VALIDATION REPORT

Validation Title: **Revalidation of screen test kits for *E. coli* O157:H7 - FOLLOW UP EVALUATION**

Validation Report Number: MIC 0910

Report prepared by: Warwick Aspin and Justine Inverarity

AsureQuality Limited
Auckland Laboratory
PO Box 41
Auckland
New Zealand

EXECUTIVE SUMMARY

A new enrichment broth was introduced in the New Zealand red meat surveillance programme for *Escherichia coli* O157:H7 in 2008. Modified TSB with novobiocin and casamino acids (mTSB+NCA) replaced Modified EC (mEC) broth as the enrichment media and the sample size to enrichment broth volume was changed from 125g per 1.125L to 375g per 1L. An evaluation study was conducted by AsureQuality to investigate method performance using the new enrichment protocol and the current approved screen methods, Tecra, VIP, Reveal and BAX MP. The results of this work highlights the difficulty of interpreting weakly positive reactions

A follow-up study was commissioned to further investigate the observations. The three strains of *E. coli* O157:H7 that were found to express weak reactions in the original work were used in the study.

The evaluation did not reproduce the findings of the original study as all samples inoculated with *E. coli* O157:H7 expressed positive reactions in the various screen methods evaluated in this study. However, false negative results were encountered for two veal samples, whereby all screen kits including Tecra, which performed well in the original work, failed to detect *E. coli* O157:H7. The cultural plating method confirmed the presence of *E. coli* O157:H7 at levels of ~$1.0X10^5$ cfu/ml of enrichment broth, however, the presence of high background flora was also noted. It was concluded that the veal matrix originally contained high levels of background microflora which "out competed" the target organisms during enrichment and led to a masking effect in the subsequent screen tests.

Additional work was then conducted to compare the selectivity of the old enrichment media (mEC) against the new media (TSB+NCA). The results obtained from this additional work demonstrate equivalence between the two enrichment media with respect to selectivity and limitations of each formulation to suppress gram negative competitive flora. All samples with high background flora that were spiked with *E. coli* O157, tested negative by all screen kits at every incubation time point. Both media failed to suppress the competitive flora present in the beef samples with high background flora, which is believed to be responsible for the false negative outcome. In contrast, every sample with low background flora that was spiked with *E. coli* O157 returned a positive result for all screen kits at the earliest time point evaluated in this study, 15 hours.

From this data, the limit for detection using these kits under these conditions appears to be in the order of 100 000 - 500 000 cells following enrichment. The onus is therefore on the enrichment broth to propagate to these numbers, which was not always achieved from the veal samples studied, particularly when high background counts were reported. Such deficiencies of enrichment protocols for *E. coli* O157:H7 have been reported previously (Grant 2005; Vimont et al, 2007) and are currently only overcome using more sensitive detection systems (e.g. IMS-PCR).

There is evidence from the New Zealand National Microbial Database microbiological monitoring programme that the level of background flora on veal is higher than on beef, although the bacterial composition of this flora has not been clearly defined. While it is possible differences in background flora occur between New Zealand and overseas countries where kits have previously been validated, evidence in support of this has not been reported.

INTRODUCTION

In late 2008, AsureQuality completed a study designed to verify the performance of four screen test kits for *E. coli* 0157:H7 following method amendments to sample size and enrichment procedures in the NZFSA *E. coli* O157:H7 monitoring programme for red meat. The study showed that some wild industry strains of *E. coli* 0157:H7, designated as N373, N427, N635, produced weak positive signals using VIP and Reveal screen tests at 15h and that the VIP kit reaction remained weak after 22h incubation. The minimum recommended incubation time of 15 hours was considered insufficient when using these kits, with the potential to generate false negative results.

A follow-up study was considered necessary to determine the significance of the observations noted in this study and the protocol was designed to determine the minimum incubation time required to obtain a positive result for these *E. coli* O157:H7 strains - refer to AsureQuality report MIC 0808V.

METHOD

Test Organism Preparation

The following organisms were used in the evaluation:

- One *E. coli* O157:H7 verocytotoxin negative reference strain (NZRM 3614).
- Three wild-type *E. coli* O157:H7 verocytotoxin positive strains that expressed weak reaction in a previous evaluation; (designated as N373, N427, N635)
- A generic non-O157:H7 *E. coli* strain (NZRM 916) was used as a negative control.

The wild type strains were provided by AgResearch and were authenticated using phenotypical expression. The NZRM cultures were resuscitated from PROTEC™ beads stored at -20°C by three passages onto Tryptose-Soy agar (TSA, Difco, Dteroit, USA) incubated at 35°C 18-24 hours, and then plated onto TSA to confirm purity.

Overnight cultures were prepared for all strains by streaking onto TSA and incubating at 37±1°C for 18-22 hours. Purity was determined and a 10^8 cfu/ml suspension was prepared using 10ml of maximum recovery diluent (MRD, Oxoid, Basingstoke, UK).
The suspension was serially diluted to prepare a cell concentration of approximately 100 cfu/ml. To assist achievement of the target inoculation for the trial (<10cfu), an

enumeration of the suspension was made using TSA and each culture suspension was refrigerated (2-5℃) overnight.

Sample Enrichment Preparation:

375g of adult beef meat was selected from a carton of product previously tested as negative for *E. coli* O157:H7. The sample portion was added to 1L of mTSB+N+C broth, and stomached according to the standard NMD protocol (NZFSA, 2009). Each of the five standardized cultures were inoculated into a separate enrichment preparation. Each enrichment was prepared in duplicate. The enrichments were mixed for 2 minutes, and then transferred to a 42℃ incubator. At 15 hours of incubation an aliquot of each enrichment was removed and tested using three screen test kits Reveal, VIP and Tecra VIA. If a negative result was obtained the enrichment was returned to the incubator and further testing at other time points was conducted, i.e. at 18 hours, 20 hours, 22 hours, up to a maximum of 22 hours, if required. Note: all enrichments were tested at 15 hours and 18 hours.

The minimum incubation time to obtain a positive expression for each of the screen kits was determined and the process was then repeated to verify the same could be achieved for bobby calf (veal) trim.

Screen Testing:

Sample enrichments were analysed using the following test kits:

- TECRA *E. coli* O157:H7 VIA (3M, Frenchs Forest, NSW, Australia)
- VIP-Gold for EHEC (BioControl, Atlanta, Georgia, USA)
- Reveal-20 for *E. coli* O157:H7 (Neogen, Lansing, MI, USA)

All tests were performed in accordance with the manufacturer's instructions.

Post Enrichment *E. coli* O157:H7 Enumeration:

The *E. coli* O157:H7 concentration was determined for each enrichment at each incubation time point using direct plating technique with CT SMAC agar plates. Enrichments were thoroughly mixed by hand shaking for 60 seconds and a 1ml aliquot was removed. Serial dilutions were performed using MRD and 0.1ml of each dilution was plated onto the surface of duplicate CT-SMAC agar plates. The inoculum was evenly distributed across the plate surface using a sterile spreader and the plates were left at room temperature for 30 minutes to allow sample to absorb into the agar. Plates were

then inverted and incubated at 37±1℃ for 22±2 hours. Typical sorbitol negative colonies were counted as *E. coli* O157:H7. Latex agglutination using Oxoid *E. coli* O157 kit was performed on a selection of colonies from each sample to confirm the sorbitol negative colonies were O157 positive.

Verification of Inoculation Dose:

At the time of enrichment seeding, each of the culture suspension doses was enumerated to quantify the level of inoculation.

Spread plate technique using TSA and incubation at 37±1℃ for 22±2 hours was used. Five replicates of each suspension dose were plated. Colony counts were made and the results were averaged to provide an estimate of the dose level achieved for each organism.

Additional methods for Part 2 - Additional Study to investigate the effects of high background flora on method performance

Sample matrix variables

1) Low background flora beef trim:
   Frozen beef was allowed to thaw in refrigerator (2-6℃) for 24hours.
2) High background flora beef trim:
   Frozen beef trim was thawed in a refrigerator (2-6℃) for 3 days to allow inherent flora levels to increase.

A coliform and generic *E. coli* count was performed on each sample matrix using *E. coli* Petrifilm, to determine the relevant baseline flora levels prior to analysis.

Enrichment media variables

Old NMD enrichment: modified EC broth + novobiocin (mEC+N),

New NMD enrichment broth: TSB + novobiocin + casamino acid (TSB+NCA)

Sample Analysis

The beef samples were enriched according to the new NMD sample to enrichment broth ratio i.e. 375g of product to 1L of enrichment broth.

Each media was used to enrich each sample matrix. Each media/sample matrix combination was spiked with a low dose (<10cfu) of each of two *E. coli* O157 strains N635 and N427. A duplicate enrichment preparation was prepared for each combination. The

enrichments were mixed for 2 minutes, and then transferred to a 42℃ incubator. At 15 hours of incubation an aliquot of each enrichment was removed and tested using three screen test kits Reveal, VIP and Tecra VIA. The enrichment was returned to the incubator and further testing was conducted if a negative result was obtained i.e. at 18 hours, 20hours, up to a maximum of 22hours, if required.

PART 1 – Determination of minimum incubation time for screen kits

RESULTS

All screen kits evaluated, successfully detected all *E. coli* O157 strains included in the study. The results of the original revalidation study conducted in 2008, whereby VIP and Reveal kit expressed weak reactions for *E. coli* O157 strains N427, N635 and N373, were not reproduced. The quality of signal expressed by both lateral flow kits, VIP and Reveal, was improved compared with the expression obtained in the earlier study. The inoculation dose levels achieved were consistent with those obtained in the previous study i.e. less than 10 cfu/enrichment.

The study also demonstrated no matrix interference for either adult beef trim or bobby calf (veal) trim sample types.

The minimum incubation time required to obtain a positive expression was determined as 15 hours for both sample matrix types and all test kit/organism combinations.

It should be noted the VIP kit evaluated in this study was a modified version of the kit evaluated in 2008. The difference between the two VIP kits is a change in the chromagen, which enhances both the positive and control line intensity to improve interpretation. Also the physical size of the device had been reduced which greatly improved the sample migration rate across the chromatography medium and as a result increased the speed of expression. The antibody complex remained unchanged; therefore no additional validation was required and AOAC first action approval status was unaffected.

Of note, two replicates from the veal matrix group spiked with *E. coli* O157 did not express positive results after 22 hours of enrichment incubation for any test kit, despite post enrichment enumeration results showing *E. coli* O157 concentrations ranging from 8.0 $x10^4$ to 5.0 x 10^5. The sorbitol negative colonies counted as *E. coli* O157 were confirmed

using latex agglutination (Oxoid). Interestingly, for each sample exhibiting an apparent false negative result, high levels of sorbitol positive colonies were also encountered suggesting background flora in these samples were “out competing” the target flora. The author recommended further work should be conducted to investigate if the enrichment media change in NZFSA NMD methodology for *E. coli* O157:H7, had introduced a reduction in the selectivity of the enrichment process. See part 2 below.

Table 1: Screen test and enumeration data for beef samples @ 15 hours incubation.

Sample	VIP	REVEAL	TECRA (OD value)	Spike dose (cfu)	Post incubation counts (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
E. coli O157 **N373**	+ +	+ +	+ (3.012) + (2.953)	7 cfu	<1000 <1000	5.4×10^8 5.4×10^8
E. coli O157 **N427**	+ +	+ +	+ (2.777) + (3.044)	10 cfu	<1000 <1000	3.2×10^8 5.4×10^8
E. coli O157 **N635**	+ +	+ +	+ (2.891) + (3.076)	12 cfu	<1000 <1000	1.5×10^8 4.7×10^8
E. coli **(NZRM 916)**	- -	- -	- (0.029) - (0.109)	22 cfu	3.2×10^6 5.0×10^4	<1000 <1000
E. coli O157 **(NZRM 3614)**	+ +	+ +	+ (2.950) + (3.027)	6 cfu	<1000 <1000	6.2×10^8 6.0×10^8
Blank	-	-	- (0.055)			
- ve kit			- (0.015)			
+ve kit			+ (1.058)			

Table 2: Screen test and enumeration data for Beef samples @ 18 hours incubation.

Sample	VIP	REVEAL	TECRA (OD value)	Spike dose (cfu)	Post incubation counts (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
E. coli O157 **N373**	+ +	+ +	+ (3.063) + (2.897)	7 cfu	<1000 <1000	6.9×10^8 4.5×10^8
E. coli O157 **N427**	+ +	+ +	+ (2.775) + (3.080)	10 cfu	<1000 <1000	4.8×10^8 6.8×10^8
E. coli O157 **N635**	+ +	+ +	+ (3.131) + (2.902)	12 cfu	<1000 <1000	2.8×10^8 5.2×10^8
E. coli **(NZRM 916)**	- -	- -	- (0.064) - (0.042)	22 cfu	8.0×10^5 2.8×10^6	<1000 <1000
E. coli O157 **(NZRM 3614)**	+ +	+ +	+ (2.826) + (2.420)	6 cfu	<1000 <1000	4.7×10^8 7.5×10^8
Blank	-	-	- (0.094)			
- ve kit			- (0.019)			
+ve kit			+ (1.336)			

Table 3: Screen test and enumeration data for Veal samples @ 15 hours incubation.

Sample	VIP	REVEAL	TECRA (OD value)	Spike dose (cfu)	Post incubation counts (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
E. coli O157 **N373**	+ +	+ +	+ (2.170) + (2.106)	5 cfu	<1000 <1000	1.1×10^8 $>2.5 \times 10^8$
E. coli O157 **N427**	+ -	+ -	+ (2.533) - (0.031)	3 cfu	<1000 TDTC*	1.6×10^8 TDTC*
E. coli O157 **N635**	- +	- +	- (0.080) + (1.701)	5 cfu	8.8×10^5 2.8×10^5	$1.0 \times 10^{5**}$ 1.3×10^6
E. coli **(NZRM 916)**	- -	- -	- (0.026) - (0.085)	3 cfu	<1000 6.0×10^5	<1000 <1000
E. coli O157 **(NZRM 3614)**	+ +	+ +	+ (2.994) + (1.785)	6 cfu	<1000 <1000	$>2.5 \times 10^8$ $>2.5 \times 10^8$
Blank	-	-	- (0.021)	N/A		
- ve kit			- (0.019)			
+ve kit			+ (1.123)			

Table 4: Screen test and enumeration data for Veal samples @ 18 hours incubation.

Sample	VIP	REVEAL	TECRA (OD value)	Spike dose (cfu)	Post incubation counts (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
E. coli O157 **N373**	+ +	+ +	+ (1.378) + (1.797)	5 cfu	<1000 <1000	1.0×10^8 1.1×10^8
E. coli O157 **N427**	+ -	+ -	+ (1.427) - (0.034)	3 cfu	<1000 2.8×10^7	1.6×10^8 <1000
E. coli O157 **N635**	- +	- +	- (0.065) + (1.431)	5 cfu	1.0×10^6 4.0×10^6	$1.1 \times 10^{5**}$ 3.0×10^6
E. coli **(NZRM 916)**	- -	- -	- (0.081) + (0.402)	3 cfu	<1000 2.4×10^7	<1000 <1000
E. coli O157 **(NZRM 3614)**	+ +	+ +	+ (2.070) - (1.594)	6 cfu	<1000 <1000	6.1×10^8 1.2×10^8
Blank	-	-	- (0.025)			
- ve kit			- (0.033)			
+ve kit			+ (1.125)			

Table 5: Screen test and enumeration data for Veal samples @ 20 hours and 22hours incubation. (Note: testing ceased 18 hours for Positive samples)

Sample	VIP	REVEAL	TECRA (OD value)	Spike dose (cfu)	Post incubation counts (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
E. coli O157 **N427 (20 hours)**	-	-	0.076	5 cfu	1.1×10^{6}	$9.0 \times 10^{4**}$
E. coli O157 **N635 (20 hours)**	-	-	0.035	3 cfu	1.6×10^{7}	TDTC*
E. coli O157 **N427 (22 hours)**	-	-	0.049	5 cfu	9.3×10^{5}	$8.0 \times 10^{4**}$
E. coli O157 **N635 (22 hours)**	-	-	0.033	3 cfu	3.6×10^{7}	$5.0 \times 10^{5**}$
Blank	-	-	- (0.025)			
- ve kit			- (0.033)			
+ve kit			+ (1.147) + (1.120)			

* To difficult to count due to the spreading growth present on the media. The dominant organism type was Sorbitol positive, however the colony appearance was variable.
** O157 latex agglutination was positive for a representative number of colonies tested.
Spike dose an estimation of cells numbers, expressed as cfu, inoculated into the enrichment broth – see verification of inoculation dose.

Part 2 – Additional study to investigate the effects of high background flora on NMD *E. coli* O157 method performance.

An additional evaluation was conducted to compare the performance of the old NMD enrichment media, modified EC broth + novobiocin (mEC+N), against the new NMD enrichment media, TSB + novobiocin + casamino acid (TSB+NCA). The work was performed to compare the selectivity of media and to evaluate if either media was better than the other when increased levels of background flora are present in meat samples.

RESULTS

The results obtained from this additional work demonstrate equivalence between the two enrichment media with respect to selectivity and limitations of each formulation to suppress gram negative competitive flora. All samples with high background flora that were spiked with *E. coli* O157, tested negative by all screen kits at every incubation time point. Both media failed to suppress the competitive flora present in the beef samples with high background flora, which is believed to be responsible for the false negative outcome. In contrast, every sample with low background flora that was spiked with *E. coli* O157 returned a positive result for all screen kits at the earliest time point evaluated in this study, 15 hours.

At 22 hours incubation, the *E. coli* O157 levels in TSB+NCA enrichment broth were slightly higher (~10^6 cfu/ml) compared to mEC+N (~10^4-10^5 cfu/ml). However, this log increase did not result in improved method performance. At the same incubation time point, the levels of competitive flora were the approximately same for both enrichment media (~10^7 cfu/ml).

The baseline level of coliforms found in the high background flora sample was 2.0 x 10^6 cfu/g, and no generic *E. coli* was found at the lowest dilution performed (10^{-3}). In comparison, the fresh beef sample had a coliform level of 1.6 x 10^3 cfu/g and an *E. coli* level <10cfu/g.

Table 1: Screen test and enumeration data for beef samples @ 15 hours incubation. (Note: testing ceased for samples expressing positive results)

Reference	VIP	Reveal	Tecra (OD Value)	Spike dose (cfu)	Post incubation (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
mEC – Aged N635 (a)		-	0.029	4	**1.45×10^7**	**$<1.0 \times 10^4$**
mEC - Aged N635 (b)		-	0.038	4	**1.46×10^7**	**1.1×10^6**
mEC - Aged N427 (a)		-	0.037	4	**1.39×10^7**	**7.0×10^5**
mEC - Aged N427 (b)		-	0.044	4	**1.71×10^7**	**1.3×10^6**
mEC – Fresh N635 (a)		-	0.165	4	****1.32×10^7**	**none**
mEC - Fresh N635 (b)	+	+	2.945	4	**not performed**	**not performed**
mEC - Fresh N427 (a)	+	+	2.595	4	**not performed**	**not performed**
mEC - Fresh N427 (b)	+	+	2.806	4	**not performed**	**not performed**
ModEc - Fresh blank		-	0.040	N/A	**not performed**	**not performed**
Mod TSB - Aged N635 (a)		-	0.033	4	**1.28×10^7**	**2.1×10^6**
Mod TSB - Aged N635 (b)		-	0.032	4	**9.2×10^6**	**11×10^6**
Mod TSB - Aged N427 (a)		-	0.034	4	**1.52×10^7**	**1.3×10^6**
Mod TSB - Aged N427 (b)		-	0.042	4	**1.52×10^7**	**1.2×10^6**
Mod TSB - Fresh N635 (a)	+	+	2.752	4	**not performed**	**not performed**
Mod TSB - Fresh N635 (b)	+	+	2.858	4	**not performed**	**not performed**
Mod TSB - Fresh N427 (a)	+	+	2.910	4	**not performed**	**not performed**
Mod TSB - Fresh N427 (b)	+	+	3.072	4	**not performed**	**not performed**
Mod TSB - Aged blank		-	0.039	N/A	**not performed**	**not performed**

** Sorbitol Positive, mixed growth of mucoid and non-mucoid colonies. No presence of translucent colonies on -4 dilution
NB: Due to a shortage of materials, VIP testing was only performed on sample that were positive for the Reveal or Tecra.

Table 2: Screen test and enumeration data for beef samples @ 18 hours incubation. (Note: testing ceased for samples expressing positive results)

Reference	VIP	Reveal	Tecra (OD Value)	Spike dose (cfu)	Post incubation (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
mEC – Aged N635 (a)	-	-	0.034	4	**not performed**	**not performed**
mEC - Aged N635 (b)	-	-	0.042	4	**not performed**	**not performed**
mEC - Aged N427 (a)	-	-	0.043	4	**not performed**	**not performed**
mEC - Aged N427 (b)	-	-	0.038	4	**not performed**	**not performed**
mEC – Fresh N635 (a)	-	-	0.036	4	****1.85 x 106**	**none**
mEC - Fresh N635 (b)				4	**not performed**	**not performed**
mEC - Fresh N427 (a)				4	**not performed**	**not performed**
mEC - Fresh N427 (b)				4	**not performed**	**not performed**
ModEc - Fresh blank				N/A	**not performed**	**not performed**
Mod TSB - Aged N635 (a)	-	-	0.030	4	**not performed**	**not performed**
Mod TSB - Aged N635 (b)	-	-	0.034	4	**not performed**	**not performed**
Mod TSB - Aged N427 (a)	-	-	0.036	4	**not performed**	**not performed**
Mod TSB - Aged N427 (b)	-	-	0.028	4	**not performed**	**not performed**
Mod TSB - Fresh N635 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N635 (b)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (b)				4	**not performed**	**not performed**
Mod TSB - Aged blank				N/A	**not performed**	**not performed**

** Sorbitol Positive, mixed growth of mucoid and non-mucoid colonies. No presence of translucent colonies on -4 dilution

Table 3: Screen test and enumeration data for beef samples @ 20 hours incubation. (Note: testing ceased for samples expressing positive results)

Reference	VIP	Reveal	Tecra (OD Value)	Spike dose (cfu)	Post incubation (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
mEC – Aged N635 (a)	-	-	0.034	4	**not performed**	**not performed**
mEC - Aged N635 (b)	-	-	0.043	4	**not performed**	**not performed**
mEC - Aged N427 (a)	-	-	0.052	4	**not performed**	**not performed**
mEC - Aged N427 (b)	-	-	0.040	4	**not performed**	**not performed**
mEC – Fresh N635 (a)	-	-	0.033	4	****1.91 x 10^6**	**none**
mEC - Fresh N635 (b)				4	**not performed**	**not performed**
mEC - Fresh N427 (a)				4	**not performed**	**not performed**
mEC - Fresh N427 (b)				4	**not performed**	**not performed**
ModEc - Fresh blank				N/A	**not performed**	**not performed**
Mod TSB - Aged N635 (a)	-	-	0.041	4	**not performed**	**not performed**
Mod TSB - Aged N635 (b)	-	-	0.023	4	**not performed**	**not performed**
Mod TSB - Aged N427 (a)	-	-	0.037	4	**not performed**	**not performed**
Mod TSB - Aged N427 (b)	-	-	0.039	4	**not performed**	**not performed**
Mod TSB - Fresh N635 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N635 (b)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (b)				4	**not performed**	**not performed**
Mod TSB - Aged blank				N/A	**not performed**	**not performed**

** Sorbitol Positive, mixed growth of mucoid and non-mucoid colonies. No presence of translucent colonies on -4 dilution

Table 4: Screen test and enumeration data for beef samples @ 22 hours incubation. (Note: testing ceased for samples expressing positive results)

Reference	VIP	Reveal	Tecra (OD Value)	Spike dose (cfu)	Post incubation (cfu/ml)	
					Sorbitol Positive	Sorbitol Negative
mEC – Aged N635 (a)	-	-	0.032	4	**9.3×10^6**	**1.0×10^4**
mEC - Aged N635 (b)	-	-	0.033	4	**1.08×10^7**	**1.4×10^6**
mEC - Aged N427 (a)	-	-	0.038	4	**9.0×10^6**	**6.0×10^5**
mEC - Aged N427 (b)	-	-	0.040	4	**1.86×10^7**	**9.0×10^5**
mEC – Fresh N635 (a)	-	-	0.029	4	****1.84×10^7**	**none**
mEC - Fresh N635 (b)				4	**not performed**	**not performed**
mEC - Fresh N427 (a)				4	**not performed**	**not performed**
mEC - Fresh N427 (b)				4	**not performed**	**not performed**
ModEc - Fresh blank				N/A	**not performed**	**not performed**
Mod TSB - Aged N635 (a)	-	-	0.042	4	**1.30×10^7**	**3.3×10^6**
Mod TSB - Aged N635 (b)	-	-	0.045	4	**1.40×10^7**	**2.0×10^6**
Mod TSB - Aged N427 (a)	-	-	0.034	4	**2.37×10^7**	**1.4×10^6**
Mod TSB - Aged N427 (b)	-	-	0.033	4	**no result**	**no result**
Mod TSB - Fresh N635 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N635 (b)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (a)				4	**not performed**	**not performed**
Mod TSB - Fresh N427 (b)				4	**not performed**	**not performed**
Mod TSB - Aged blank				N/A	**not performed**	**not performed**

** Sorbitol Positive, mixed growth of mucoid and non-mucoid colonies. No presence of translucent colonies on -4 dilution

INTERPRETATION

From this data, the limit for detection using these kits under these conditions appears to be in the order of 100 000 - 500 000 cells. The onus is therefore on the enrichment broth to propagate to these numbers, which was not always achieved from the veal samples studied, particularly when high background counts were reported. Such deficiencies of enrichment protocols for *E. coli* O157:H7 have been reported previously (Grant 2005; Vimont et al, 2007) and are currently only overcome using more sensitive detection systems (e.g. IMS-PCR).

There is evidence from the New Zealand National Microbial Database microbiological monitoring programme that the level of background flora on veal is higher than on beef, although the bacterial composition of this flora has not been clearly defined. While it is possible differences in background flora occur between New Zealand and overseas countries where kits have previously been validated, evidence in support of this has not been reported.

CONCLUSION

The data demonstrates all *E. coli* O157:H7 screen kits approved for use in New Zealand Food Safety Authority (NZFSA) *E. coli* O157:H7 monitoring programme are compatible with the new enrichment and sampling protocol adopted by NZFSA.

REFERENCES

1. US OMAR - Part 12 United States of America: Technical Procedures for Monitoring Escherichia coli O157:H7 Programme in Bulk Manufacturing Beef, Amendment 4, September 2009
2. US OMAR - Part 13 United States of America: Technical Procedures for Monitoring Escherichia coli O157:H7 Programme in Bulk Manufacturing Bobby Veal, Amendment 4, September 2009
3. Vimont, A., Vernozy-Rozand, C., Monent, M. P., Bavai, C., Fremaux, B., Delignette-Muller, M.-L. 2007. Growth of Shiga-Toxin producing *Escherichia coli* (STEC) and bovine feces background microflora in various enrichment protocols. Vet. Microbiol. 123, 274-281.

4. Grant, M. A. 2005. Comparison of a new enrichment procedure for Shiga-Toxin-Producing *Escherichia coli* with five standard methods.
5. Aspin W., Inverarity J. 2008. Revalidation of screen test kits for *Escherichia coli* O157:H7. Report to NZFSA.

ACKNOWLEDGEMENTS

The evaluation was conducted at AsureQuality Auckland Laboratory by Warwick Aspin, Justine Inverarity, and Rodolfo Cabuang.

The protocol was designed with the assistance of Dr Roger Cook, NZFSA, David Tisdall, NZFSA and John Mills, AgResearch.

Wild E. coli O157:H7 strains were obtained from AgResearch culture collection.
The study was funded by NZFSA