LB	42°C for 24h	20	4.96	5.08	5.64	4.93	4.89	4.76	4.40	4.16
26a	mEHEc	LB	42°C for 24h	4	5.54	5.32	5.45	5.35	5.29	5.32	5.15	5.19
26b	mEHEc	LB	42°C for 24h	10	5.54	5.64	5.36	5.35	5.19	5.32	5.24	5.18
26c	mEHEc	LB	42°C for 24h	20	5.54	5.48	5.43	5.26	5.45	5.30	5.16	5.09
27a	Bax	LB	42°C for 24h	4	5.20	5.26	5.09	5.03	5.00	5.19	5.14	5.09
27b	Bax	LB	42°C for 24h	10	5.20	5.15	5.20	5.09	5.11	5.10	5.06	5.05

27c	Bax	LB	42°C for 24h	20	5.20	5.30	5.09	5.12	5.16	5.13	5.15	5.10
28a	FSIS	LB	42°C for 12h	4	6.51	6.18	6.40	6.24	6.31	6.15	6.09	6.00
28b	FSIS	LB	42°C for 12h	10	6.51	6.32	6.60	6.31	6.29	6.21	6.06	5.43
28c	FSIS	LB	42°C for 12h	20	6.51	6.53	6.53	6.30	6.01	5.85	5.57	5.43
29a	mEHEc	LB	42°C for 12h	4	7.88	7.91	7.91	7.90	7.88	7.87	7.99	7.80
29b	mEHEc	LB	42°C for 12h	10	7.88	7.96	7.93	7.89	7.74	7.91	7.80	7.74
29c	mEHEc	LB	42°C for 12h	20	7.88	8.04	8.13	7.82	7.88	7.94	7.79	7.87
30a	Bax	LB	42°C for 12h	4	6.86	6.95	6.85	6.72	6.77	6.76	6.77	6.75
30b	Bax	LB	42°C for 12h	10	6.86	6.72	6.82	6.72	6.72	6.92	6.79	6.70
30c	Bax	LB	42°C for 12h	20	6.86	7.00	6.76	6.76	6.72	6.75	6.79	6.84
31a	FSIS	HB	42°C for 24h	4	3.52	3.45	3.40	3.43	3.46	3.46	3.26	3.41
31b	FSIS	HB	42°C for 24h	10	3.52	3.32	3.40	3.45	3.51	3.48	3.36	3.40
31c	FSIS	HB	42°C for 24h	20	3.52	3.54	3.31	3.00	1.95	1.00	1.00	1.00
32a	mEHEc	HB	42°C for 24h	4	4.34	4.08	4.49	4.23	4.21	4.21	3.87	4.12
32b	mEHEc	HB	42°C for 24h	10	4.34	4.40	4.41	4.13	4.28	4.24	4.11	4.17
32c	mEHEc	HB	42°C for 24h	20	4.34	4.36	4.40	4.19	4.28	4.22	4.01	4.04
33a	Bax	HB	42°C for 24h	4	3.53	3.77	3.65	3.71	3.63	3.68	3.45	3.60
33b	Bax	HB	42°C for 24h	10	3.53	3.58	3.63	3.56	3.63	3.49	3.71	3.60
33c	Bax	HB	42°C for 24h	20	3.53	3.53	3.66	3.63	3.54	3.71	3.97	3.48
34a	FSIS	HB	42°C for 12h	4	5.03	5.04	5.17	5.08	5.05	4.98	5.08	5.06
34b	FSIS	HB	42°C for 12h	10	5.03	5.06	5.10	5.06	5.10	4.96	5.04	4.99
34c	FSIS	HB	42°C for 12h	20	5.03	5.22	5.02	4.91	4.63	4.34	3.92	3.34
35a	mEHEc	HB	42°C for 12h	4	4.66	4.76	4.81	4.72	4.76	4.61	4.67	4.56
35b	mEHEc	HB	42°C for 12h	10	4.66	4.83	4.66	4.67	4.73	4.59	4.79	4.71
35c	mEHEc	HB	42°C for 12h	20	4.66	4.69	4.63	4.71	4.69	4.46	4.61	4.49
36a	Bax	HB	42°C for 12h	4	4.06	3.95	3.70	3.58	3.77	3.57	3.76	3.52
36b	Bax	HB	42°C for 12h	10	4.06	4.04	3.72	3.72	3.64	3.66	3.67	3.54
36c	Bax	HB	42°C for 12h	20	4.06	3.83	3.72	3.68	3.61	3.46	3.61	3.46