Method Comparison Study Report for the ISO 16140-2:2016 validation of Compact Dry ETC, for the enumeration of *Enterococcus* in a broad range of foods and environmental samples

MicroVal study number: 2014LR48 extension

Method/Kit name: CompactDry ETC

Report version: extension merged report v2, 19/10/23

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Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v.2.5.

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Method/Kit name: CompactDry ETC

Validation standard: Microbiology of the food chain— Method validation

Part 1: Vocabulary (ISO 16140-1:2016) and

Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)

Reference methods:

NMKL Method No. 68 5th Edition 2011: *Enterococcus.* Determination in foods and feeds.

Scope of validation: broad range of foods and environmental samples

Certification organization: LRQA

List of abbreviations

- AL Acceptability Limit
- AP Accuracy Profile
- Art. Cont. Artificial contamination
- CFU Colony Forming Units
- CL confidence limit (usually 95%)
- EL Expert Laboratory
- $\bar{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Inc/Ex Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- EL Expert Laboratory
- n number of samples
- na not applicable
- neg negative (target not detected)
- NG no growth
- nt not tested
- RT Relative Trueness
- SD standard deviation of differences
- 10^{-1} dilution 10-fold dilution of original food
- 10^{-2} dilution 100-fold dilution of original food

- BPW Buffered Peptone Water
- PSD Peptone Salt Diluent
- MRD Maximum Recovery Diluent
- NA Nutrient Agar
- PCA Plate Count Agar

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of *Enterococcus* in a broad range of foods was carried out by Campden BRI as the MicroVal Expert Laboratory.

An extension study was performed at the request of the manufacturer to include a further category (environmental samples) to extend the scope of the validation for this media. This study was completed in June 2023 by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used is:

Enumeration of *Enterococcus* on Compact Dry ETC, incubated at 37°C±1°C for 20 -24h.

The Compact Dry ETC plates contain a chromogenic medium and selective agents for the detection and enumeration of *Enterococcus* which according to the manufacturer's instructions appear as blue colonies after 20-24hr incubation at 37±1ºC. The minimum incubation time of 20h was used in this study for the Compact Dry ETC plates.

Reference method is:

NMKL Method No. 68 5th Edition 2011: Enterococcus. Determination in foods and feeds.

Scope of the validation study is: 1 further category (Environmental samples)

Categories included:

- Environmental samples

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Inclusivity and exclusivity
- Interlaboratory Study (ILS)[1]

The final conclusion on the Method Comparison study is summarized below:

The alternative method CD ETC shows comparable performance to the reference method NMKL Method No. 68 5th Edition 2011 for the enumeration of *Enterococcus* in environmental samples.

2 Method protocols

The Method Comparison study was carried out using 10g portions of sample material.

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 6887-series parts 1, 2, 3, 4 and 5. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results. Depending on the sample being tested and the expected contamination level, single or multiple dilutions were used with single or duplicate plates if considered necessary to improve the reliability of the calculated result and ensure at least two relevant plates were available for use in calculations

2.2 Alternative method

See the flow diagram in Annex A

See the Kit insert in Annex B.

The Compact Dry ETC plates contain chromogenic medium and selective agents for the detection and enumeration of *Enterococcus* which according to the manufacturer's instructions appear as blue colonies after 20-24hr incubation at 37±1ºC. The minimum incubation time of 20h was used in this study for the Compact Dry ETC plates.

2.3 Study design

The reference method and alternative methods were performed with, as far as possible, exactly the same sample.

The Method Comparison Study was carried out using 10 gram test portions of the sample.

The samples were prepared for analysis and diluted in accordance with ISO 6887 (all parts) unless specified differently in the alternative method.

3 Method comparison study

3.1 Relative trueness study

The trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study was conducted using naturally or artificially contaminated samples. Different categories, types and items were tested for this.

A total of 1 category was included in this validation study in addition to the orignal 5 categories. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category.

Each category was made up of 3 types, with at least 5 Items representative for each type.

In accordance with ISO 16140-2, for each category, a minimum of 15 individual samples was tested, made up of at least three types with at least 5 samples representative for each type.

Table 1. List of Categories, Types, and examples of Items tested within the relative trueness study.

Category	Types	Items	No of samples	Number of interpretable results	ISO 6887
Dairy products	Dairy desserts	chilled custard, trifle, ice cream	8	5	6887-5
	Soft cheese	Soft cheese	11	5	6887-5
	Hard cheese	cheddar	9	5	6887-5
Fruits and vegetables	Seasonings	spices	5	5	6887-4
	Sprouts	mung beans	5	5	6887-4
	Leafy greens	parsley, lettuce	7	5	6887-4
Raw poultry and meats	Fresh poultry cuts	turkey breast, turkey fillet	6	5	6887-2
	Fresh mince	lamb, beef, pork	7	5	6887-2
	Processed ready to cook	Frozen and fresh patties	7	6	6887-2
Ready to eat foods	Ready to eat poultry	turkey fillet	5	5	6887-2
	Cooked fish products	prawns	9	6	6887-3
	Cooked meat	ham	10	6	6887-2
Multi component foods	Composite foods with raw ingredients	e.g. sandwiches, pasta salads.	5	5	6887-1, 6887-4
	Mayonnaise based salads	Mayonnaise based salads	7	5	6887-1, 6887-4
	Cooked chilled foods	e.g. rice products	7	5	6887-1, 6887-4
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	Equipment, floors, walls	5	5	ISO 18593
	Process water	Wash water, cooling water	5	5	6887-6
	Dust	Bakery and food manufacturing environment	5	5	6887-1, 6887-4

90 samples were analyzed, leading to 90 exploitable results.

3.1.1 Test sample preparation

No naturally contaminated samples were found in pre-screening studies. It was therefore necessary to use artificial contamination procedures. Artificial procedures used a range of seeding protocols and strains in order to examine a wide range of different conditions.

Further details of the artificial inoculation used in the studies is given in the table below

Sample type	Procedure for artificial contamination
process water	Seeding and storage of samples post inoculation for 48h ±2h at 2-8°C to chill stress the cells
Surfaces and dust samples	Seeding and storage of samples post inoculation and storage for 2 weeks at ambient
Dry animal feed	Seeding with lyophilised cells and storage for 2 weeks at ambient

Inoculation of samples was at the range usually associated with the test organisms and within the capabilities of the test methods. Enumeration methods generally cover the range 10^2cfu/g to 10^7cfu/g

0% of the samples were naturally contaminated.

3.1.2 Protocols applied during the validation study

Incubation time

The minimum incubation time of 20h was used in this study for the Compact Dry ETC plates.

Confirmations if required for the alternative method

No confirmation step was carried out, as it is not required by the manufactuer and the reference method.

3.1.3 Test results

All raw data per category are given in Annex C.

The samples were analyzed by the reference and the alternative methods in order to have 15 interpretable results per incubation protocol, and 5 interpretable results per tested type.

3.1.4 Calculation and interpretation of relative trueness study

The calculations are provided in Annex D.

The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity (y = x).

Figure 1 shows the scatter plot for Dairy products.

Figure 2 shows the scatter plot for Fruits and vegetables

Figure 3 shows the scatter plot for Raw meat and poultry

Figure 4 shows the scatter plot for ready to meat foods

Figure 5 shows the scatter plot for multicomponent foods

Figure 6 shows the scatter plot for the environmental category

Figure 7 shows the scatter plot for all the categories.

Figure 1 - Scatter plot of the reference method versus alternative method results for Dairy products

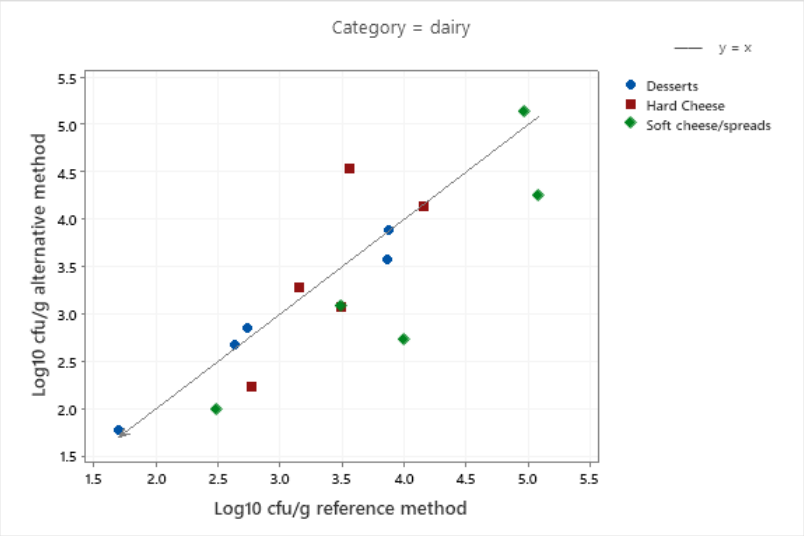


Figure 2 - Scatter plot of the reference method versus alternative method results for fruits and vegetables

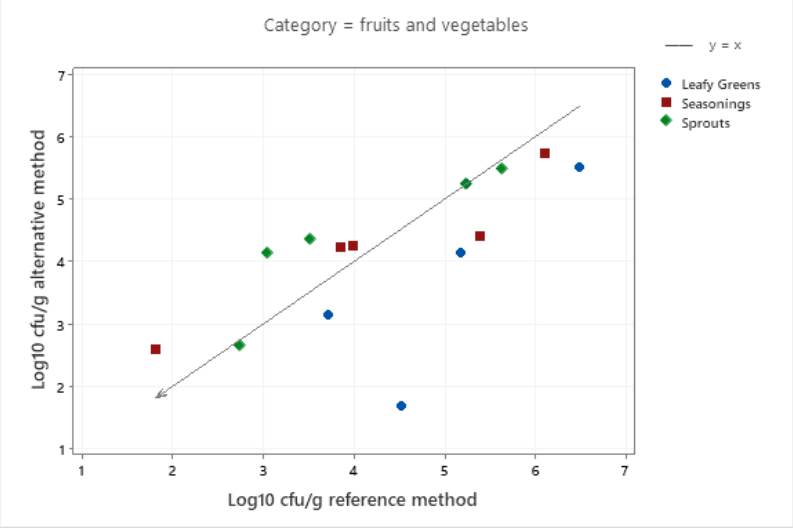


Figure 3 - Scatter plot of the reference method versus alternative method results for raw meat and poultry

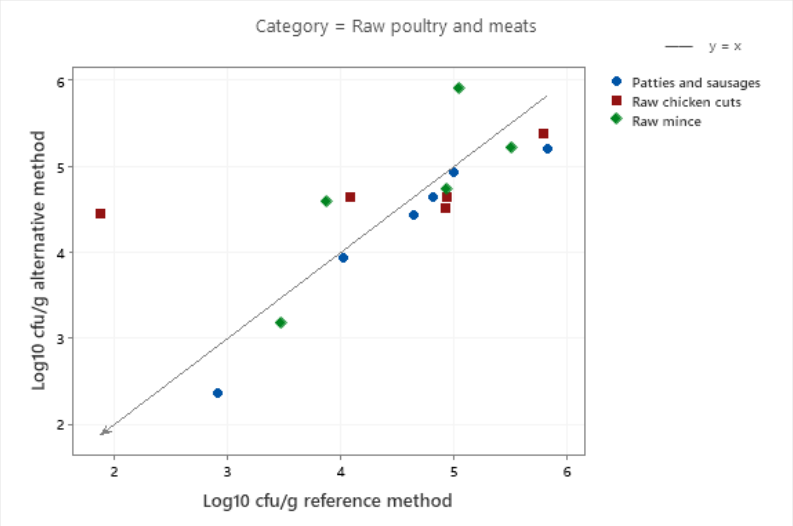


Figure 4 - Scatter plot of the reference method versus alternative method results for ready to eat foods

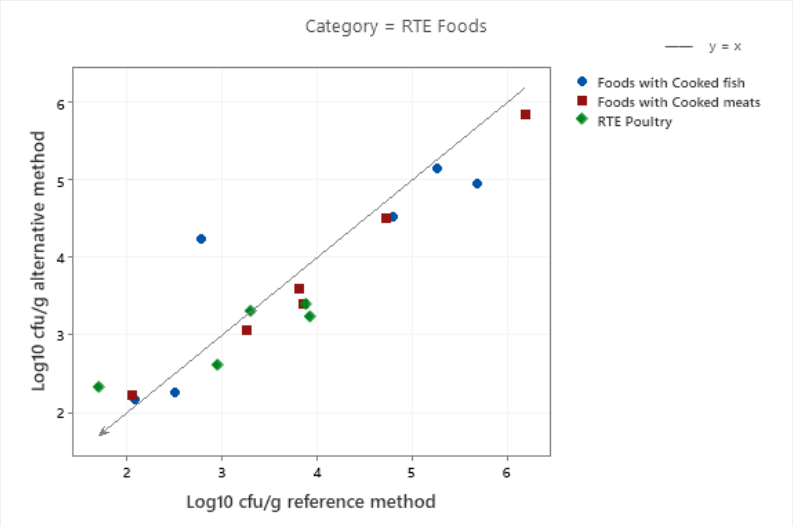


Figure 5 - Scatter plot of the reference method versus alternative method results for multicomponent foods

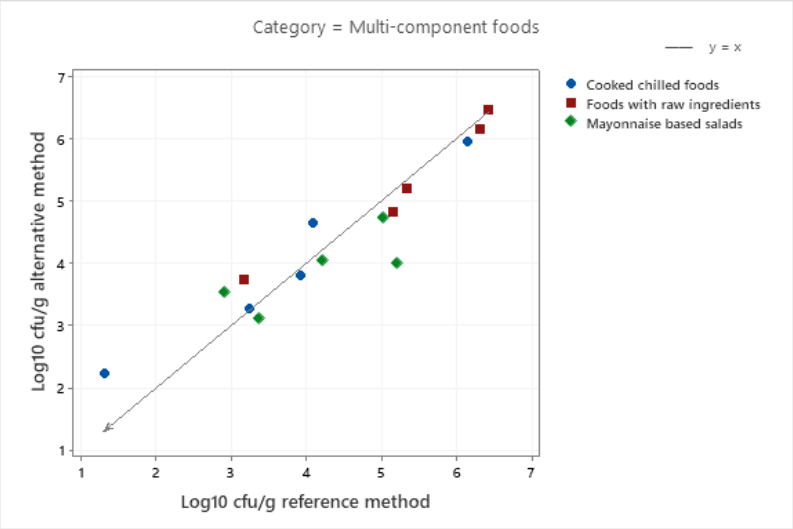


Figure 6 - Scatter plot of the reference method versus alternative method results for the environmental category

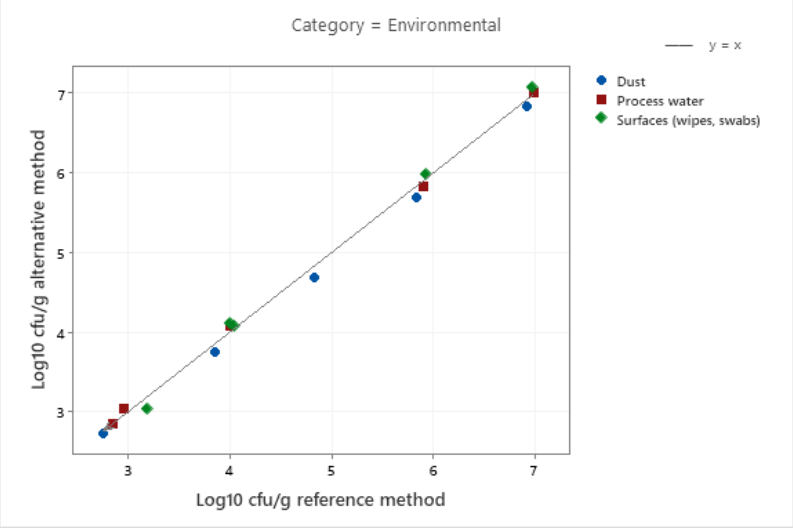
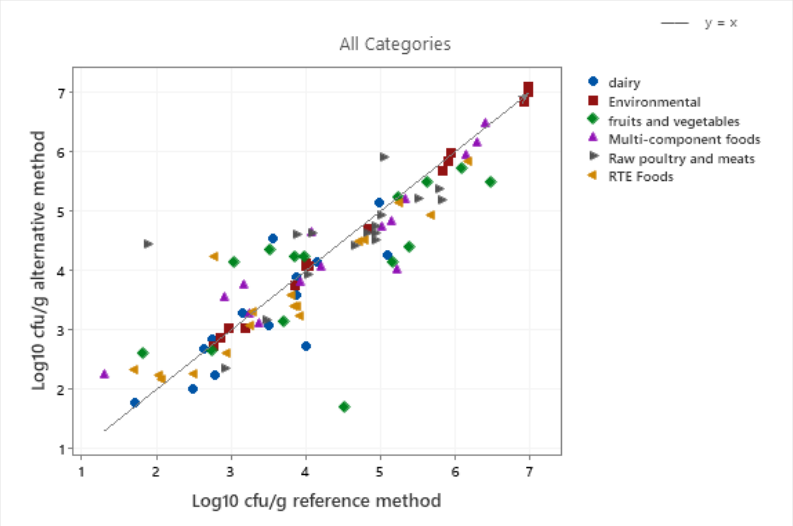


Figure 7 - Scatter plot of the reference method versus alternative method results for all the categories



The data in the scatter plots show good agreement between the reference and alternative methods with no obvious disagreement.

A summary of the calculated values per category is provided in Table 2.

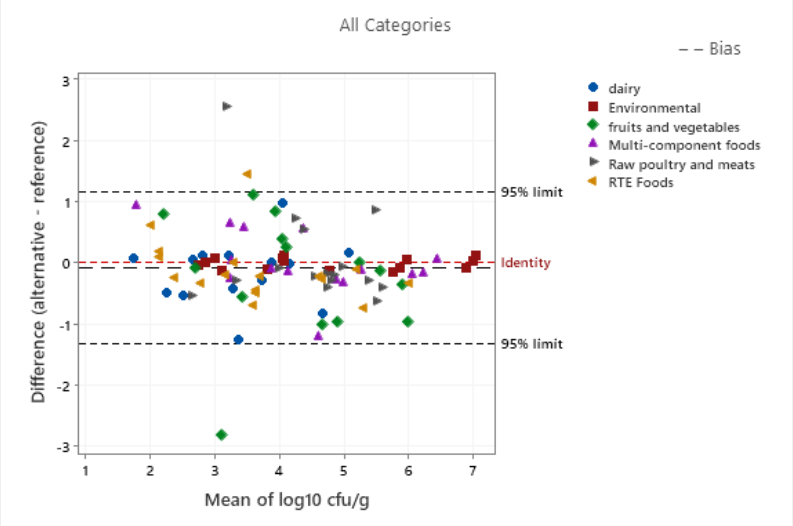
The Bland-Altman difference plot for all the samples is given Figure 8.

Table 2. Summary of calculated values per category.

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Dairy	15	-0.180	0.515	-1.321	0.962
Environmental	15	-0.015	0.094	-0.224	0.193
fruits and vegetables	15	-0.233	0.975	-2.393	1.926
Multi-component foods	15	-0.005	0.520	-1.157	1.147
Raw poultry and meats	16	0.074	0.796	-1.676	1.823
RTE Foods	17	-0.114	0.519	-1.246	1.019
All Categories	93	-0.078	0.624	-1.324	1.168

$\overline{D}$: Average difference SD: standard deviation of differences n: number of samples

Figure 8 – Bland-Altman difference plot for all the samples



The results of difference and the scatter plots were interpreted according to ISO 16140-2:2015 section 6.1.2.3 based on a visual observation on the amount of bias and extreme result. It is expected that not more than one in 20 data values will lie outside the CLs. Any disagreements with the expectation should be recorded.

For this data set there are 3 in 90 data values which lie outside the CLs. There is no disagreement with the expectation of less than one in 20 and therefore the relative trueness of the alternative method is considered to be satisfactory.

The three results which fall outside the CL's are listed in Table 3.

Table 3: Results falling outside the confidence limits

Food Category	**Food type**	**Sample code**	**Food item**	**Strain**	**Spiking/ seeding protocol**	**Difference log cfu/g (alternative – reference)**
RTE Foods	Foods with Cooked fish	49	Seafood terrine	*E. faecium* 9645	55°C/5min	1.46
Raw poultry and meats	Raw chicken cuts	153	Chicken mini fillets	*E.faecium* NCIMB 700580	Chill storage for 4 days	2.57
Fruits and vegetables	Seasonings	157	Whole cardamon pods	*E.faecalis* 12672	Storage at ambient for 10days	-2.82

There is no trend in product type, inoculated strain or seeding/spiking protocol between these three data points and these differences are considered to be due to non-identifiable causes and are of no practical significance.

The low recovery on the ETC for whole cardamon pods could be due to inhibitory effects by plating a larger volume (1ml) of a -1 dilution.

3.1.5 Discordant results

It is commonly recognized that a bias higher than 0.5 Log CFU/g difference between the compared methods should be explained if possible. It is the case for 26 samples, 14 with positive bias and or 12 with negative bias. There was no pattern to the data in terms of the inoculated organism, spiking/seeding protocol used, or product category. Apart from the 3 samples highlighted in Table 3 above, all other data were within the CL's of the Bland Altman difference plot.

Positive bias higher than 0.5 Log CFU/g

The results showing a higher enumeration with the alternative method than with the reference method are observed. (See Table 4).

Table 4 – Discordant results with a positive bias

Sample n°	Product category	Products	Biaslog Alt - log Ref(log CFU/g)	Inoculated Organism	Stress applied
165	Raw meat and poultry	Whole chicken	0.55	*E.faecium* 9645	Chlorine
109	Multi component foods	Feta and chickpea salad	0.58	*E.faecium* 9645	Chlorine
77	RTE foods	Breaded chicken strips	0.62	*E.mundtii* 16812	Freezing
128	Fruit and veg	Dried cumin	0.63	*E.faecium* 9645	Ambient
168	Raw meat and poultry	Lamb mince	0.87	*E.faecalis* 12672	Chilling
132	RTE foods	Singapore noodles	0.55	*E.faecalis* 12672	Chilling
157	Fruit and Veg	Organic kale	2.82	*E.faecalis* 1993	Chilling
168	Raw meat and poultry	Beef mince, 15% fat	0.87	*E.faecalis* 1993	Freezing
86	Multi component foods	Chinese rice	0.94	*E.faecalis* 7297	Freezing
125	Dairy	Grated cheddar cheese	0.97	E.casseliflavus 16811	Chilling
40	Fruit and Veg	Fresh beansprouts	1.11	*E.hirae* 15939	Acidity
49	RTE foods	Seafood terrine	1.46	*E.faecium* 9645	Heating
153	Raw meat and poultry	Chicken mini fillets	2.57	*E.faecium* 700580	Chilling
151	Fruit and Veg	Wild rosemary	0.79	*E.faecalis* 12672	Ambient

Negative bias higher than 0.5 Log CFU/g

The results showing a higher enumeration with the reference method than with the alternative method are observed (See Table 5).

Table 5 – Discordant results with a negative bias

Sample n°	Product category	Products	Bias log Alt - log Ref (log CFU/g)	Inoculated Organism	Stress applied
157	Fruit and Veg	Organic whole cardamom	-2.82	*E.faecalis* 12672	Ambient
57	Dairy	Boursin black pepper cream	-1.26	*E.faecium* 9645	Acidity
131	Multicompoment foods	Celery, fruit and peanut salad	-1.21	*E.faecium* 9645	Chlorine
67	Fruit and Veg	Fresh beansprouts	-1.01	*E.hirae* 15939	Acidity
43	RTE foods	Sweet chilli cooked turkey bites	-0.97	*E.avium* 701605	Heating
1	RTE foods	Frozen prawns	-0.74	*E.mundtii* 16812	Freezing
147	Raw meat and poultry	Chicken liver pate	-0.69	*E.mundtii* 16812	Freezing
70	Raw meat and poultry	Chorizo	-0.63	*E.mundtii* 16812	Freezing
67	Fruit and Veg	Fresh coriander	-1.01	*E.avium* 701605	Acidity
83	Raw meat and poultry	Persian spiced chicken burgers	-0.55	*E.faecalis* 12672	Chilling
63	Dairy	Pineapple and almond cream cheese roll	-0.531	*E.avium* 701605	Heating
115	Dairy	Cheese roll	-0.83	*E.avium* 701605	Heating

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method is satisfied as it shows comparative performance to the reference method.

The relative trueness of the Alternative method is satisfied as the expectation of not more than 1 in 20 data points outside of the acceptability limits is met.

3.2 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. This study is conducted using artificially contaminated samples, using one type per category.

3.2.1 Categories, sample types and strains

For each of 5 food categories, one type of food was tested using 6 samples per type. Of the 6 samples, there were 2 at a low level, 2 at a medium level and 2 at a high level of contamination. For each of the 6 samples per category, 5 replicate test portions were tested.

According to ISO16140-2:2015 6.1.3.2, for each category being tested, at least one food type shall be tested but the six samples tested might belong to the same food item or to different food items. According to MicroVal discussions there are 2 options that may be used here. Either a single food item is used per type but 2 batches are tested, or 2 different food items are tested with one batch per item. So for example, for dairy desserts, it would be possible to test:

- chilled custard batch 1 and chilled custard batch 2, or
- chilled custard batch 1 and whipped cream batch 1

The choice of number of food items is important if the data from the study is to be used for a joint MicroVal /AOAC validation. For AOAC studies, only the data from the accuracy profile studies can be used and for the certificate claims only the food items tested here can be claimed. So, if only 1 food item is used per category then the AOAC food matrix claim will be limited to 5 food items and if 2 food items are used per category then the AOAC food matrix claim will include 10 food items.

In order to evaluate the difference between the 2 options on the statistical analysis, this study tested both approaches.

Other matrices

The environmental category was tested with a single batch of two different surface types, using 6 samples per type.

Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analyzed per food type. The following food type/strain pairs were studied (See Table 6):

Environmental surfaces were indiviuallly inoculated using an appropriate volume and concentration of inoculum. The inoculum will be evenly distributed over the test area without excessive accumulation that may cause uneven drying. Following inoculation, the surfaces were dried at room temperature (18-25°C) until the surface is visibly dry.

Table 6. Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Types	Inoculated Strain ***Enterococcus***	Item	Inoculation levels
Dairy products	Dairy desserts	*E.mundtii* CRA 16812 from soil	Chilled custard Batch 1	Low:100cf/g
				Medium : 1000cfu/g
				High : 10,000cfu/g
			Chilled custard Batch 2	Low:100cf/g
				Medium : 1000cfu/g
				High : 10,000cfu/g
			Whipped cream	Low:100cf/g
				Medium : 1000cfu/g
				High : 10,000cfu/g
Fruits and vegetables	Leafy greens e.g.	*E.faecium* NCIMB 9645 from grass silage	Parsley Batch 1	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g

Category	Types	Inoculated Strain *Enterococcus*	Item	Inoculation levels
	parsley, lettuce		Parsley Batch 2	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g
			Shredded lettuce	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g
Raw poultry and meats	Fresh beef	*E.avium* NCIMB 702366, source unknown	Fresh steak Batch 1	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g
			Fresh steak Batch 2	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g
			Patties	Low: 50cf/g
				Medium : 1000cfu/g
				High : 50,000cfu/g
Ready to eat foods	Cooked fish products e.g. prawns	*E. casseliflavus* CRA 16811 from plants	Tuna pate Batch 1	Low: 50cf/g
				Medium : 100cfu/g
				High : 1000cfu/g
			Tuna pate Batch 2	Low: 50cf/g
				Medium : 100cfu/g
				High : 1000cfu/g
			Fresh cooked prawns	Low: 50cf/g
				Medium : 100cfu/g
				High : 1000cfu/g
Multi component foods	Composite foods with raw ingredients	*E.hirae* CRA 15939, industrial isolate	Pasta salad Batch 1	Low 500cf/g
				Medium : 5000cfu/g
				High : 50,000cfu/g
			Pasta salad Batch 1	Low 500cf/g
				Medium : 5000cfu/g
				High : 50,000cfu/g
			Sandwiches	Low 500cf/g
				Medium : 5000cfu/g
				High : 50,000cfu/g
Environmental samples	Surfaces	*Enterococcus faecalis* CRA 16049 human isolate (NCIMB 13280)	Stainless steel (4" x 4")	100/test area
				1000/test area
				10000/test area
			Plastic chopping board (4" x 4")	100/test area
				1000/test area
				10000/test area

Preparation of samples was done as a bulk inoculation. A 100g sample was inoculated with 1ml of appropriate dilution of inoculating strain and homogenised by hand massaging or stomaching to evenly distribute the inoculum. For all matrices, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis.

Environmental surfaces were indiviuallly inoculated using an appropriate volume and concentration of inoculum. The inoculum will be evenly distributed over the test area without excessive accumulation that may cause uneven drying. Following inoculation, the surfaces were dried at room temperature (18-25°C) until the surface is visibly dry.

3.2.2 Calculations and interpretation of accuracy profile study

The statistical results and the accuracy profiles are provided Figure 9 to 14. Because the study design included 9 samples per category instead of 6, the statistical analysis was carried out 3 times for each category instead of once. For example for dairy products the analysis was carried out for

(i) custard batch 1 (low, medium, high) and custard batch 2 (low, medium, high)

(ii) custard batch 1 (low, medium, high) and cream

(iii) custard batch 2 (low, medium, high) and cream

If any of the upper or lower limits exceeded the limits and the standard deviation of the reference method was >0.125, additional evaluation procedure were followed, as described in ISO 16140-2 (F/DIS, 16140) and the new acceptability limits were calculated as a function of the standard deviation $AL_s = 4 \cdot s_{ref}$.

The new AL's are shown in the statistical analysis in Figure 9 to 14.

Other matrices

The raw data are provided Annex E and the summary tables (in log CFU/g) in Annex F

The statistical results and the accuracy profiles are provided Figure 4.

The calculations were done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) available on http://standards.iso.org/iso/16140

Figure 9 – Accuracy profile for dairy products

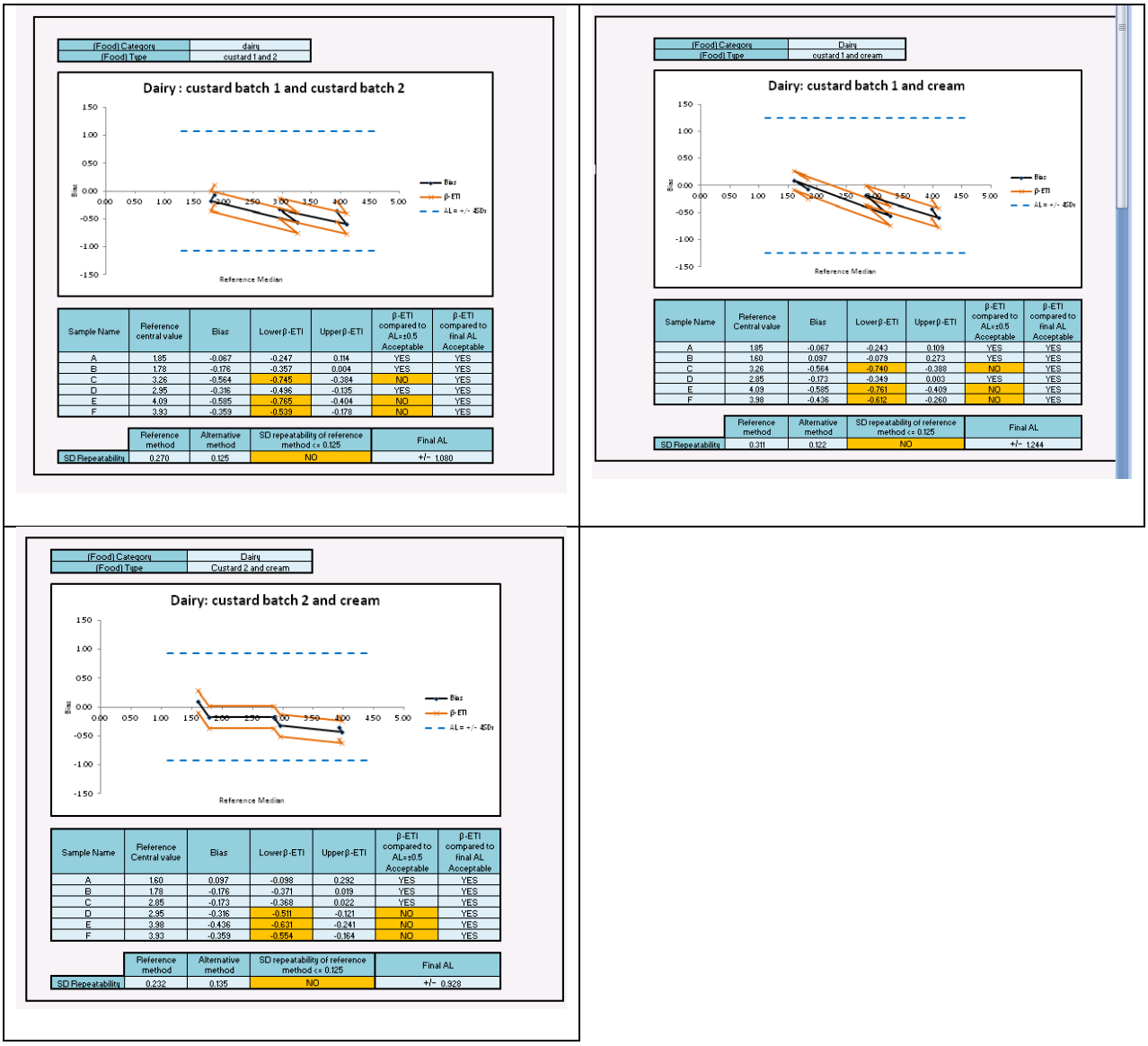
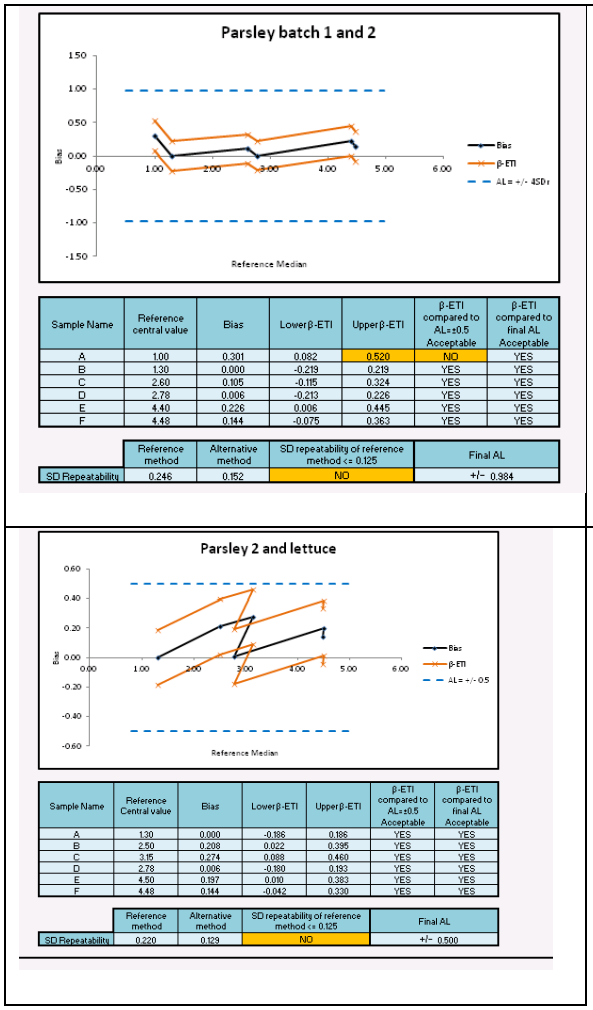
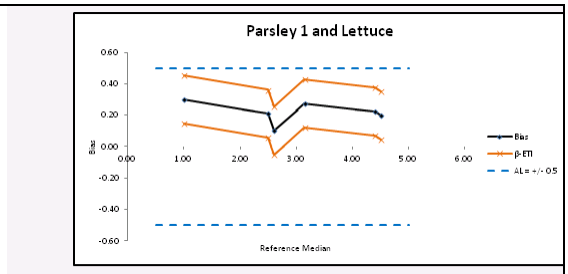


Figure 10 – Accuracy profile for fruits and vegetables



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.00	0.301	0.082	0.520	NO	YES
B	1.30	0.000	-0.219	0.219	YES	YES
C	2.60	0.105	-0.115	0.324	YES	YES
D	2.78	0.006	-0.213	0.226	YES	YES
E	4.40	0.226	0.006	0.445	YES	YES
F	4.48	0.144	-0.075	0.363	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.246	0.152	NO	+/- 0.384



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.00	0.301	0.148	0.454	YES	YES
B	2.50	0.208	0.055	0.361	YES	YES
C	2.60	0.105	-0.048	0.258	YES	YES
D	3.15	0.274	0.121	0.427	YES	YES
E	4.40	0.226	0.073	0.379	YES	YES
F	4.50	0.197	0.044	0.350	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.215	0.106	NO	+/- 0.500

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.30	0.000	-0.186	0.186	YES	YES
B	2.50	0.208	0.022	0.395	YES	YES
C	3.15	0.274	0.088	0.460	YES	YES
D	2.78	0.006	-0.180	0.193	YES	YES
E	4.50	0.197	0.010	0.383	YES	YES
F	4.48	0.144	-0.042	0.330	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.220	0.129	NO	+/- 0.500

Figure 11 – Accuracy profile for multi component foods

(Food) Category	Pasta
(Food) Type	Pasta 1 and 2

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.98	0.136	0.058	0.214	YES	YES
B	1.78	0.222	0.144	0.300	YES	YES
C	3.95	-0.057	-0.135	0.021	YES	YES
D	4.03	-0.158	-0.236	-0.080	YES	YES
E	4.95	-0.078	-0.156	0.000	YES	YES
F	4.99	-0.131	-0.209	-0.053	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.170	0.054	NO	+/- 0.500

(Food) Category	Pasta
(Food) Type	Pasta 1 and sandwich

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.98	0.136	-0.004	0.276	YES	YES
B	1.60	0.301	0.161	0.441	YES	YES
C	3.95	-0.057	-0.197	0.083	YES	YES
D	3.78	0.072	-0.068	0.213	YES	YES
E	4.95	-0.078	-0.218	0.062	YES	YES
F	4.96	-0.131	-0.271	0.009	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.179	0.097	NO	+/- 0.500

(Food) Category	Pasta
(Food) Type	Pasta 2 and Sandwich

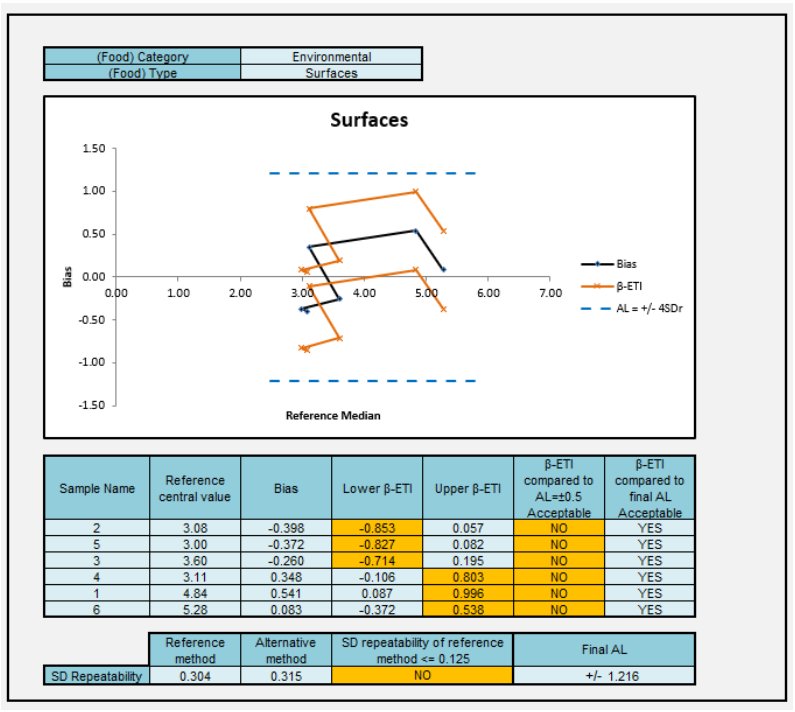
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
A	1.60	0.301	0.167	0.435	YES	YES
B	1.78	0.222	0.088	0.356	YES	YES
C	3.78	0.072	-0.062	0.207	YES	YES
D	4.03	-0.158	-0.292	-0.024	YES	YES
E	4.96	-0.131	-0.265	0.003	YES	YES
F	4.99	-0.131	-0.265	0.003	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.240	0.093	NO	+/- 0.500

Figure 12 – Accuracy profile for raw meat and poultry

Figure 13 – Accuracy profile for RTE foods

Figure 14 – Accuracy profile for environmental samples



(Food) Category	Environmental
(Food) Type	Surfaces

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
2	3.08	-0.398	-0.853	0.057	NO	YES
5	3.00	-0.372	-0.827	0.082	NO	YES
3	3.60	-0.260	-0.714	0.195	NO	YES
4	3.11	0.348	-0.106	0.803	NO	YES
1	4.84	0.541	0.087	0.996	NO	YES
6	5.28	0.083	-0.372	0.538	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.304	0.315	NO	+/- 1.216

For some of the food categories the additional AL calculation was required. This was for the dairy products, fruit and vegetables products and RTE foods.

For the dairy product, 5 of the 9 samples showed an AL above 0.5 logcfu/g. These were for custard batch 1 medium level, custard batch 1 high level, custard batch 2 medium level, custard batch 2 high level, and cream high level. These levels showed a negative bias i.e. a lower level on the alternative method compared to the reference method. The samples were inoculated with *E.mundtii* 16812.

For the fruit and vegetables, only 1 of the 9 samples (parsley batch 1 low level inoculated with *E.faecium* NCIMB 9645) had a slight positive bias of 0.520. All other samples were within the recalculated ALs

For the RTE foods, only 1 of the 9 samples (tuna pate batch 2 low level inoculated with *E. casseliflavus* CRA 16811) had a positive bias of 0.660. All other samples were within the ALs.

After the AL values were recalculated, all the data for the dairy, fruit and vegetables and RTE foods fell within the new ALs the alternative method was accepted as being equivalent to the reference method.

For 2 categories, multi-component foods and raw meat and poultry the AL of 0.5 was achieved and the alternative method was accepted as being equivalent to the reference method without the need for the additional calculation.

Other matrices

In this study, the environmental category required the new AL to be calculated. All of these categories met the new AL value of 1.216.

The accuracy of the Alternative method is satisfied as the environmental category met the recalculated AL.

The large re-calculated AL is due to a high reproducibility for the reference method. Analysis of the data showed that the reproducibility of both the reference and alternative methods was relatively high for the surface samples. There are several possible reasons for differences between replicates when using surfaces samples including:

- Individual inoculation of surfaces
- Uneven drying of the inoculum on surfaces
- Variability in die-off of the organism on each surface replicate

3.3 Inclusivity / exclusivity

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method.

The exclusivity study is a study involving pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.

3.3.1 Protocol
After being grown according to appropriate conditions, decimal dilutions were made and the 50 target strains and 30 non-target strains were enumerated by the alternative method, the reference method and a non selective agar (TSA).

3.3.2 Results
Of the 50 inclusivity strains tested 36 strains were detected and 14 were not detected using the alternative method. For the reference method 33 of the strains were detected and 17 were not detected.

The strains not detected for either method were*: E. cecorum*, 16849; *E. aquamarinus*, 16813; *E. dispar*, 16850, *E. columbae*, 16851; *E. pseudoavium*, 16852; *E. sulfureus*, 16853; *E. seriolicida*, 16854; *E. flavescens*, 16855; *E. sacharolyticus*, 16863; *E. dispar*, 16864; *E. xiangfangensis*,16865; *E. solitarus*, 16867.

Those not detected by the alternative method but detected by the reference method were: *E. durans*, 16810; *E. porcinus*16857.

Those not detected by the reference method but detected by the alternative method were: *E. durans*, 16464; *E. haemoperoxidus*, 16858; *E. thailandicus*, 16859; *E.malodoratus*, 16860; *E. gallinarum*, 16861.

It would appear that both methods were good at detecting the more usual Enterococcus species, i.e. *E. faecalis* and *E. faecium*, but less good at detecting other species. In the inclusivity study there were 50 strains of Enterococci covering 23 different species. The Compact Dry ETC method was more specific as it detected 11 of the 23 different species where as the reference method only detected 8 of the different species.

Of the 30 exclusivity strains tested, 28 were not detected and 2 were detected using both the reference and alternative methods. The 2 detected cultures were *Lactobacillus gasseri CRA 6804* and *Streptococcus lactis* CRA 527.

3.4 Limit of quantification (LOQ)

The LOQ applies only to instrumental methods. It does not apply to methods based on counting visible colonies. It may also not apply to instrumental methods where it is not possible to get blank samples e.g. instrumental methods for total plate counts.

The alternate method is based on visible colonies therefore the LOQ does not have to be calculated for the alternative method in this study.

3.5 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The Compact Dry ETC for enumeration of Enterococci in foods method shows satisfying trueness
- The Compact Dry ETC for enumeration of Enterococci in foods method shows satisfactory and accuracy profile.
- The Compact Dry ETC for enumeration of Enterococci in foods method was shown to be specific and selective. Compared to the Reference method it was able to detect more inclusivity cultures covering a wider range of species).

4 Interlaboratory study

The Inter laboratory study was prepared during week commencing 21st November 2016 and run during week commencing 28th November 2016.

4.1 Organisation

There were 5 organisations used in this study representing 3 different countries. The number of collaborators from each organisation varied from 1 to 3 (according to ISO16140-2:2016 6.2.2) giving a maximum of 11 potential data sets. Three of the data sets (f, g, h) were not used in the analysis due to incomplete data for the reference method, even though the alternative method performed well. So finally, there were 8 valid data sets from 4 different organisations and 3 different countries

Matrix and strain used

Chilled salmon pâté was inoculated with *Enterococcus faecalis* NCIMB 775.

Samples

For each of the 11 collaborators participating in the interlaboratory study 7 x 10g samples of salmon pâté were weighed into sterile stomach bags. One sample of pâté remained uninoculated. For the remaining six samples, appropriate dilutions of the *E.faecalis* culture were used to individually inoculate 2 x 10g samples at the low (~10^2 cfu/ml), middle (~10^4cfu/ml) and high (~10^6cfu/ml) contamination levels.

Labelling

The samples were blind-coded (as shown in Table 7). Where more than 1 collaborator was used at an organization, different blind coding numbers were used for the replicate sets of samples. After inoculation, the samples were frozen for 72 hours prior to despatch Stability test were done to establish the effect of freeze thawing on the levels of *E.faecalis* contained in samples and the stability of the inoculated during chilled 72 hours chilled transportation was tested. Additionally, a set of samples was prepared at the same time, for the Expert laboratory (Campden BRI) organising the trial to confirm the presence of the target organism and the contamination levels. These data were not used in the analysis

Table 7 Sample codes and contamination levels of E.faecalis in salmon pâté samples used in the collaborative study

Contamination level	**Sample code set 1**	**Sample code set 2**	**Sample code set 3**
Uninoculated	7	14	18
Low (10^2 cfu/g)	1	8	21
Low (10^2 cfu/g)	9	2	15
Medium (10^4 cfu/g)	11	3	17
Medium (10^4 cfu/g)	10	4	19
High (10^6 cfu/g)	12	5	20
High (10^6 cfu/g)	6	13	16

Shipping

Prior to despatch, each set of samples was removed from the freezer and packed into plastic containers (Air-Sea Containers Limited, code 490). These plastic containers were then placed inside a thermal control unit (Air-Sea Containers Limited, TC-20 code 802) with cool packs (Air-Sea Containers Limited, CP-20 code 405). Each laboratory also received an additional vial containing water “temperature control sample” which was packed with the test samples.

This was used to enable the laboratory to take a temperature measurement, representative of the samples, upon receipt. In addition to this a continuous electronic temperature monitor (Thermochron iButton) was placed in the sample packages. The laboratories were requested to return the ibuttons to the expert laboratory upon receipt. The target storage conditions were for the temperature to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

Shipping was arranged so that each laboratory would receive their samples within 24 to 72h dependent on location and speed of the International courier service. The condition of the samples was recorded by each laboratory on a supplied form.

The analyses were started on Tuesday 29th November 2016.

Stability testing:

Stability testing was done prior to despatch of the samples. A set of samples was produced at the highest inoculation level and was tested immediately after inoculation, and 24 h, 48 h and 72h after removal from the freezer and storage at 8±°C.

Study protocol

The protocol for the Interlaboratory study was sent to the collaborative laboratories prior to the study date. The protocol gives detailed instructions for the method analysis and reporting of results.

4.2 Study results

The collaborative study was analysed in accordance with the protocol in ISO 16140 (6.3) to comparatively determine the performance characteristics of the Compact Dry ETC method against the reference method for the enumeration of Enterococcus in foods. The raw data from the inter-laboratory study is given in Appendix 5.

Data set H is for samples analysed by the Expert lab on 29/11/2016. These were not included in any statistical analysis.

Comments from Collaborative laboratories

Comments were received from some of the laboratories about the ease of interpretation of the reference method. Several laboratories noted that the colonies were pale and difficult to count on the reference media and in some cases there were no colonies detected on the reference medium at all.

For the ETC, there were no comments recorded and all collaborators were able to enumerate the levels of Enterococcus in all inoculated samples.

So it would appear that ETC plates were easier to count than the reference agar.

Receipt of samples

Three laboratories (1, 4, and 5) received their samples on 28/11/2016 and 1 laboratory (3) received their samples on 29/11/2016. Labs receiving samples on 28/11/2016 stored their samples refrigerated (<8°C) over night and all labs tested the samples on 29/11/2016. The last lab (2) did not receive their samples until 02/12/16 as they had been held up in customs. They refrigerated them at <8°C for a further 3 days and then tested them finally on 5/12/16. Whilst the sample were tested on a different day to the other laboratories, it is considered that the

samples remained sufficiently stable during this period for use in the final analysis as the data obtained was not substantially different from that obtained by the other labs used in the study (see 3.5)

Condition of samples

The temperature range of the samples upon receipt by the collaborative laboratories (Table 8) was variable. It ranged from 4.1 to 15°C for lab 2 which had a delay in the samples being held at customs. The Ibutton data shows the temperature profile of the samples throughout transport. Despite any deviations in temperatures, all samples were received in good condition by the labs and any storage of samples above 8°C did not appear to adversely affect the samples as seen in the analysis of the differences in data between labs in section (3.5). It was therefore decided on this occasion to accept complete data sets from all the labs even if the water vial temperature exceeded 8°C on receipt

Table 8 Temperature of control sample upon receipt

Laboratory	Date received	Temperature of control sample upon receipt (°C)	Average storage temperature (°C) over entire transport period
1	28/11/2016	4.1	1.9
2	02/12/2016	15	6.4
3	29/11/2016	10.5	8.2
4	28/11/2016	5.7	2.3
5	28/11/2016	7.3	5.9
Expert lab	28/11/2016	6.5	6.7

Stability of samples

Four replicate samples of the high inoculation level salmon pâté samples were enumerated on ETC at time zero (immediately after inoculation and prior to freezing) and after 24h, 48h and 72h storage after removal from the freezer and placing into an incubator set at 8±1°C.

Table 9: Levels of Enterococcus (Log10 cfu/g) in stability samples stored at 8±1°C

Replicate	0h	24h	48h	72h
1	6.20	6.15	6.34	6.48
2	6.11	6.26	6.34	6.43
3	6.15	6.28	6.28	6.45
4	6.16	6.23	6.32	6.45
Mean value	**6.16**	**6.23**	**6.32**	**6.45**

The data showed that the levels of *Enterococcus* were not affected by the freezing process and were stable during chill storage with a mean increase in level of 0.3 logs after 72h at 8±1°C.

Analysis of between- laboratory differences

The storage temperatures of the samples transported to the different laboratories was variable and one laboratory received and tested their samples after the other laboratories. In order to visualise whether this was likely to have a substantial impact on the study, the data was plotted using Minitab 17.3.1

Figures 15 and 16 show the individual value plot of the data by labs and by level for the Reference method and the ETC alternative method respectively. A visual check of this data does not indicate any unusual patterns in the data.

Figure 17 summarises the differences between each labs results, and the mean across all labs for the same level. The symbols show the mean of the six differences for each lab (3 levels x 2 replicates). The bars show 95% confidence intervals on those means, corrected (Bonferroni) for the multiple comparisons implicit in each plot. For the ETC data, collaborators a, b, i, j, k are slightly below the collaborators c, d, e, and expert.

Although the data for sets for c, d and e which were obtained from laboratories with a temperature on receipt above 8°C show a positive deviation from the zero line , they show a similar level to the data from the expert laboratory which had been stored at the correct temperature. In addition the confidence intervals for the data set overlap for some of the groups with a positive deviation from zero and some of the groups with a negative deviation from zero. Deviations were all <0.5logs from the zero line. Based on the spread of the data and the magnitude of the residuals it seems reasonable to include all data in the subsequent analysis.

Figure 15: Visual analysis of data for the reference method

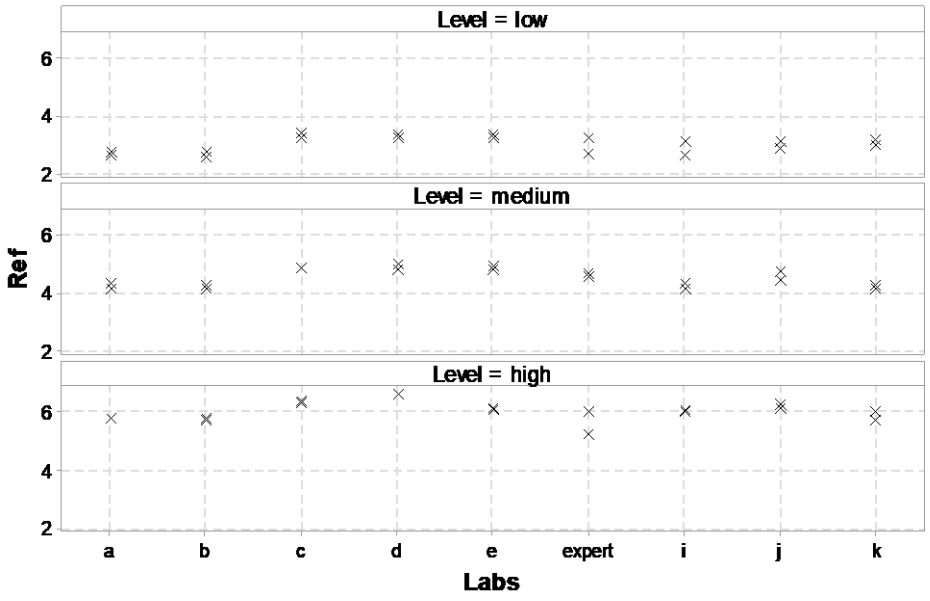


Figure 16: Visual analysis of data for the alternative (ETC) method

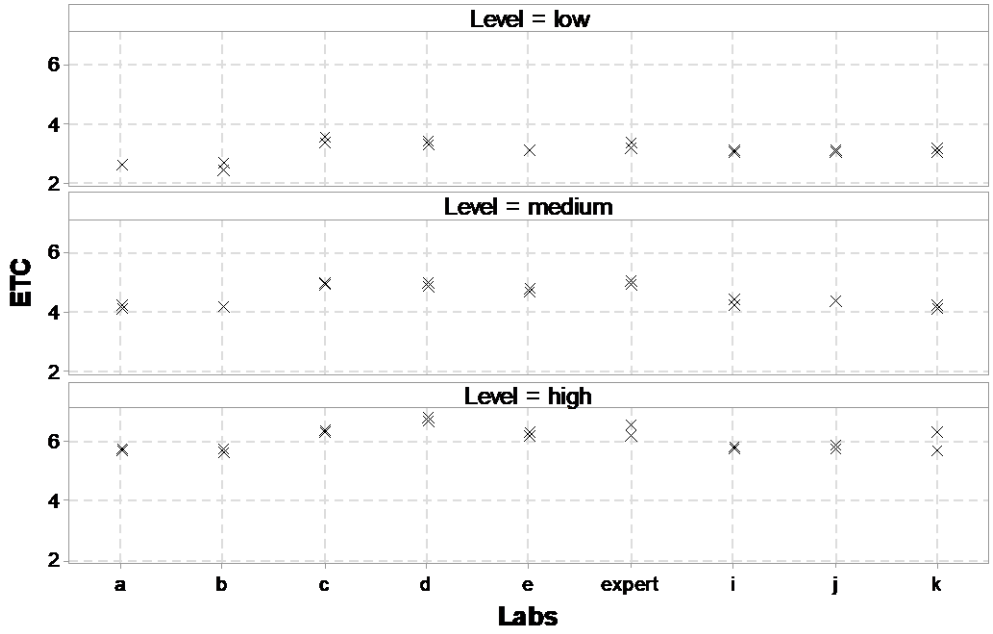
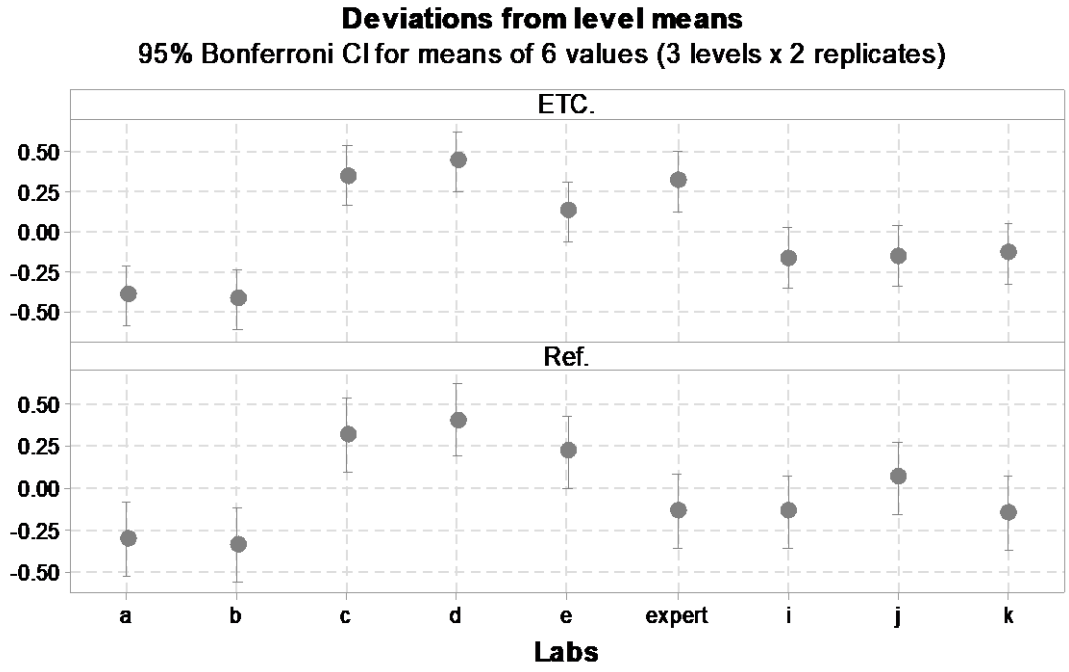


Figure 17: Visual analysis of the deviations for each laboratory from the level means



The pooled standard deviation is used to calculate the intervals.

4.3 Calculation and interpretation of data

The data from the collaborative trial were calculated and interpreted according to section 6.2.3 of ISO 16140-2:2016 using the freely available Excel® spreadsheet (http://standards.iso.org/iso/16140).

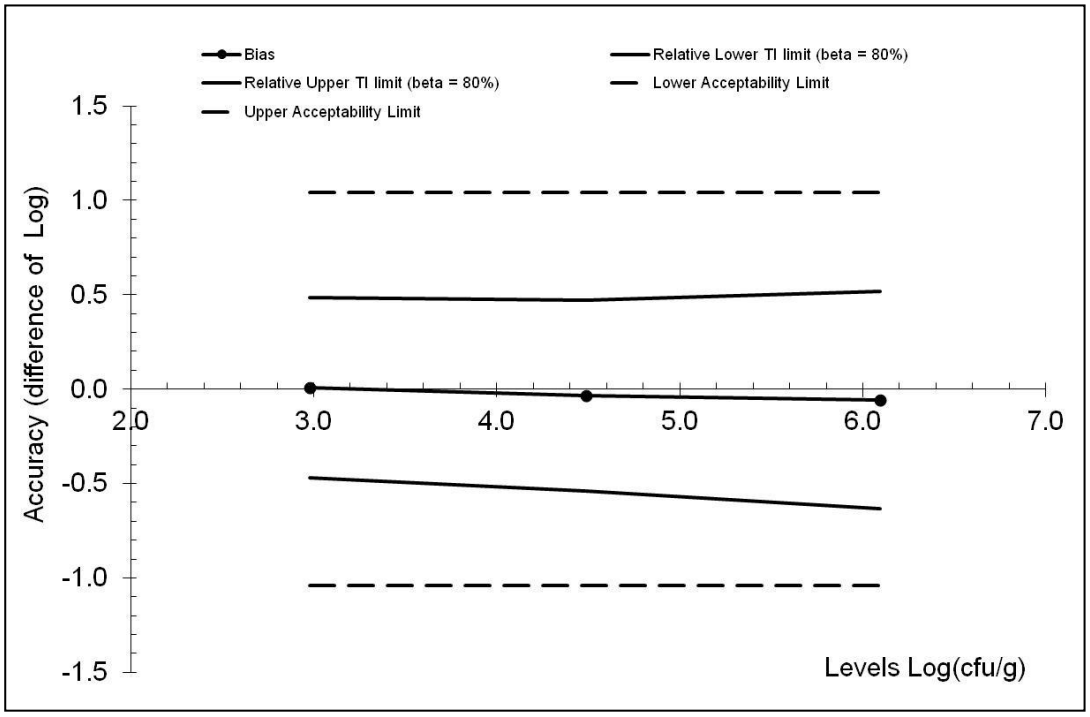
The log transformed data from the existing trial is shown in Table 10 below and the Accuracy profile graph is shown in Figure 18.

Table 10: Summary of the results of the interlaboratory study per analyte level (k)

		Reference method x_{ijk}		**Alternative method k_{ijk}**	
Collaborators (i)	**Level (k)**				
A	Blank	<10		<10	
B	Blank	<10		<10	
C	Blank	<10		<10	
D	Blank	<10		<10	
E	Blank	<10		<10	
I	Blank	<10		<10	
J	Blank	<10		<10	
K	Blank	<10		<10	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	Low	2.699	2.568	2.550	2.561
B	Low	2.491	2.672	2.380	2.630
C	Low	3.204	3.369	3.320	3.490
D	Low	3.196	3.294	3.339	3.249
E	Low	3.324	3.163	3.031	3.048
I	Low	2.602	3.076	3.000	3.059
J	Low	2.845	3.072	3.038	3.000
K	Low	2.954	3.134	3.114	2.963
A	Medium	4.111	4.277	4.079	4.194
B	Medium	4.140	4.244	4.123	4.173
C	Medium	4.862	4.834	4.862	4.959
D	Medium	4.963	4.778	4.967	4.810
E	Medium	4.765	4.878	4.649	4.785
I	Medium	4.138	4.287	4.214	4.406
J	Medium	4.436	4.699	4.320	4.357
K	Medium	4.260	4.105	4.226	4.102
A	High	5.778	5.791	5.699	5.751
B	High	5.751	5.737	5.631	5.744
C	High	6.342	6.362	6.350	6.322
D	High	6.633	6.643	6.826	6.663

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
E	High	6.102	6.152	6.186	6.279
I	High	6.105	6.008	5.729	5.822
J	High	6.135	6.260	5.751	5.839
K	High	6.041	5.691	6.301	5.707

Figure 18: Accuracy profile of the alternative method (ETC) in the Inter laboratory study



The statistical analysis of the ILS data is shown in Table 5 below. It can be seen that the repeatability standard deviation (S_r) was similar for the alternative and reference method ranging from 0.087 to 0.162 for ETC and 0.097 to 0.162 for the reference method.

The between-labs standard deviation (S_L) was microbiologically similar for the alternative method (0.309 to 0.355) and the reference method (0.252 to 0.315) as was the reproducibility standard deviation (S_R) showing (0.321 to 0.391) for the alternative method and (0.300 to 0.312) for the reference method.

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the Acceptability Limits AL (±0.5log units)then a further calculation is done to calculate the pooled average SR of the reference method. This was done and gave an SR value of 0.315. This value was used to recalculate the new AL as a function of the standard deviation (ALs) using the formula 3.3 x SR,ref which gives new ALs values of +1.04 and -1.04.

Whilst quite large, the re-calculated AL is similar to those found in the methods comparison study where the AL's ranged from 0.500 to 1.244 for the 5 different product categories, with an average of 0.7

Looking at Figure 4, it can be seen that no values lie outside of these new ALs values and therefore the alternative method is accepted as being equivalent to the reference method.

5 Overall conclusions of the validation study

- The alternative method CD ETC for enumeration of *Enterococcus* shows satisfactory results for relative trueness;
- The alternative method CD ETC for enumeration of *Enterococcus* shows satisfactory results for accuracy profile;
- The alternative method CD ETC for enumeration of *Enterococcus* is selective and specific, as shown in the results of the initial study ref 2014LR48.
- The alternative method CD ETC for enumeration of *Enterococcus* shows satisfactory performance in the ILS as shown in the results of the initial study ref 2014LR48.
- The alternative CD ETC for enumeration of *Enterococcus* shows comparable performance to the reference method NMKL Method No. 68 5th Edition 2011: Enterococcus. Determination in foods and feeds.

Date, 12/09/23

Signature Suzanne Jordan

6 References

Nissui Compact Dry ETC Kit insert version February 2020

NMKL Method No. 68 5th Edition 2011: Enterococcus. Determination in foods and feeds.

ISO 6887; Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – All parts.

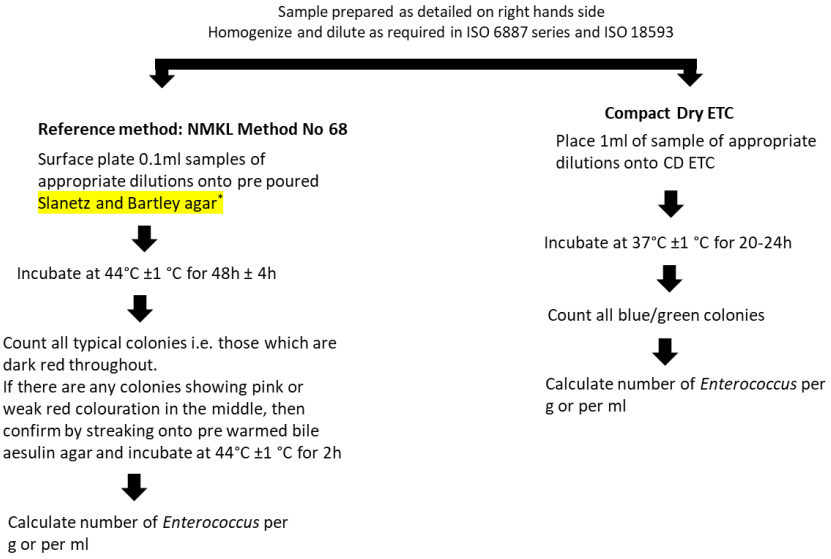
ISO 7218; Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations.

ISO 16140-2:2016; Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

2016-028 (GC) Proposed MicroVal Technical committee interpretation of ISO 16140-2 and ISO

2017-063 (TC) The MicroVal Process

ANNEX A: Flow diagram of the reference method and alternative methods



ANNEX B: Kit insert(s)

Refer to separate pdf

ANNEX C: Raw data per category relative trueness

Artificial contamination of the samples

Food Category	Sample code	Food item	strain	spiking protocol	seeding protocol	injury level
RTE Foods	7	Breaded chicken strips	*E.mundtii* 16812		Freezing for 10 days	
RTE Foods	129	Cheese and bacon quiche	*E.faecalis* 7297		Chilled 4 days	
RTE Foods	147	Chicken liver pate	*E.mundtii* 16812		Freezing for 10 days	
RTE Foods	146	Salmon pate	*E.mundtii* 16812		Chilled 4 days	
Raw poultry and meats	70	Chorizo	*E.mundtii* 16812		Freezing for 10 days	
RTE Foods	62	Cooked cocktail sausages	*E. faecalis* 4132		Chilled 4 days	
Multi-component foods	109	Feta and chickpea salad	*E. faecium* 9645	6ppm chlorine 1min		0.34
Multi-component foods	85	Microwave frozen rice	*E. faecalis* 4132		Freezing for 10 days	
Dairy	125	Grated cheddar cheese	E. casseliflavus 16811		Chilled 4 days	
Multi-component foods	80	Potato salad	*E. casseliflavus* 16811		Chilled 4 days	
RTE Foods	58	Hot smoked salmon	*E. faecalis* 4132		Freezing for 10 days	
Dairy	138	Jarlsberg cheese	*E. hirae* 16809		Chilled 4 days	
Dairy	65	Emmental cheese	*E. hirae* 16809		Chilled 4 days	
Multi-component foods	56	Ham sandwich	*E.faecalis* 7297		Chilled 4 days	
Multi-component foods	112	Egg and cress sandwich	*E. hirae* 16809		Chilled 4 days	
RTE Foods	5	Frozen king prawns	*E. faecalis* 4132		Freezing for 10 days	
Dairy	111	Olive spread	*E.mundtii* 16812		Freezing for 10 days	
RTE Foods	31	Parma ham	*E.faecalis* 7297		Chilled 4 days	
RTE Foods	140	Pastrami	*E. casseliflavus* 16811		Chilled 4 days	
Multi-component foods	3	Pilau rice	*E. faecalis* 4132		Freezing for 10 days	
Multi-component foods	86	Chinese rice	*E.faecalis* 7297		Freezing for 10 days	
Multi-component foods	12	Prawn pasta salad	*E. hirae* 16809		Chilled 4 days	
RTE Foods	48	Savoury eggs	*E. casseliflavus* 16811		Freezing for 10 days	
RTE Foods	61	Salami	*E. faecalis* 4132		Freezing for 10 days	

Food Category	Sample code	Food item	strain	spiking protocol	seeding protocol	injury level
RTE Foods	82	Seafood sticks	*E.faecalis* 7297		Freezing for 10 days	
Dairy	45	Cookie dough ice-cream	*E. casseliflavus* 16811		Freezing for 10 days	
Dairy	35	Caramel ice cream	*E. casseliflavus* 16811		Freezing for 10 days	
Multi-component foods	17	Coleslaw	*E. hirae* 16809		Chilled 4 days	
Dairy	92	Butter	*E.faecalis* 7297		Chilled 4 days	
RTE Foods	1	Frozen prawns	*E.mundtii* 16812		Freezing for 10 days	
RTE Foods	49	Seafood terrine	*E. faecium* 9645	55C/5minutes		0.99
Dairy	57	Boursin black pepper cream cheese	*E.faecalis* 1528	pH2 60 min		2.16
Dairy	41	Cheesy bacon cheese spread	*E.hirae* 15939	pH2 60min		0.85
Dairy	115	Pineapple and almond cream cheese roll	*E. avium* 702366	55C/5minutes		0.54
Dairy	63	Cheddar and onion	*E.avium* 701605	55C/5minutes		0.39
Dairy	55	Apricot and wednesleydale	*E.avium* 701605	pH 2 15minutes		0.34
Dairy	94	Caramel and chocolate dessert	*E. avium* 702366	pH2 15minutes		0.31
Dairy	114	Tiramisu	*E.avium* 701605	50C/5minutes		0.349
Multi-component foods	131	Celery, fruit and peanut salad	*E. faecium* 9645	6ppm chlorine 1min		0.34
Dairy	52	Apricot fool	*E. faecium* 9645	55C/5minutes		0.99
Fruit and Vegetables	40	Fresh beansprouts	*E.hirae* 15939	pH2 60min		1.1
Fruit and Vegetables	67	Fresh coriander	*E.avium* 701605	pH 2 15minutes		0.34
Fruit and Vegetables	28	Fresh parsley	*E. avium* 702366	pH2 15minutes		0.31
RTE Foods	25	Cooked seasoned chicken bites	*E. avium* 702366	55C/5minutes		0.54
RTE Foods	30	Cooked chicken breast	*E.avium* 701605	55C/5minutes		0.39
RTE Foods	43	Sweet chilli cooked turkey bites	*E.avium* 701605	50C/5minutes		0.39
Fruit and Vegetables	123	Black peppercorns	*E. faecium* 9645	50C/5minutes		0.32
Fruit and Vegetables	2	Dried mixed herbs	*E. faecium* 9645	55C/5minutes		0.99
Fruit and Vegetables	128	Dried cumin	*E. faecium* 9645	50C/5minutes		0.99
Multi-component foods	77	Florida salad	*E. avium* 702366	55C/5minutes		0.54
Multi-component foods	18	Morrocan cous cous	*E.hirae* 15939	pH2 60min		0.85
Raw poultry and meats	149	Minted lamb grills	*E.faecium* NCIMB 700580		Freezing for 10 days	

Food Category	Sample code	Food item	strain	spiking protocol	seeding protocol	injury level
Raw poultry and meats	101	Beef quarter pounders	*E.faecalis* NCIMB1993		Freezing for 10 days	
Raw poultry and meats	23	Persian spiced chicken burgers	*E.faecalis* 12672		Chilled 4 days	
Raw poultry and meats	83	Moroccan spiced lamb burgers	*E.faecium* NCIMB 700580		Chilled 4 days	
Raw poultry and meats	162	Pulled pork shoulder burgers	*E.faecalis* NCIMB1993		Chilled 4 days	
Raw poultry and meats	91	Lean mince beef, 5% fat	*E. faecium* 9645	6ppm chlorine 1min		0.34
Raw poultry and meats	88	Veal mince (typically 15% fat)	*E. faecium* 9645	6ppm chlorine 1min		0.34
Raw poultry and meats	50	Beef mince, 15% fat	*E.faecalis* NCIMB1993		Freezing for 10 days	
Raw poultry and meats	168	Lamb mince	*E.faecalis* 12672		Chilled 4 days	
Raw poultry and meats	103	Pork mince	*E.faecalis* 12672		Chilled 4 days	
Raw poultry and meats	46	Chicken mini fillets	*E.faecium* NCIMB 700580		Chilled 4 days	
Raw poultry and meats	153	Chicken breast in, lemon & apricot marinade	*E.faecium* NCIMB 700580		Freezing for 10 days	
Raw poultry and meats	89	Green Thai chicken kebabs	*E.faecalis* NCIMB1993		Freezing for 10 days	
Raw poultry and meats	165	Whole chicken	*E. faecium* 9645	6ppm chlorine 1min		0.34
Raw poultry and meats	152	Boneless chicken breasts	*E. faecium* 9645	6ppm chlorine 1min		0.34
Multi-component foods	132	Singapore noodles	*E.faecalis* 12672		Chilled 4 days	
Fruit and Vegetables	90	Organic whole cardamom	*E.faecalis* 12672		Leave at room temperature 2 weeks	
Fruit and Vegetables	151	Dried rosemary	*E.faecalis* 12672		Leave at room temperature 2 weeks	
Fruit and Vegetables	157	Organic kale	*E.faecalis* NCIMB1993		Chilled 4 days	
Fruit and Vegetables	33	Organic spinach	*E. faecium* 9645	6ppm chlorine 1min		0.34
Fruit and Vegetables	13	Sliced raw british greens	*E. faecium* 9645	6ppm chlorine 1min		0.34
Fruit and Vegetables	22	Frozen bean sprout stir-fry	*E. faecium* 9645	6ppm chlorine 1min		0.34
Fruit and Vegetables	116	Cress	*E. faecium* 9645	6ppm chlorine 1min		0.34
Fruit and Vegetables	9	Pea shoots	*E. faecium* 9645	6ppm chlorine 1min		0.34

Food Category	Sample code	Food item	strain	spiking protocol	seeding protocol	injury level
Fruit and Vegetables	16	Organic alfalfa sprouts	*E.faecalis* NCIMB1993		Chilled 4 days	
Multi-component foods	64	Sweet chilli chicken wrap	*E.faecalis* NCIMB1993		Chilled 4 days	
Multi-component foods	39	Salmon and King Prawn sandwich	*E.faecium* NCIMB 700580		Chilled 4 days	

	NMKL Method 68					Compact Dry ETC			
Food item	**Dilution(0.1ml)**	**cfu/plate**	**cfu/g**	**Log cfu/g**		**Dilution (1ml)**	**cfu/plate**	**cfu/g**	**Log cfu/g**
Breaded chicken strips	-1 *	3	50	1.70		-1	25	210	2.32
	-1*	2				-1	17		
Cheese and bacon quiche	-1	18	1800	3.26		-1	114	1163	3.07
	-2	0				-2	13		
Chicken liver pate	-1	86	8454	3.93		-2	17	1727	3.24
	-2	7				-3	2		
Salmon pate	-2	64	62727	4.80		-3	25	33636	4.53
	-3	5				-4	2		
Chorizo	-3	68	663636	5.82		-3	158	156363	5.19
	-4	5				-4	14		
Cooked cocktail sausages	-1*	6	110	2.04		-1	17	170	2.23
	-1*	5				-1	0		
Feta and chickpea salad	-1	14	1455	3.16		-2	57	5545	3.74
	-2	2				-3	4		
Microwave frozen rice	-1	83	8272	3.92		-2	63	6363	3.80
	-2	3				-3	7		
Grated cheddar cheese	-1	31	3636	3.56		-2	38	33636	4.53
	-2	9				-3	2		
Potato salad	-2	107	104545	5.02		-3	57	54545	4.74
	-3	8				-4	3		
Hot smoked salmon	-1*	21	310	2.49		-1	18	180	2.26
	-1*	10				-1	18		
Jarlsberg cheese	-1*	36,39	1425	3.15		-1	>200	1900	3.28
	-1	21				-2	19		
Emmental cheese	-1	142	14272	4.15		-2	140	13636	4.13
	-2	15				-3	10		
Ham sandwich	-2	143	139000	5.143		-3	69	64545	4.81
	-3	10				-4	2		
Egg and cress sandwich	-4	26	2600000	6.415		-5	29	2900000	6.46
	-5	0				-6	0		

Frozen king prawns	-1*	9	120	2.079		-1	14	150	2.18
	-1*	3				-1	16		
Olive spread	-1*	9,12	305	2.484		-1	10	100	2.00
	-1*	4				-2	0		
Parma ham	-1	66	7181	3.856		-2	24	2545	3.41
	-2	13				-3	4		
Pastrami	-2	55	53636	4.729		-3	32	31818	4.50
	-3	4				-4	3		
Pilau rice	-4	14	1400000	6.146		-4	92	881818	5.95
	-4	0				-5	7		
Chinese rice	-1*	1	20	1.301		-1	22	175	2.24
	-1*	1				-1	13		
Prawn pasta salad	-1	23	2300	3.362		-1	133	1281	3.11
	-2	0				-2	8		
Savoury eggs	-1	66	6454	3.810		-2	39	3909	3.59
	-2	5				-3	4		
Salami	-2	>150	1550000	6.190		-3	>200	710000	5.85
	-3	155				-4	71		
Seafood sticks	-3	19	181818	5.260		-3	143	139091	5.14
	-4	1				-4	10		
Cookie dough ice-cream	-1*	4	50	1.699		-1	5	60	1.78
	-1*	1				-1	7		
Caramel ice cream	-1*	12,33	425	2.628		-1	49	481	2.42
	-1	4				-2	4		
Coleslaw	-1	162	16000	4.204		-2	104	11300	4.05
	-2	14				-3	9		
Butter	-2	95	93636	4.971		-3	143	139090	5.14
	-3	8				-4	10		
Frozen prawns	-3	51	481818	5.683		-3	90	88181	4.95
	-4	2				-4	7		
Seafood terrine	-1	6	600	2.778		-2	17	17273	4.24
	-2	0				-3	2		
Boursin black pepper cream	-1	91	9818	3.992		-1	54	545	2.74

Cheese									
	-2	17				-2	6		
Cheesy bacon cheese	-1	>150	121000	5.083		-2	178	18000	4.26
	-2	121				-3	20		
Pineapple and almond cream cheese roll	-1	31	3091	3.490		-2	12	1200	3.08
	-2	3				-3	0		
Cheddar and onion	-1*	30	590	2.771		-1	14	173	2.24
	-1*	29				-2	5		
Apricot and wednesleydale	-1	76	7273	3.862		-1	>200	3800	3.58
	-2	4				-2	38		
Caramel and chocolate	-1	77	7455	3.872		-2	75	7727	3.89
	-2	5				-3	10		
Tiramisu	-3	16	163636	5.214		-3	10	10000	4.00
	-4	2				-4	0		
Celery, fruit and peanut	-1	4	545	2.736		-1	75	718	2.86
	-2	2				-2	4		
Apricot fool	-1	11	1100	3.041		-2	155	14273	4.15
	-2	0				-3	2		
Fresh beansprouts	-3	>150	146000	5.164		-2	130	14182	4.15
	-4	146				-3	26		
Fresh coriander	-1	51	5100	3.708		-1	148	1400	3.15
	-2	0				-2	6		
Fresh parsley	-1	82	7636	3.883		-2	24	2455	3.39
	-2	2				-3	3		
Cooked seasoned chicken	-2	2	2000	3.301		-3	2	2000	3.30
	-3	0				-4	0		
Cooked chicken breast	-1*	61,56	885	2.947		-1	43	409	2.61
	1	6				-2	2		
Sweet chilli cooked turkey	-3	24	240000	5.380		-3	25	25455	4.41
	-4	1				-4	3		
Black peppercorns	-1	99	9545	3.980		-2	172	17545	4.24
	-2	6				-3	21		

Dried mixed herbs	-2	7	7000	3.845		-3	18	17273	4.24
	-3	0				-4	1		
Dried cumin	-1*	36,35	805	2.906		-2	37	3455	3.54
	-1	9				-3	1		
Florida salad	-1*	33.37	1750	3.243		-1	189	1827	3.26
	-1	28				-2	12		
Morrocan cous cous	-2	44	43636	4.640		-2	>20	27000	4.43
	-3	4				-3	27		
Minted lamb grills	-1	>150	64000	4.81		-2	>200	43000	4.63
	-2	64				-3	43		
Beef quarter pounders	-2	91	98182	4.99		-2	>200	86000	4.93
	-3	11				-3	86		
Persian spiced chicken burgers	-1	>150	800	2.903		-1	16	227	2.36
	-2	8				-2	9		
Moroccan spiced lamb	-1	104	10364	4.016		-2	85	8455	3.93
	-2	10				-3	8		
Pulled pork shoulder	-3	34	318182	5.503		-3	160	168182	5.23
	-4	1				-4	25		
Lean mince beef, 5% fat	-1	26	2909	3.464		-1	150	1500	3.18
	-2	6				-2	2		
Veal mince (15% fat)	-2	29	85455	4.932		-3	56	55455	4.74
	-3	5				-4	5		
Beef mince, 15% fat	-2	>150	110000	5.041		-3	>200	820000	5.91
	-3	111				-4	82		
Lamb mince	-1	76	7455	3.872		-3	42	40000	4.60
	-2	6				-4	2		
Pork mince	-2	87	85455	4.932		-2	>200	44000	4.64
	-3	7				-3	44		
Chicken mini fillets	-1*	3,2	75	1.875		-1	>200	28000	4.45
	-1*	1				-2	28		
Chicken breast in, lemon & apricot marinade	-2	82	83636	4.922		-2	>200	33000	4.52
	-3	10				-3	33		

Green Thai chicken kebabs	-2	>150	610000	5.785		-3	>200	240000	5.38
	-3	61				-4	24		
Whole chicken	-2	114	12000	4.079		-2	>200	43000	4.63
	-3	18				-3	43		
Boneless chicken breasts	-2	122	1245455	6.095		-4	51	536364	5.73
	-3	15				-5	8		
Singapore noodles	-1*	2,1	65	1.813		-2	4	400	2.60
	-1	1				-3	0		
Organic whole cardamom	-2	35	32727	4.515		-1	5	50	1.70
	-3	1				-2	0		
Waitrose cooks' ingredients Wild rosemary	-3	>150	3020000	6.480		-3	>200	320000	5.51
	-4	30				-4	32		
Organic kale	-1	29	3273	3.515		-3	21	22727	4.36
	-2	7				-4	4		
Organic spinach	-3	43	427273	5.631		-4	33	318182	5.50
	-4	4				-5	2		
Sliced raw british greens	-3	17	172727	5.237		-3	179	175455	5.24
	-4	2				-4	14		
Frozen bean sprout stir-fry	-1	4	545	2.736		-1	48	455	2.66
	-2	2				-2	2		
Cress	-3	>150	2000000	6.301		-4	131	1363636	6.13
	-4	20				-5	19		
Pea shoots	-3	22	218182	5.339		-3	153	158182	5.20
	-4	2				-4	21		
Organic alfalfa sprouts	-1	13	1300	3.114		-2	17	1636	3.21
	-2	0				-3	1		
Sweet chilli chicken wrap	-1*	11,11	160	2.204		-1	23	236	2.37
	-1	1				-2	3		
Salmon and King Prawn sandwich	-2	50	51818	4.714		-3	56	54545	4.74
	-3	7				-4	4		
Spring onion cream cheese	-1*	0	<10	<1		-1	0	<10	<1

	-1*	0				-1	0		
Low fat cream cheese	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Garlic and herbs cream	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cheese strings spread	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Stilton (pasteurised)	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Brie (pasteurised)	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Red leicester (pasteurised)	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Wednesleydale	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cheddar (pasteurised)	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Trifle	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Fudge fool	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cottage cheese	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Lemon cheescake	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Parsley	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Lettuce	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Tomato and tuna pasta	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cheese and spring onion	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		

Sweet chilli noodle pasta	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Pilau rice	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Frozen beef burgers	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Raw chicken fillets	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Raw beef mince	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Raw lamb mince	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cooked chicken roll	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cooked breaded ham	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cooked sliced ham	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Liver and bacon pate	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Tuna pate batch 1	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Tuna pate batch 2	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		
Cooked chilled prawns	-1*	0	<10	<1		-1	0	<10	<1
	-1*	0				-1	0		

ANNEX D: Calculation and interpretation of relative trueness

Type	Item	Sample code	log(Ref)	log(Alt)	Mean	Difference
Dust	Metal - industrial kitchen sink	311	2.8	2.7	2.7	-0.03
Dust	Ceramic - sink splashback	312	3.9	3.8	3.8	-0.10
Dust	Rubber - equipment piping	313	4.8	4.7	4.8	-0.14
Dust	Plastic - equipment surface	314	5.8	5.7	5.8	-0.15
Dust	MDF - lab bench	315	6.9	6.8	6.9	-0.09
Process water	Cooling water	306	3.0	3.0	3.0	0.08
Process water	Wash water	307	4.0	4.1	4.0	0.08
Process water	Cooling water 2	309	5.9	5.8	5.9	-0.08
Process water	Surface run off - industrial kitchen	310	7.0	7.0	7.0	0.02
Process water	High pressure cooling water	34B	2.9	2.9	2.9	0.01
Surfaces (wipes, swabs)	Industrial kitchen sink corner	301	3.2	3.0	3.1	-0.13
Surfaces (wipes, swabs)	Radiator	302	4.0	4.1	4.1	0.11
Surfaces (wipes, swabs)	Oven top	303	4.0	4.1	4.1	0.04
Surfaces (wipes, swabs)	Base of equipment	304	5.9	6.0	6.0	0.05
Surfaces (wipes, swabs)	Bakery sink corner	305	7.0	7.1	7.0	0.11

ANNEX E: Raw data accuracy profile study

MICROVAL® NEN

DAIRY PRODUCTS									
			NMKL Method 68				Compact Dry ETC		
Food item	Inoculum	Sample number	Dilution (0.1ml) cfu/ plate	cfu/g	Log cfu/g		Dilution(1ml) cfu/ plate	cfu/g	Log cfu/g
Custard batch 1	Low	84a	(-1)* 2,5	70	1.85		(-1),3 (-2) 0	30	1.48
		84b	(-1)* 4,8	120	2.08		(-1),3 (-2) 0	30	1.48
		84c	(-1)* 1,3	40	1.6		(-1),6 (-2) 0	60	1.78
		84d	(-1)* 3,3	60	1.78		(-1),6 (-2) 0	60	1.78
		84e	(-1)* 13,8	210	2.32		(-1),5 (-2) 0	50	1.70
	Medium	10a	(-1) 19 (-2) 1	1800	3.26		(-1),63 (-2) 10	600	2.78
		10b	(-1) 18 (-2) 5	2090	3.32		(-1),37 (-2) 2	355	2.55
		10c	(-1) 13 (-2) 0	1300	3.11		(-1),55 (-2) 4	536	2.73
		10d	(-1) 7 (-2) 0	700	2.85		(-1),48 (-2) 6	491	2.69
		10e	(-1)19 (-2) 2	1900	3.28		(-1)50 (-2) 2	473	2.67
	High	147a	(-1) 124 (-2) 11	12300	4.09		(-2),26 (-3) 4	2700	3.43
		147b	(-1) 101 (-2) 9	1000	3.00		(-2),32 (-3) 4	3300	3.52
		147c	(-1) 134 (-2) 10	13090	4.12		(-2),32 (-3) 2	3100	3.49
		147d	(-1) 105 (-2) 2	9727	3.99		(-2),35 (-3) 3	3500	3.54
		147e	(-1)123 (-2) 13	12363	4.09		(-2)31 (-3) 4	3200	3.51
Custard batch 2	Low	15a	(-1)* 3,2	50	1.70		(-1),9 (-2) 0	90	1.95
		15b	(-1)* 2,2	40	1.60		(-1),3 (-2) 0	30	1.48
		15c	(-1)* 1,6	70	1.85		(-1),7 (-2) 0	70	1.85
		15d	(-1)* 1,5	60	1.78		(-1),4 (-2) 0	40	1.60
		15e	(-1)* 6,8	140	2.15		(-1),3 (-2) 0	30	1.48
	Medium	25a	(-1)* 45,45 (-1)90	900	2.95		(-1),49 (-2) 3	470	2.67
		25b	(-1)* 15,15 (-1) 12	750	2.88		(-1),42 (-2) 2	400	2.60
		25c	(-1)*34,34 (-1)11	890	2.95		(-1),40 (-2) 4	400	2.60
		25d	(-1)* 42,21 (-1)9	765	2.88		(-1),43 (-2) 0	430	2.63
		25e	(-1)* 31,31 (-1)13	960	2.98		(-1) 58 (-2) 6	580	2.76
	High	133a	(-1) 41 (-2) 4	4100	3.61		(-2),51 (-3) 2	4800	3.68
		133b	(-1)45 (-2) 1	4180	3.62		(-2),38 (-3) 3	3700	3.57
		133c	(-1)>150 (-2) 12	12000	4.08		(-2),33 (-3) 1	3100	3.49
		133d	(-1) 90 (-2) 9	9000	3.95		(-2),35 (-3) 5	3600	3.56
		133e	(-1)78 (-2) 15	8450	3.93		(-2)36 (-3) 6	3800	3.58
Whipped cream	Low	31a	(-1)* 4,0	40	1.60		(-1),5 (-2) 0	50	1.70
		31b	(-1)* 2,1	30	1.48		(-1),5 (-2) 0	50	1.70
		31c	(-1)* 2,1	30	1.48		(-1),8 (-2) 0	80	1.90
		31d	(-1)* 4,2	60	1.78		(-1),4 (-2) 0	40	1.60
		31e	(-1)* 2,4	60	1.78		(-1),2 (-2) 0	20	1.30
	Medium	85a	(-1) 7 (-2) 0	700	2.85		(-1),46 (-2) 2	440	2.64
		85b	(-1) 12 (-2) 0	1200	3.08		(-1),50 (-2) 3	480	2.68
		85c	(-1) 8 (-2) 0	800	2.90		(-1),48 (-2) 0	480	2.68
		85d	(-1) 4 (-2) 0	400	2.60		(-1),41 (-2) 1	380	2.58
		85e	(-1)1 (-2) 0	100	2.00		(-1) 47 (-2) 5	470	2.67
	High	190a	(-1) 110 (-2) 10	10900	4.04		(-2),38 (-3) 1	3500	3.54
		190b	(-1) 67 (-2) 3	6360	3.80		(-2),36 (-3) 2	3450	3.54
		190c	(-1) 45 (-2) 5	4545	3.66		(-2),38 (-3) 5	3900	3.59
		190d	(-1) 127 (-2) 13	12909	4.11		(-2),45 (-3) 1	4180	3.62
		190e	(-1)98 (-2) 7	9545	3.98		(-2)30 (-3) 4	3090	3.49

*0.5ml plated

MICROVAL® NEN

FRUIT & VEGETABLE PRODUCTS									
			NMKL Method 68				Compact Dry ETC		
Food item	Inoculum	Sample number	Dilution (0.1ml) cfu/ plate	cfu/g	Log cfu/g		Dilution(1ml) cfu/ plate	cfu/g	Log cfu/g
Parsley 1	Low	2a	(-1)* 2,0	20	1.30		(-1) 1 (-2) 0	10	1.00
		2b	(-1)* 1,0	10	1.00		(-1) 2 (-2) 0	20	1.30
		2c	(-1)* 1,0	10	1.00		(-1) 2 (-2) 0	20	1.30
		2d	(-1)* 1,0	10	1.00		(-1)1 (-2) 0	10	1.00
		2e	(-1)* 1,0	10	1.00		(-1) 3 (-2) 0	30	1.48
	Medium	115a	(-1) 2 (-2) 0	200	2.30		(-1) 67 (-2) 10	700	2.85
		115b	(-1) 2 (-2) 0	200	2.30		(-1) 49 (-2) 7	509	2.71
		115c	(-1) 4 (-2) 0	400	2.60		(-1) 47 (-2) 9	509	2.71
		115d	(-1) 13 (-2) 0	1300	3.11		(-1),45 (-2) 5	455	2.66
		115e	(-1) 4 (-2) 0	400	2.60		(-1) 50 (-2) 5	500	2.70
	High	134a	(-2) 40 (-3) 0	40000	4.60		(-3)36 (-4) 5	37000	4.57
		134b	(-2) 43 (-3) 2	40900	4.61		(-3) 43 (-4) 4	42700	4.63
		134c	(-2) 25 (-3) 3	25400	4.40		(-3) 38 (-4) 9	43600	4.64
		134d	(-2),11 (-3) 3	12700	4.10		(-3) 37 (-4) 4	37200	4.57
		134e	(-2) 16 (-3) 6	20000	4.30		(-3) 54 (-4) 5	53600	4.73
Parsley 2	Low	165a	(-1)* 3,0	30	1.48		(-1) 1(-2) 0	10	1.00
		165b	(-1)* 2,0	20	1.30		(-1) 1 (-2) 0	10	1.00
		165c	(-1)* 3,0	30	1.48		(-1)4 (-2) 0	40	1.60
		165d	(-1)* 1,0	10	1.00		(-1) 3(-2) 0	30	1.48
		165e	(-1)* 2,0	20	1.30		(-1) 2 (-2) 0	20	1.30
	Medium	186a	(-1) 13 (-2) 0	1300	3.11		(-1),50 (-2) 4	490	2.69
		186b	(-1) 4 (-2) 0	400	2.60		(-1) 76 (-2) 8	765	2.88
		186c	(-1) 7 (-2) 0	700	2.85		(-1) 51 (-2) 13	581	2.76
		186d	(-1) 6 (-2) 0	500	2.70		(-1) 72 (-2) 10	745	2.87
		186e	(-1)1 (-2) 0	600	2.78		(-1) 58 (-2) 9	609	2.78
	High	64a	(-1) 64 (-2) 11	30000	4.48		(-3)37 (-4) 6	3900	3.59
		64b	(-1) 61 (-2) 12	12000	4.08		(-3) 41 (-4) 5	54500	4.74
		64c	(-2) 25 (-3) 5	50000	4.70		(-3) 38 (-4) 2	36000	4.56
		64d	(-1) 48 (-2) 10	10000	4.00		(-3) 43 (-4) 3	41800	4.62
		64e	(-1)50 (-2) 10	5000	3.70		(-3) 44 (-4) 3	42700	4.63
Lettuce	Low	197a	(-1)* 12,11 (-1)4	315	2.50		(-1),54 (-2) 2	509	2.71
		197b	(-1)* 6,8 (-1) 9	520	2.72		(-1) 57 (-2) 5	564	2.75
		197c	(-1)*6,8 (-1)5	950	2.98		(-1) 47 (-2) 6	482	2.68
		197d	(-1)* 20,6 (-1)2	230	2.35		(-1),50 (-2) 6	509	2.71
		197e	(-1)* 18,20 (-1)2	290	2.46		(-1) 40 (-2) 4	400	2.60
	Medium	68a	(-1) 16 (-2) 2	1630	3.21		(-2) 37 (-3) 2	3500	3.54
		68b	(-1) 14 (-2) 0	1400	3.15		(-2) 24 (-3) 4	2550	3.41
		68c	(-1) 11 (-2) 0	1100	3.04		(-2) 26 (-3) 2	2550	3.41
		68d	(-1) 18 (-2) 2	1818	3.25		(-2),27 (-3) 2	2630	3.42
		68e	(-1)10 (-2) 0	1000	3.00		(-2) 34 (-3) 6	3630	3.56
	High	23a	(-2) 36 (-3) 4	36300	4.56		(-3) 58 (-4) 4	56400	4.75
		23b	(-2) 23 (-3) 2	22700	4.36		(-3) 43 (-4) 1	40000	4.60
		23c	(-2) 44 (-3) 2	41800	4.62		(-3) 49 (-4) 6	50000	4.70
		23d	(-2) 15 (-3) 2	15400	4.19		(-3) 49 (-4) 3	47300	4.67
		23e	(-2) 32 (-3) 3	31800	4.50		(-3) 55 (-4) 10	59000	4.77

*0.5ml plated

MICROVAL® NEN

RAW MEAT & POULTRY									
			NMKL Method 68				Compact Dry ETC		
Food item	Inoculum	Sample number	Dilution (0.1ml) cfu/ plate	cfu/g	Log cfu/g		Dilution(1ml) cfu/ plate	cfu/g	Log cfu/g
Steak 1	Low	200a	(-1)* 20,24 (-1)4	920	2.96		(-1) 51 (-2)9	545	2.74
		200b	(-1)*20,6 (-1) 2	230	2.36		(-1)53 (-2) 5	527	2.72
		200c	(-1)*23,21 (-1)0	440	2.64		(-1)75 (-2) 9	764	2.88
		200d	(-1)* 14,18 (-1)7	510	2.71		(-1 79 (-2) 15	855	2.93
		200e	(-1)* 24,26 (-1)7	600	2.78		(-1) 61 (-2) 5	600	2.78
	Medium	180a	(-1) 136 (-2) 9	13152	4.12		(-2) 111 (-3) 14	11364	4.06
		180b	(-1) 117 (-2) 7	11273	4.05		(-2) 95 (-3) 7	9273	3.97
		180c	(-1) 147 (-2) 10	14273	4.15		(-2) 106 (-3) 12	10727	4.03
		180d	(-1) 118 (-2) 9	10818	4.03		(-2) 102(-3) 6	9818	3.99
		180e	(-1)122 (-2) 8	11818	4.07		(-2)89 (-3) 9	8909	3.95
	High	6a	(-3) 44 (-4) 6	4545453	4.66		(-4) 63 (-5) 3	600000	5.78
		6b	(-3) 33 (-4) 3	327273	5.51		(-4) 61 (-5) 4	590909	5.77
		6c	(-3) 80 (-4) 6	781818	5.89		(-4) 40 (-5) 6	418182	5.62
		6d	(-3) 79 (-4) 2	736364	5.87		(-4) 57 (-5) 4	554545	5.74
		6e	(-3) 83 (-4) 6	809091	5.91		(-4) 72 (-5) 9	736364	5.87
Steak 2	Low	79a	(-1)* 3,2	340	2.53		(-1) 57 (-2) 9	600	2.78
		79b	(-1)* 2,2	300	2.48		(-1) 61 (-2) 3	582	2.76
		79c	(-1)* 1,6	395	2.60		(-1),85(-2) 2	791	2.90
		79d	(-1)* 1,5	485	2.69		(-1) 60 (-2) 8	618	2.79
		79e	(-1)* 6,8	695	2.84		(-1) 61 (-2) 7	618	2.79
	Medium	76a	(-1)* 19,19 (-1)3	7364	3.87		(-2) 96 (-3) 12	9818	3.99
		76b	(-1)*22,8 (-1) 3	9091	3.96		(-2) 114 (-3) 14	11636	4.07
		76c	(-1)*17,24 (-1)4	13455	4.13		(-2) 113 (-3) 13	11454	4.06
		76d	(-1)* 30,24 (-1)7	15000	4.18		(-2) 112(-3) 10	11091	4.04
		76e	(-1)* 17,22 (-1)10	8364	3.92		(-2)120 (-3) 16	12364	4.09
	High	111a	(-3) 42 (-4) 1	390909	5.59		(-4) 53 (-5) 11	518181	5.71
		111b	(-3) 37 (-4) 6	390909	5.59		(-4) 40 (-5) 4	400000	5.60
		111c	(-3) 41 (-4) 3	400000	5.60		(-4) 42 (-5) 7	445455	5.65
		111d	(-3) 58 (-4) 9	609091	5.78		(-4) 42 (-5) 4	418182	5.62
		111e	(-3) 56 (-4) 5	554545	5.74		(-4) 44 (-5) 1	409090	5.61
Patties	Low	100a	(-1)* 13,16 (-1)0	645	2.81		(-1) 67 (-2) 8	682	2.83
		100b	(-1)*29,16 (-1) 11	775	2.89		(-1) 79 (-2) 6	773	2.89
		100c	(-1)*12,16 (-1)9	590	2.77		(-1) 165 (-2)6	1555	3.19
		100d	(-1)* 15,,23 (-1)8	590	2.77		(-1) 58 (-2) 8	600	2.78
		100e	(-1)* 11,22 (-1)2	265	2.42		(-1) 81 (-2) 7	800	2.90
	Medium	90a	(-1) 105 (-2) 8	10273	4.01		(-2) 90 (-3) 12	9273	3.97
		90b	(-1) 103 (-2) 10	10273	4.01		(-2) 121 (-3) 5	11455	4.06
		90c	(-1) 121 (-2) 8	1127	3.05		(-2) 111 (-3) 10	11000	4.04
		90d	(-1) 112 (-2) 10	11091	4.04		(-2) 125 (-3) 10	12273	4.09
		90e	(-1)127 (-2) 11	12545	4.10		(-2)89 (-3) 8	8818	3.95
	High	9a	(-3) 60 (-4) 8	618182	5.79		(-4) 44 (-5) 4	436364	5.64
		9b	(-3) 83 (-4) 6	809091	5.91		(-4) 57 (-5) 4	554545	5.74
		9c	(-3) 65 (-4) 10	681818	5.83		(-4) 82 (-5) 2	763636	5.88
		9d	(-3) 69 (-4) 6	681818	5.83		(-4) 85 (-5) 8	845455	5.98
		9e	(-3) 65 (-4) 7	6545454	5.82		(-4) 44 (-5) 8	472727	5.67

*0.5ml plated

MICROVAL® NEN

RTE PRODUCTS									
			NMKL Method 68				Compact Dry ETC		
Food item	Inoculum	Sample number	Dilution (0.1ml) cfu/ plate	cfu/g	Log cfu/g		Dilution(1ml) cfu/ plate	cfu/g	Log cfu/g
Tuna pate 1	Low	159a	(-1)* 1,4	50	1.70		(-1) 8,5	65	1.81
		159b	(-1)* 0,5	50	1.70		(-1)10,8	90	1.95
		159c	(-1)* 2,4	60	1.78		(-1) 10,10	100	2.00
		159d	(-1)* 2,4	60	1.78		(-1) 6,8	70	1.85
		159e	(-1)* 3,4	70	1.85		(-1) 7,5	60	1.78
	Medium	4a	(-1)* 11,11 (-1)3	260	2.41		(-1) 25 (-2) 2	240	2.38
		4b	(-1)* 615 (-1) 1	155	2.19		(-1) 19 (-2) 7	240	2.38
		4c	(-1)*6,9 (-1)4	500	2.70		(-1) 21 (-2) 4	230	2.36
		4d	(-1)* 5,6 (-1)0	110	2.04		(-1) 26 (-2) 3	270	2.43
		4e	(-1)* 9,13 (-1)5	360	2.56		(-1)24 (-2) 4	250	2.40
	High	127a	(-1) 15 (-2) 0	1500	3.18		(-1) 75 (-2) 9	760	2.88
		127b	(-1) 14 (-2) 0	1400	3.15		(-1) 73 (-2) 4	700	2.85
		127c	(-1) 20 (-2) 0	2000	3.30		(-1) 69 (-2) 11	730	2.85
		127d	(-1) 14 (-2) 0	1400	3.15		(-1) 63 (-2) 10	660	2.82
		127e	(-1)10 (-2) 0	1000	3.00		(-1) 67 (-2) 7	670	2.83
Tuna pate 2	Low	193a	(-1)* 9,3	120	2.08		(-1) 11,7	180	2.26
		193b	(-1)* 5,0	50	1.70		(-1)13,11	240	2.38
		193c	(-1)* 4,3	70	1.85		(-1) 8,6	140	2.15
		193d	(-1)* 2,3	50	1.70		(-1) 13,13	260	2.41
		193e	(-1)* 1,2	30	1.48		(-1) 14,4	180	2.26
	Medium	50a	(-1)* 8,12 (-1)3	250	2.40		(-1)26 (-2) 2	250	2.40
		50b	(-1)* 14,3 (-1) 0	170	2.23		(-1)19 (-2) 2	190	2.28
		50c	(-1)*9,9 (-1)2	190	2.28		(-1) 27 (-2) 0	270	2.43
		50d	(-1)* 8,8 (-1)1	130	2.11		(-1) 21 (-2) 1	200	2.30
		50e	(-1)* 11,5 (-1)2	180	2.26		(-1) 17 (-2) 1	160	2.20
	High	83a	(-1) 7 (-2) 0	700	2.85		(-1)77 (-2) 6	750	2.88
		83b	(-1)10 (-2) 0	1000	3.00		(-1)80 (-2) 6	780	2.89
		83c	(-1)19 (-2) 0	1900	3.28		(-1) 64 (-2) 7	650	2.81
		83d	(-1) 12 (-2) 0	1200	3.08		(-1) 83 (-2) 8	830	2.92
		83e	(-1)7 (-2) 0	700	2.85		(-1) 72 (-2) 4	690	2.84
Prawns	Low	121a	(-1)* 7,3 (-1)1	100	2.00		(-1) 11,10	105	2.02
		121b	(-1)* 7,4 (-1) 4	255	2.41		(-1)12,9	105	2.02
		121c	(-1)*5,0 (-1)0	50	1.70		(-1) 10,7	85	1.93
		121d	(-1)* 7,1 (-1)3	190	2.28		(-1) 14,10	120	2.08
		121e	(-1)* 4,1 (-1)0	50	1.70		(-1) 13,11	120	2.08
	Medium	176a	(-1)* 17,6 (-1)1	165	2.22		(-1),48 (-2) 1	450	2.65
		176b	(-1)* 20,15 (-1) 3	325	2.51		(-1)31 (-2) 3	310	2.49
		176c	(-1)*6,3 (-1)6	345	2.54		(-1) 41(-2) 4	410	2.61
		176d	(-1)* 16,12 (-1)1	140	2.15		(-1)31 (-2) 6	340	2.53
		176e	(-1)* 20,12 (-1)6	460	2.66		(-1) 33 (-2) 4	340	2.53
	High	152a	(-1) 6(-2) 0	600	2.78		(-1),72 (-2) 7	720	2.86
		152b	(-1) 9 (-2) 0	900	2.95		(-1)81 (-2) 6	790	2.90
		152c	(-1) 5 (-2) 0	500	2.70		(-1) 79(-2) 5	760	2.88
		152d	(-1) 10 (-2) 0	1000	3.00		(-1)68 (-2) 3	650	2.81
		152e	(-1)7 (-2) 0	700	2.85		(-1) 83 (-2) 4	790	2.90

*0.5ml plated

MICROVAL® NEN

MULTI-COMPONENT FOODS									
			NMKL Method 68				Compact Dry ETC		
Food item	Inoculum	Sample number	Dilution (0.1ml) cfu/ plate	cfu/g	Log cfu/g		Dilution(1ml) cfu/ plate	cfu/g	Log cfu/g
Pasts 1	Low	60a	(-1)* 6,3(-1)1	95	1.98		(-1)13 (-2) 0	130	2.11
		60b	(-1)* 5,3 (-1) 4	80	1.90		(-1)12 (-2) 0	120	2.08
		60c	(-1)*6,6 (-1)0	120	2.08		(-1)12 (-2) 0	120	2.08
		60d	(-1)* 6,5 (-1)0	110	2.04		(-1)14 (-2) 0	140	2.15
		60e	(-1)* 5,4 (-1)0	90	1.95		(-1)13 (-2) 0	130	2.11
	Medium	169a	(-1) 102 (-2) 9	10091	4.00		(-2)88 (-3) 6	8545	3.93
		169b	(-1) 75 (-2) 5	7273	3.86		(-2)61 (-3) 12	6636	3.82
		169c	(-1) 110 (-2) 12	11091	4.04		(-2)70 (-3) 8	7090	3.85
		169d	(-1) 92 (-2) 5	8818	3.95		(-2)79 (-3) 6	7727	3.89
		169e	(-1)82 (-2) 9	8273	3.92		(-2)78 (-3) 7	7727	3.89
	High	43a	(-2)84 (-3) 6	81818	4.91		(-3) 56 (-4) 2	52727	4.72
		43b	(-2)89 (-3) 6	86363	4.94		(-3) 67 (-4) 5	65445	4.82
		43c	(-2)94(-3) 3	88182	4.95		(-3) 93 (-4) 10	93636	4.97
		43d	(-2)97 (-3) 11	98182	4.99		(-3) 78 (-4) 12	81818	4.91
		43e	(-2)93 (-3) 9	92727	4.97		(-3) 70 (-4) 11	73636	4.87
Pasta 2	Low	137a	(-1)* 5,2	70	1.85		(-1),11 (-2) 0	110	2.04
		137b	(-1)* 3,2	50	1.70		(-1)10(-2) 0	100	2.00
		137c	(-1)* 4,2	60	1.78		(-1)9 (-2) 0	90	1.95
		137d	(-1)* 4,2	60	1.78		(-1)9 (-2) 0	90	1.95
		137e	(-1)* 1,0	60	1.78		(-1)12 (-2) 0	120	2.08
	Medium	46a	(-1) 107 (-2) 11	10727	4.03		(-2)80 (-3) 6	7818	3.89
		46b	(-1) 106 (-2) 7	10272	4.01		(-2)74 (-3) 8	7454	3.87
		46c	(-1) 130 (-2) 8	12545	4.10		(-2)72 (-3) 4	6909	3.84
		46d	(-1) >150 (-2) 9	9000	3.95		(-2)85 (-3) 6	8212	3.91
		46e	(-1)>150 (-2) 20	20000	4.30		(-2)66 (-3) 6	6545	3.82
	High	102a	(-2)93 (-3) 14	97273	4.99		(-3) 71 (-4) 10	73636	4.87
		102b	(-2)102 (-3) 10	10818	4.03		(-3) 63 (-4) 7	63636	4.80
		102c	(-2)99(-3) 5	94545	4.98		(-3) 65 (-4) 5	63636	4.80
		102d	(-2)94 (-3) 13	97272	4.99		(-3) 74 (-4) 10	76363	4.88
		102e	(-2)>150 (-3) 21	21000	5.32		(-3) 71 (-4) 7	71909	4.86
Sandwich	Low	141a	(-1)* 2,1	30	1.48		(-1)9 (-2) 0	90	1.95
		141b	(-1)* 6,1	70	1.85		(-1)6 (-2) 0	60	1.78
		141c	(-1)* 1,0	10	1.00		(-1)8 (-2) 0	80	1.90
		141d	(-1)* 4,0	40	1.60		(-1)6 (-2) 0	60	1.78
		141e	(-1)* 2,4	60	1.78		(-1)19 (-2) 1	181	2.26
	Medium	41a	(-1) 77 (-2) 12	8090	3.91		(-2)68(-3) 8	6909	3.84
		41b	(-1) >150(-2) 9	9000	3.95		(-2)68 (-3) 6	6727	3.83
		41c	(-1) >150 (-2) 6	6000	3.78		(-2)67 (-3) 7	7636	3.88
		41d	(-1) >150 (-2) 5	5000	3.70		(-2)68 (-3) 10	7090	3.85
		41e	(-1)30 (-2) 2	2000	3.30		(-2)84 (-3) 9	8454	3.93
	High	39a	(-2)95 (-3) 14	90000	4.95		(-3) 53 (-4) 3	50909	4.71
		39b	(-2)95 (-3) 6	91818	4.96		(-3) 52 (-4) 7	53636	4.73
		39c	(-2)89(-3) 8	88182	4.95		(-3) 68 (-4) 6	67273	4.83
		39d	(-2)114 (-3) 12	11454	5.06		(-3) 70 (-4) 12	74545	4.87
		39e	(-2)88 (-3) 12	90909	4.96		(-3) 68 (-4) 6	67273	4.83

*0.5ml plated

Sample code	Category	Type	Item	Strain	Source	Level (aim)	Alternate method (ETC)		Reference method (S&B)		log difference
							cfu/ml	log cfu/ml	cfu/ml	log cfu/ml	
2A	Environmental samples	Surface samples	Stainless steel	16049	human isolate	Low	2.09E+02	2.32	7.45E+02	2.87	0.55
2B							6.00E+02	2.78	1.20E+03	3.08	0.30
2C							5.09E+02	2.71	1.20E+03	3.08	0.37
2D							4.82E+02	2.68	1.40E+03	3.15	0.46
2E							3.00E+02	2.48	1.40E+03	3.15	0.67
3A						Medium	9.45E+02	2.98	4.00E+03	3.60	0.63
3B							3.50E+03	3.54	7.70E+03	3.89	0.34
3C							1.60E+03	3.20	3.20E+03	3.51	0.30
3D							2.20E+03	3.34	3.10E+03	3.49	0.15
3E							2.00E+03	3.30	5.80E+03	3.76	0.46
1A						High	2.90E+05	5.46	3.70E+05	5.57	0.11
1B							7.00E+04	4.85	6.90E+04	4.84	-0.01
1C							4.90E+04	4.69	6.50E+04	4.81	0.12
1D							2.40E+05	5.38	3.80E+05	5.58	0.20
1E							3.40E+05	5.53	3.50E+05	5.54	0.01
5A			Plastic chopping board			Low	6.45E+02	2.81	1.20E+03	3.08	0.27
5B							3.45E+02	2.54	9.91E+02	3.00	0.46
5C							4.18E+02	2.62	7.82E+02	2.89	0.27
5D							5.00E+02	2.70	4.73E+02	2.67	-0.02
5E							2.45E+02	2.39	1.50E+03	3.18	0.79

Sample code	Category	Type	Item	Strain	Source	Level (aim)	Alternate method (ETC)		Reference method (S&B)		log difference
							cfu/ml	log cfu/ml	cfu/ml	log cfu/ml	
4A						Medium	4.30E+03	3.63	1.30E+03	3.11	-0.52
4B							2.90E+03	3.46	1.30E+03	3.11	-0.35
4C							4.90E+03	3.69	1.40E+03	3.15	-0.54
4D							2.30E+03	3.36	2.60E+03	3.41	0.05
4E							2.50E+03	3.40	7.00E+02	2.85	-0.55
6A						High	2.40E+05	5.38	1.90E+05	5.28	-0.10
6B							2.30E+05	5.36	3.00E+05	5.48	0.12
6C							3.50E+05	5.54	4.30E+05	5.63	0.09
6D							1.40E+04	4.15	3.90E+04	4.59	0.44
6E							8.80E+04	4.94	4.60E+04	4.66	-0.28

ANNEX F: Summary tables accuracy profile study.

(Food) Category 1	dairy
(Food) Type 1	dairy desserts

			Reference method (log cfu/g) result					Alternative method (log cfu/g) result				
Samples	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
84a-e	custard 1	Low	1.85	2.08	1.60	1.78	2.32	1.48	1.48	1.78	1.78	1.78
15a-e	custard 2	Low	1.70	1.60	1.85	1.78	2.15	1.95	1.48	1.85	1.60	1.48
31a-e	whipped cream	Low	1.60	1.48	1.48	1.78	1.78	1.70	1.70	1.90	1.60	1.30
10a-e	custard 1	Medium	3.26	3.32	3.11	2.85	3.28	2.78	2.55	2.73	2.69	2.67
25a-e	custard 2	Medium	2.95	2.88	2.95	2.88	2.98	2.67	2.60	2.60	2.63	2.76
85a-e	whipped cream	Medium	2.85	3.08	2.90	2.60	2.00	2.64	2.68	2.68	2.58	2.67
147a-e	custard 1	High	4.09	3.00	4.12	3.99	4.09	3.43	3.52	3.49	3.54	3.51
133a-e	custard 2	High	3.61	3.62	4.08	3.95	3.93	3.68	3.57	3.49	3.56	3.58
190a-e	whipped cream	High	4.04	3.80	3.66	4.11	3.98	3.54	3.54	3.59	3.62	3.49

(Food) Category 2	Fruits and vegetables
(Food) Type 3	leafy greens

			Reference method (log cfu/g) result					Alternative method (log cfu/g) result				
Samples	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
2a-e	Parsley 1	Low	1.30	1.00	1.00	1.00	1.00	1.00	1.30	1.30	1.00	1.48
115a-e	Parsley 2	Low	1.48	1.30	1.48	1.00	1.30	1.00	1.00	1.60	1.48	1.30
134a-e	lettuce	Low	2.50	2.72	2.98	2.36	2.46	2.71	2.75	2.68	2.71	2.60
165a-e	Parsley 1	Medium	2.30	2.30	2.60	3.11	2.60	2.85	2.71	2.71	2.66	2.70
186a-e	Parsley 2	Medium	3.11	2.60	2.85	2.70	2.78	2.69	2.88	2.76	2.87	2.78
64a-e	lettuce	Medium	3.21	3.15	3.04	3.26	3.00	3.54	3.41	3.41	3.42	3.56
197a-e	Parsley 1	High	4.60	4.61	4.40	4.10	4.30	4.57	4.63	4.64	4.57	4.73
68a-e	Parsley 2	High	4.48	4.08	4.70	4.00	4.70	4.59	4.74	4.56	4.62	4.63
23a-e	lettuce	High	4.56	4.36	4.62	4.19	4.50	4.75	4.60	4.70	4.67	4.77

(Food) Category 3	Raw meat and poultry
(Food) Type 3	raw beef

			Reference method (log cfu/g) result					Alternative method (log cfu/g) result				
Samples	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
200a-e	steak 1	Low	2.96	2.36	2.64	2.71	2.78	2.74	2.72	2.88	2.93	2.78
180a-e	steak 2	Low	2.53	2.48	2.60	2.69	2.84	2.78	2.76	2.90	2.79	2.79
6a-e	patties	Low	2.81	2.89	2.77	2.77	2.42	2.83	2.89	3.19	2.78	2.90
79a-e	steak 1	Medium	4.12	4.05	4.15	4.03	4.07	4.06	3.97	4.03	3.99	3.95
76a-e	steak 2	Medium	3.87	3.96	4.13	4.18	3.92	3.99	4.07	4.06	4.04	4.09
111a-e	patties	Medium	4.01	4.01	4.07	4.04	4.10	3.97	4.06	4.04	4.09	3.95
100a-e	steak 1	High	5.66	5.51	5.89	5.87	5.91	5.78	5.77	5.62	5.74	5.87
90a-e	steak 2	High	5.59	5.59	5.60	5.78	5.74	5.71	5.60	5.65	5.62	5.61
9a-e	patties	High	5.79	5.91	5.83	5.83	5.82	5.64	5.74	5.88	5.93	5.67

(Food) Category 4	RTE Foods
(Food) Type 2	Cooked fish

			Reference method (log cfu/g) result					Alternative method (log cfu/g) result				
Samples	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
159a-e	tuna pate 1	Low	1.70	1.70	1.78	1.78	1.85	1.81	1.95	2.00	1.85	1.78
4a-e	tuna pate 2	Low	2.08	1.70	1.85	1.70	1.48	2.26	2.38	2.15	2.41	2.26
127a-e	prawns	Low	2.00	2.41	1.70	2.28	1.70	2.02	2.02	1.93	2.08	2.08
193a-e	tuna pate 1	Medium	2.41	2.19	2.70	2.04	2.56	2.38	2.38	2.36	2.43	2.40
50a-e	tuna pate 2	Medium	2.40	2.23	2.28	2.11	2.26	2.40	2.28	2.43	2.30	2.20
83a-e	prawns	Medium	2.22	2.51	2.54	2.15	2.66	2.65	2.49	2.61	2.53	2.53
121a-e	tuna pate 1	High	3.18	3.15	3.30	3.15	3.00	2.88	2.85	2.86	2.82	2.83
176a-e	tuna pate 2	High	2.85	3.00	3.28	3.08	2.85	2.88	2.89	2.81	2.92	2.84
152a-e	prawns	High	2.78	2.95	2.70	3.00	2.85	2.86	2.90	2.88	2.81	2.90

(Food) Category 5	Multi-component foods
(Food) Type 1	with raw ingredients

			Reference method (log cfu/g) result					Alternative method (log cfu/g) result				
Samples	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
60a-e	pasta 1	Low	1.98	1.90	2.08	2.04	1.95	2.11	2.08	2.08	2.15	2.11
169a-e	pasta 2	Low	1.85	1.70	1.78	1.78	1.00	2.04	2.00	1.95	1.95	2.08
43a-e	sandwich	Low	1.48	1.85	1.00	1.60	1.78	1.95	1.78	1.90	1.78	2.26
137a-e	pasta 1	Medium	4.00	3.86	4.04	3.95	3.92	3.93	3.82	3.85	3.89	3.89
46a-e	pasta 2	Medium	4.03	4.01	4.10	3.95	4.30	3.89	3.87	3.84	3.92	3.82
102a-e	sandwich	Medium	3.91	3.95	3.78	3.70	3.30	3.84	3.83	3.88	3.85	3.93
141a-e	pasta 1	High	4.91	4.94	4.95	4.99	4.97	4.72	4.82	4.97	4.91	4.87
41a-e	pasta 2	High	4.99	5.01	4.98	4.99	5.32	4.87	4.80	4.80	4.88	4.86
39a-e	sandwich	High	4.95	4.96	4.95	5.06	4.96	4.71	4.73	4.83	4.87	4.83

(Food) Category 1	Environmental
(Food) Type 1	Surfaces

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
2	Stainless steel	low	750	1200	1200	1400	1400	210	600	510	480	300
5	Plastic chopping board	low	1200	990	780	470	1500	650	350	420	500	250
3	Stainless steel	interme diate	4000	7700	3200	3100	5800	950	3500	1600	2200	2500
4	Plastic chopping board	interme diate	1300	1300	1400	2600	700	4300	2900	4900	2300	2500
1	Stainless steel	high	370000	69000	65000	38000	350000	290000	70000	49000	240000	340000
6	Plastic chopping board	high	190000	300000	430000	39000	46000	240000	230000	350000	14000	88000

ANNEX G Inclusivity / Exclusivity: raw data

Inclusivity strains

Species	Campden culture collection (CRA)	Other culture collection code	Source	Compact Dry ETC +/- detection (count – dilution))	NMKL 68 +/- detection (count – dilution)	TSA Count dilution
Enterococcus durans	16464	NCTC 662	Milk	+ 63 (-6)	- 0 (-5)	36 (-7)
Enterococcus faecalis	5395	n/a	Industrial isolate	+ 45	+ 46	48
Enterococcus faecalis	5447	NCIMB775	N/a	+ 39	+ 44	46
Enterococcus faecium	16844	NCIMB 2699	Cheese	+ 10	+ 8	9
Enterococcus faecalis	5723	n/a	Industrial isolate	+ 34	+ 56	53
Enterococcus faecium	16845	NCIMB 2702	Cheese	+ 8	+ 12	11
Enterococcus faecium	16846	NCIMB 700580	Commercial milk	+ 9	+ 8	65
Enterococcus faecalis	6369	NCIMB1993	N/a	+ 70	+ 65	101
Enterococcus faecalis	6635	n/a	Industrial isolate	+ 15	+ 42	25
Enterococcus faecium	16847	NCIMB 700594	Cheddar cheese	+ 12	+ 45	32
Enterococcus faecalis	7068	n/a	Industrial isolate	+ 73	+ 58	64
Enterococcus faecium	16848	NCIMB 9645	Grass silage	+ 17	+ 12	24
Enterococcus faecalis	7296	n/a	Industrial isolate	+ 32	+ 1	26
Enterococcus faecalis	7297	n/a	Industrial isolate	+ 4	+ 41	28
Enterococcus faecalis	16049	NCIMB 13280	Human isolate	+ 28	+ 33	28
Enterococcus faecalis	1513	n/a	Dried milk powder	+ 23	+ 31	43
Enterococcus faecalis	1528	n/a	Dried milk powder	+ 36	+ 41	25
Enterococcus faecalis	4113	NCTC 775	N/a	+ 30	+ 29	33
Enterococcus faecalis	4132	n/a	Cheese	+ 15	+ 21	23

Species	Campden culture collection (CRA)	Other culture collection code	Source	Compact Dry ETC +/- detection (count – dilution))	NMKL 68 +/- detection (count – dilution)	TSA Count dilution
Enterococcus faecalis	16408	n/a	Industrial isolate	+ 38	+ 37	36
Enterococcus faecalis	16481	ATCC 29212	Human isolate	+ 35	+ 30	25
Enterococcus faecalis	1528	n/a	Dried milk	+ 13	+ 8	12
Enterococcus faecium	7865	n/a	Industrial isolate	+ 45	+ 90	48
Enterococcus faecium	16465	ATCC 8459	Cheese	+ 19	+ 30	26
Enterococcus hirae	15939	ATCC 8043	N/a	+ 30	+ 35	23
Enterococcus cecorum	16849	NCTC 12421	Raw chicken	- 0 (-6)	- 0 (-5)	45 (-7)
Enterococcus durans	16810	NCTC 8130	Cheese	- 0(-6)	+ 28 (-5)	28 (-7)
Enterococcus casseliflavus	16811	NCTC 12361	Plants	+ 14	+ 40	51
Enterococcus mundtii	16812	NCTC 12363	Soil	+ 23	+ 42	67
Enterococcus aquamarinus	16813	NCIMB 14241	Sea water	- 0(-6)	- 0 (-5)	27 (-7)
Enterococcus hirae	16809	n/a	Industrial isolate	+ 37	+ 43	35
Enterococcus dispar	16850	NCIMB 13000	Human isolate	- 0 (-6)	- 0 (-5)	52 (-7)
Enterococcus columbae	16851	NCIMB 13013	Pigeon liver	- 0 (-6)	- 0 (-5)	15 (-7)
Enterococcus pseudoavium	16852	NCIMB 13084	Cow udder	- 0 (-6)	- 0 (-5)	21 (-7)
Enterococcus sulfureus	16853	NCIMB13117	Plant	- 0 (-6)	- 0 (-5)	38 (-7)
Enterococcus seriolicida	16854	NCIMB 13208	Bird	- 0 (-6)	- 0 (-5)	35 (-7)
Enterococcus flavescens	16855	NCIMB 13326	Bird	- 0 (-6)	- 0 (-5)	81 (-7)
Enterococcus faecium	16856	NCIMB 700502	Dried milk powder	+ 35	+ 87	56
Enterococcus porcinus	16857	NCIMB 13634	Pig	- 0 (-6)	+ 45 (-6)	31 (-7)
Enterococcus haemoperoxidus	16858	NCIMB 14071	Water	+ 48 (-7)	- 0 (-5)	59 (-7)

Species	Campden culture collection (CRA)	Other culture collection code	Source	Compact Dry ETC +/- detection (count – dilution))	NMKL 68 +/- detection (count – dilution)	TSA Count dilution
Enterococcus thailandicus	16859	NCIMB 14560	Sausage	+ 79 (-6)	- 0(-5)	37 (-7)
Enteroccus malodoratus	16860	NCIMB 700846	Gouda cheese	+ 4 (-5)	- 0(-5)	40(-7)
Enterococcus gallinarum	16861	NCIMB 701229	N/a	+ 24(-7)	- 0 (-5)	34(-7)
Enterococcus avium	16862	NCIMB 701605	N/a	+ 2	+ 2	5
Enterococcus sacharolyticus	16863	NCIMB 702614	Bovine	- 0(-6)	- 0(-5)	80(-6)
Enterococcus dispar	16864	NCIMB 702829	Human isolate	- 0(-6)	- 0(-5)	57(-7)
Enterococcus xiangfangensis	16865	NCIMB 14834	Pickle	- 0 (-6)	- 0(-5)	55(-6)
Enterococcus pseudoavium	16869	NCIMB 2366		+ 37	+ 75	73
Enterococcus faecium	16866	NCIMB12672	Undercooked sausage	+ 7	+ 17	6
Enterococcus solitarus	16867	NCIMB 12902	Human isolate	- 0(-6)	- 0(-5)	17(-7)

Note: Shaded cells represent non-detection of an inclusivity strain by one or both methods

Exclusivity strains

Species	Campden culture collection code (CRA)	Source	Compact Dry ETC +/- detection (count)	NMKL 68 +/- detection (count)	TSA count
Bacillus cereus	1549	Unknown	0 (-4)	0 (-4)	13 (-4)
Bacillus subtilis	16597	UHT custard	0 (-4)	0 (-4)	190 (-5)
Brochothrix thermospacta	16019	Unknown	0 (-4)	0 (-4)	31 (-4)
Carnobacterium divergens	2072	Industrial isolate	0 (-2)	0 (-2)	30 (-2)
Citrobacter freundii	1266	Sausage	0 (-4)	0 (-4)	30 (-4)
Enterobacter agglomerans	490	Raw mince	0 (-3)	0 (-3)	30 (-3)
Enterobacter cloacae	4772	Environmental	0 (-4)	0 (-4)	81 (-4)
Erwinia herbicola	5442	Industrial isolate	0 (-4)	0 (-4)	73 (-4)
Escherichia coli	545	Raw mince	0 (-4)	0 (-4)	55 (-4)
Hafnia alvei	3996	Chicken giblets	0 (-4)	0 (-4)	17 (-4)
Lactobacillus brevis	3169	Sileage	0 (-4)	0 (-4)	140 (-4)
Lactobacillus casei	533	Industrial isolate	0 (-4)	0 (-4)	190 (-4)
Lactobacillus gasseri	6804	Human	1 (-4)	2 (-4)	70 (-4)
Lactococcus lactis	5396	Food factory isolate	0 (-4)	0 (-4)	65 (-4)
Leuconostoc mesenteroides	16022	Ham	0 (-4)	0 (-4)	18 (-4)
Listeria innocua	115	Beefburger	0 (-3)	0 (-3)	7 (-3)
Listeria monocytogenes	1105	Raw milk	0 (-4)	0 (-4)	95 (-4)
Micrococcus luteus	3503	Tea factory	0 (-4)	0 (-4)	38 (-4)

Species	Campden culture collection code (CRA)	Source	Compact Dry ETC +/- detection (count)	NMKL 68 +/- detection (count)	TSA count
Proteus mirabilis	586	Poultry	0 (-4)	0 (-4)	47 (-4)
Pseudomonas aeruginosa	8299	Ncimb 10753	0 (-4)	0 (-4)	11 (-4)
Pseudomonas fluorescens	5361	Environmental	0 (-4)	0 (-4)	27 (-4)
Salmonella Enteritidis	3505	Fish cakes	0 (-4)	0 (-4)	100 (-4)
Serratia liquefaciens	504	Raw mince	0 (-4)	0 (-4)	48 (-4)
Staphylococcus aureus	1224	Margarine	0 (-4)	0 (-4)	110 (-4)
Staphylococcus carnosus	4134	Fermented sausage	0 (-3)	0 (-3)	9 (-3)
Staphylococcus hominis	529	Milk powder	0 (-4)	0 (-4)	23 (-4)
Streptococcus cremoris	534	Raw mince	0 (-4)	0 (-4)	64 (-4)
Streptococcus cremoris	556	Raw mince	0 (-3)	0 (-3)	40 (-3)
Streptococcus lactis	527	Milk powder	>150 (-3)	>150 (-3)	76 (-4)
Streptococcus lactis	1487	Raw mince	0 (-4)	0 (-4)	90 (-4)
Streptococcus thermophilus	5683	Industrial isolate	0 (-4)	0 (-4)	52 (-6)
Streptococcus thermophilus	16868	Ncimb 8510	0 (-4)	0 (-4)	13 (-4)

Note: Shaded cells represent detection of an exclusivity strain by one or both method