Method Comparison Study Report for the ISO 16140-2:2016 validation of Compact Dry ETB, for the detection of Enterobacteriaceae in a broad range of foods

This a renewal report containing additional analysis required highlighted in yellow

MicroVal study number: 2007LR02

Method/Kit name: Compact Dry ETB

Report version: MCS/ILS renewal report v1, 26/09/2024

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Foreword

The report is prepared in accordance with ISO 16140-2:20016 and the most recent version of the MicroVal Technical Committee for interpretation on ISO 16140-2.

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Method/Kit name: Compact Dry ETB

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 method:

ISO 21528-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony- count technique

Scope of validation: A broad range of foods

Certification orgnization: Lloyd's Register

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

Contents

1 Introduction

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

The original study was conducted following ISO16140:2003 and on renewal, additional work on the original ILS was required to fulfil the requirements of ISO 16140-2:2016. As the design of the ILS remained unchanged between the 2 versions of the 16140 protocol the orignal data was analysed according to the new statistical approach outlined in ISO16140-2:2016.

On renewal in September 2024, further analysis was needed to align with the MicroVal requirements for the Enterobacteriaceae inclusivity panel. The inclusivity panel has been extended to include the newly described order Enterobacterales comprising the Enterobacteriaceae and 6 other closely related families. Two additional inclusivity isolates were tested as representative strains to cover the missing families from the Enterobacterales. This study was completed in September 2024 by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was: Compact Dry ETB

Compact Dry ETB (Shimadzu Diagnostics Corporation) are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the centre of the self-diffusible medium. This is a ready to use, selective plate containing glucose for the enumeration of Enterobacteriaceae. Colonies are red/purple after the required incubation period.

The reference method used is:

ISO 21528-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony- count technique

The flow diagram is given in Appendix A.

Scope of the validation study is: A broad range of foods

Categories included:

- Heat processed milk and dairy products
- Fresh produce and fruits
- Raw poultry and meats (Combined category raw/ RTC meats and poultry)
- Ready to eat foods (Combined category RTE/RTRH meats and poultry)
- Multi component foods or meal components

Criteria evaluated during the study have been:

- Relative trueness study;

- Accuracy profiles;
- Limits of quantification (LOQ);
- Inclusivity and exclusivity.

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

The alternative method Compact Dry ETB shows comparable performance to the reference method ISO 21528-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony- count technique.

Conclusions for the study

Overall, the conclusions for the Method Comparison are:

- The alternative method Compact Dry ETB for enumeration of Enterobacteriaceae shows satisfactory results for relative trueness;
- The alternative Compact Dry EC ETB for enumeration of Enterobacteriaceae shows satisfactory results for accuracy profile;
- The ETB for enumeration of Enterobacteriaceae in foods method was shown to be specific and selective.
- The alternative method ETB for enumeration of Enterobacteriaceae shows satisfactory results for the ILS
- The alternative method ETB for enumeration of Enterobacteriaceae shows comparable performance to the reference method. ISO 21528-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony- count technique

- **2.** Method protocols

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

1.1 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 was used, then two plates of this dilution were 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

1.2 Alternative method

See the flow diagram of the alternative method in Annex A.

See the Compact Dry ETB kit insert in Annex C.

Compact Dry ETB (Shimadzu Diagnostics Corporation) are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the centre of the self-diffusible medium. This is a ready to use, selective plate containing glucose for the enumeration of Enterobacteriaceae. Colonies are red/purple after the required incubation period.

1.3 Study design

According to ISO 16140-2 the reference method and alternative methods were performed with, as far as possible , exactly the same sample

Samples of product containing the target organism were diluted 1 in 10 with an appropriate diluent according to ISO 6887 and homonegised in a stomacher. Appropriate serial dilutions were made and all relevant dilutions were analysed using the reference method and alternative method.

2 Method comparison study

2.1 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 wasconducted using naturally or artificially contaminated samples. Different categories, types and items were tested for this.

A total of 5 categories were included in this validation study. 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.

2.1.1 Number of samples

The categories, the types and the number of samples analyzed are presented in Table 1.

Table 1 – Categories, types and number of samples analyzed

Category	Types	Number of samples
Heat processed milk and dairy products	Dry milk product e.g. milk powder, powder for milk based desserts, dried infant formula	5
	Dairy products e.g. ice-cream, yogurts, cream, hard cheese, soft cheese, raw milk cheese	5
	Pasteurised milk products e.g. skimmed, semi-skimmed, full fat and flavoured milks	5
Fresh produce and fruits	Cut ready to eat fruit e.g. fruit mixes, fruit juices	5
	Cut ready to eat vegetables e.g. Bagged pre-cut salads and shredded carrot, cabbage, vegetable juices	5
	Leafy greens/Sprouts e.g. soy, mung, alfalfa,	5

Category	Types	Number of samples
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh poultry cuts e.g. turkey breast, turkey fillet	5
	Fresh mince e.g. lamb, beef, pork	5
	Processed ready to cook e.g. frozen patties, marinated kebabs, seasoned chicken breasts	5
Ready to eat foods (Combined category RTE/RTRH meats and poultry)	Ready to eat poultry e.g. turkey fillet, chicken sausage, pate	5
	Cooked fish products e.g. prawns, terrine, pate, smoked fish	5
	Cooked meat e.g. ham, salami, pate, corned beef	5
Multi component foods or meal components	Ready to re-heat refrigerated food e.g. cooked chilled foods, rice and pasta, products	5
	Ready to re-heat food frozen e.g. fries, pizza	5
	Composite foods with substantial raw ingredients e.g. .pasta salads, sandwiches, deli-salads	5

75 samples were analysed, leading to 75 interpretable results

2.1.2 Test sample preparation

Artificial contamination was carried out by seeding protocols. The inoculated strains, the contamination protocols, are provided in Appendix C.

60 samples were artificially contaminated in this study because it was not possible to find all naturally contaminated samples, 15 samples were naturally contaminated.

2.1.3 Test results

All raw data per category are given in Annex D.

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.

2.1.4 Calculation and interpretation of relative trueness study

The data for each sample per category and for each sample in all categories were plotted. The line of identity was drawn on which all points would lie if the two methods gave identical results for each sample analysed.

The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity (y = x). The Figures 1a to 1e shows the data plotted per category and Figure 1f summarises all the data.

Figure 1 a: Dairy products

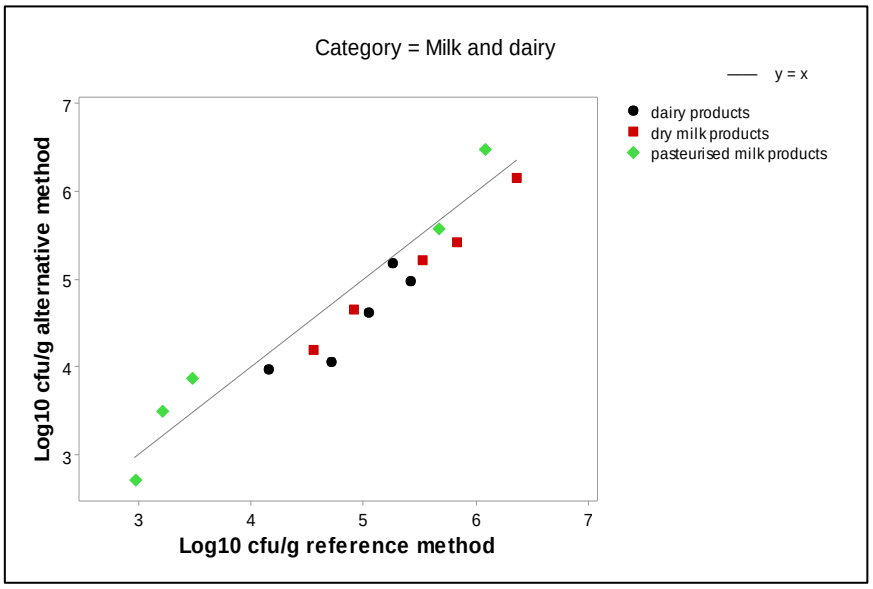


Figure 1b: Fresh produce and fruits

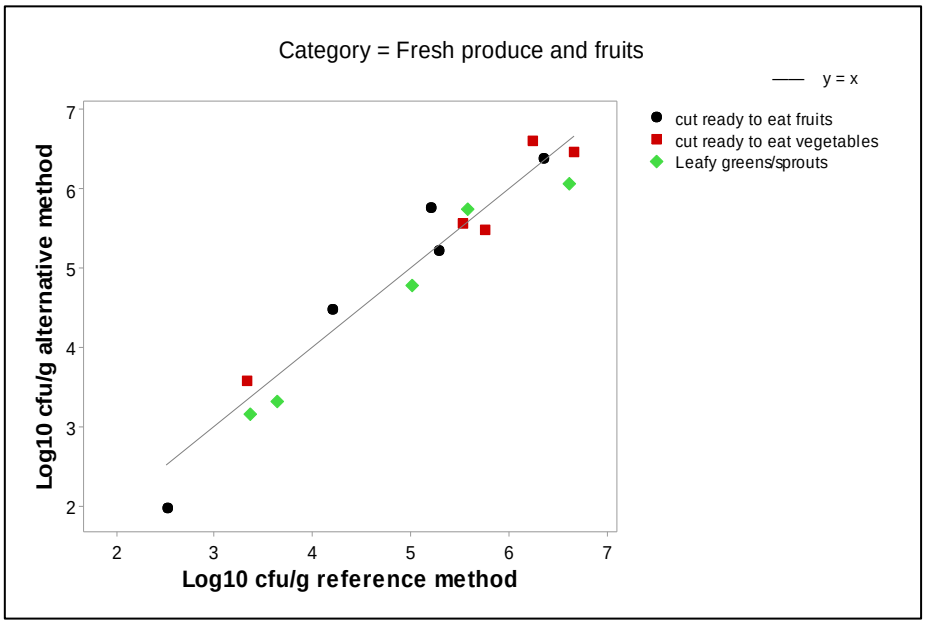


Figure 1c: Raw Meat and poultry

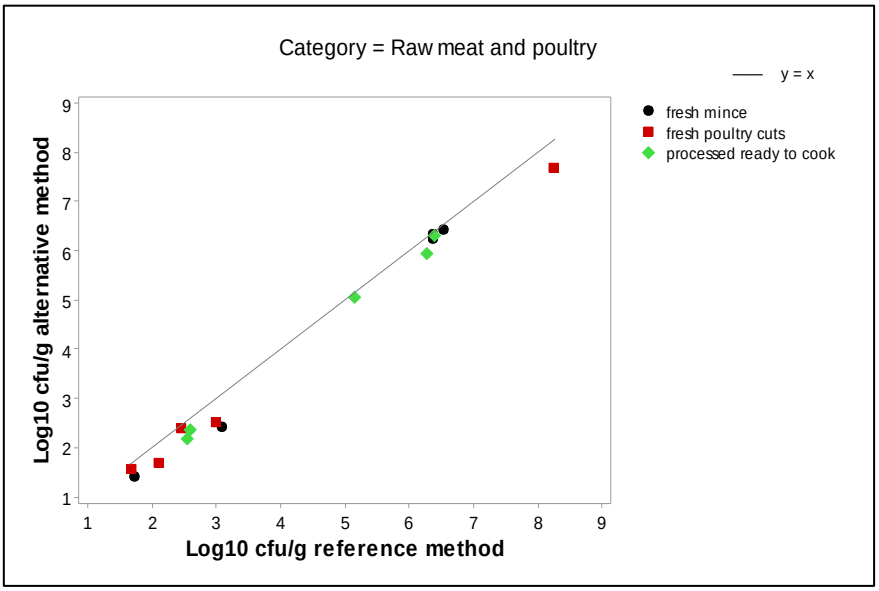


Figure 1d: Ready to eat Foods

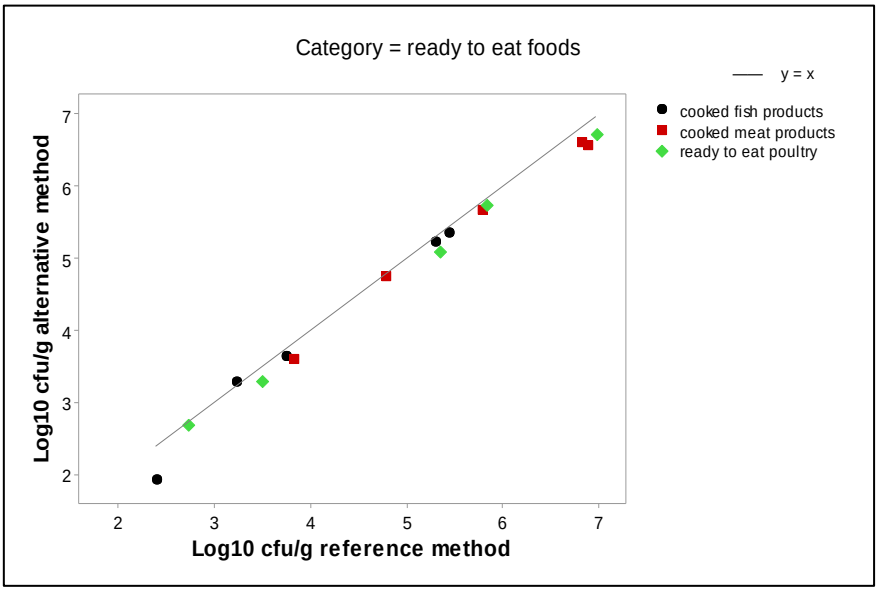


Figure 1e: Multi-component Foods

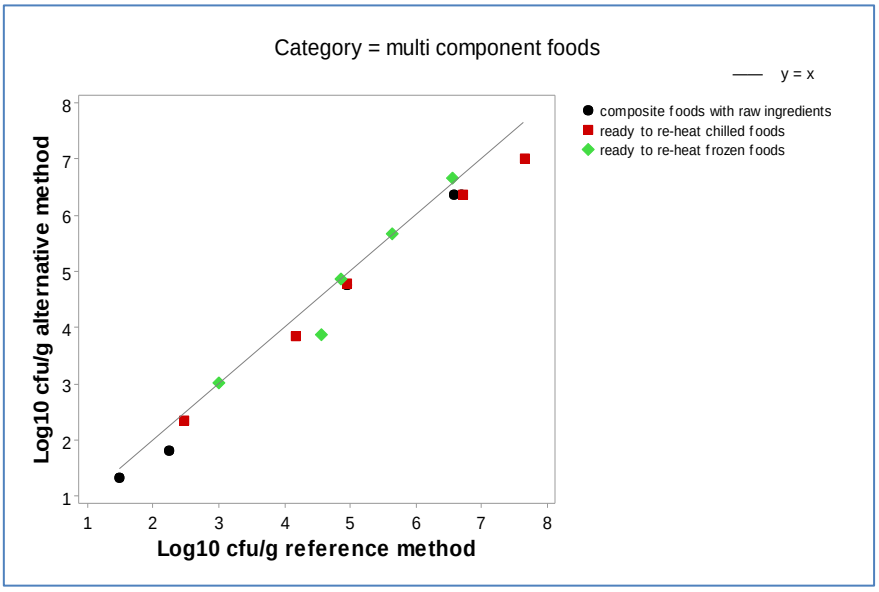
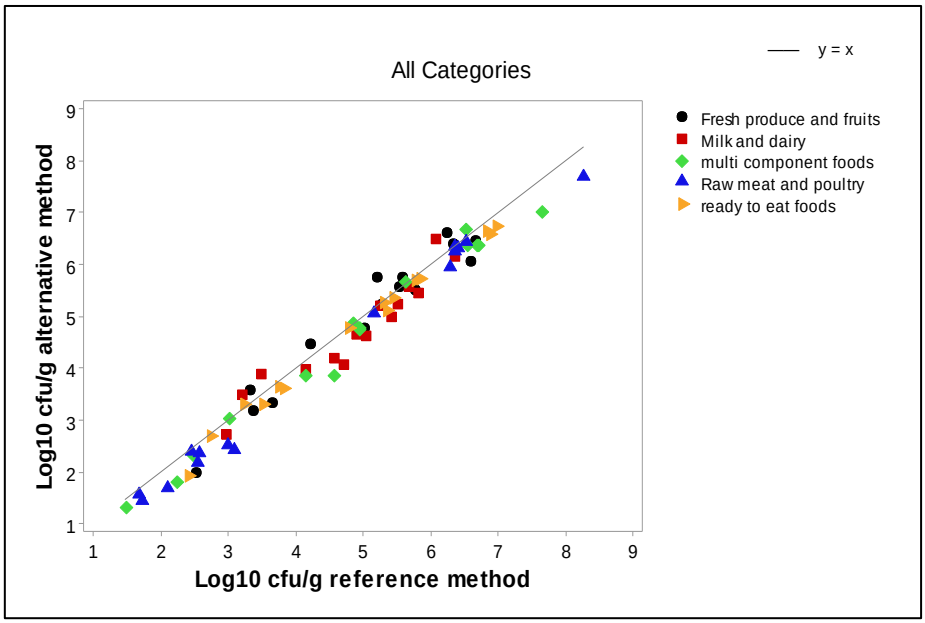


Figure 1f: All categories plot



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results. The data appears acceptable on the whole but there is some evidence of a negative bias for the alternate method for multicomponent foods and for milk and dairy products, particularly the dairy products and dried milk products. This can be seen from the individual product Figures (1a and 1e) and from the all categories Figure (1f). These products were spiked with strains of *Cronobacter*, *E.coli* and *Citrobacter*. These maybe under recovered on the reference method. This can be seen in Table 4 showing discordant results with a negative bias.

The data was analysed as described in ISO 16140-2:2016 section 6.1.2.3 in order to produce the Bland – Altman difference plot.

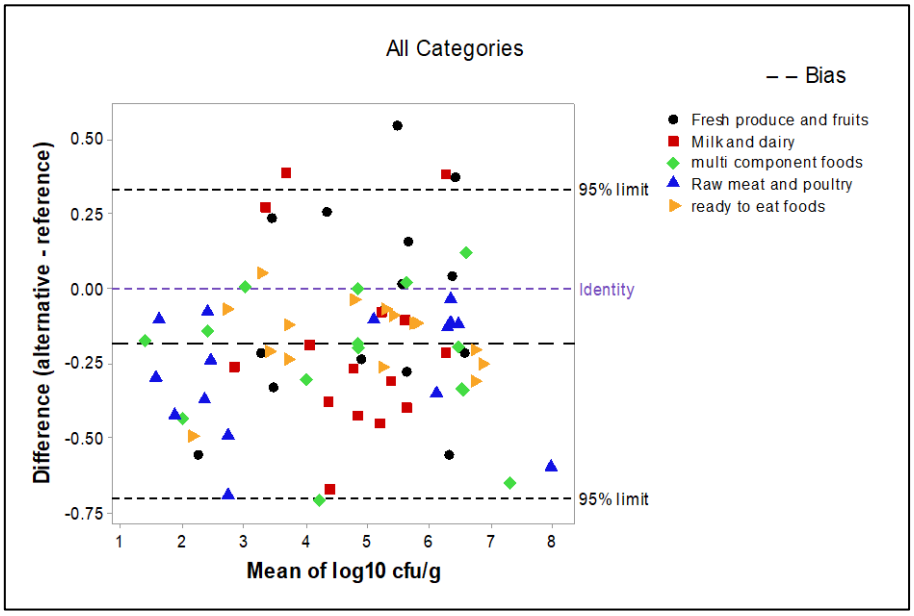
The average difference $\overline{D}$, the standard deviation of difference s_D and the limits of agreement were calculated per category and for all categories (Table 2). There was a slight negative bias for the 'all categories' data and a slightly larger negative bias for the multi-component foods which supports the visual observations from Figures 1a and 1e.

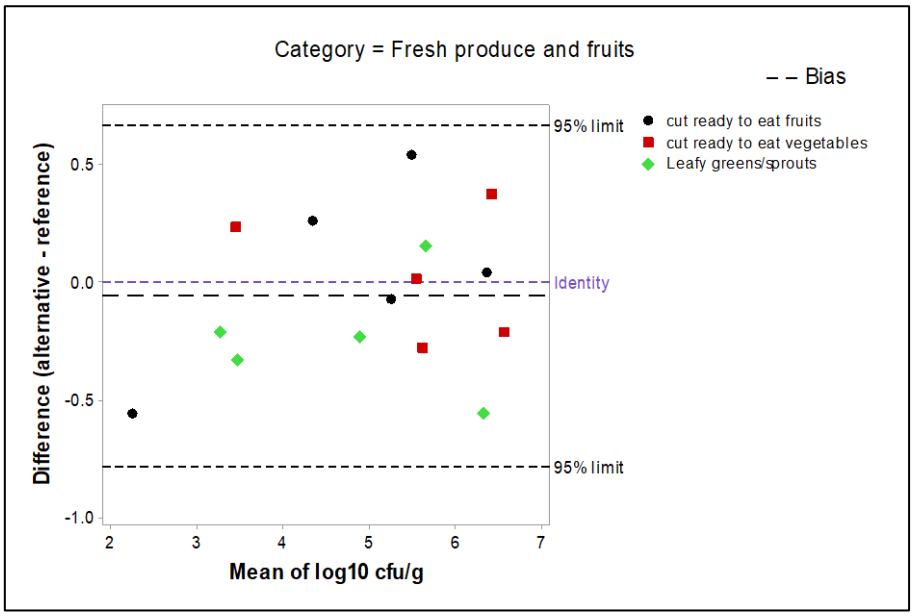
Table 2: Summary of calculated differences

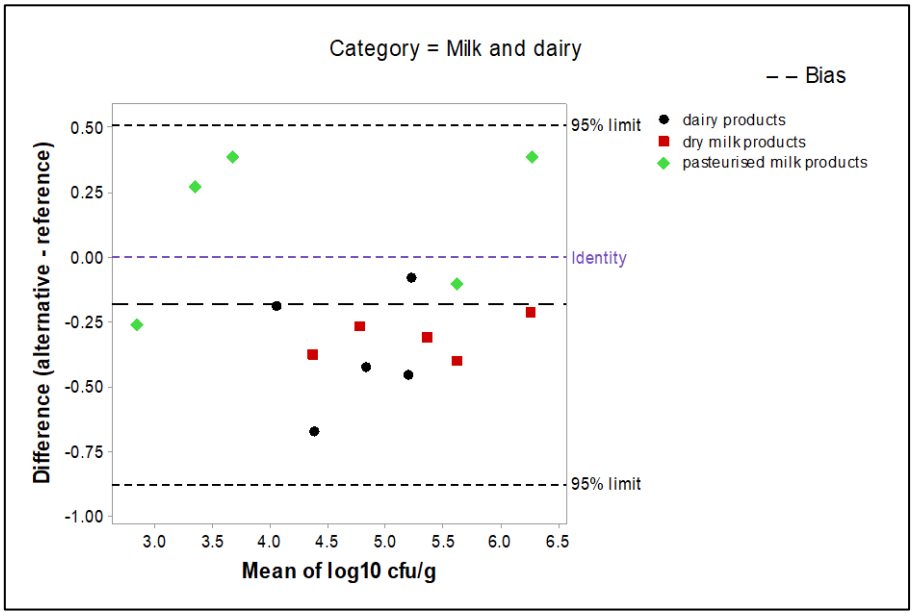
Category	n	$\overline{D}$	s_D	95% Lower limit	95% Upper limit
Fresh produce and fruits	15	-0.057339	0.327681	-0.783194	0.668517
Milk and dairy	15	-0.182829	0.312374	-0.874777	0.509118
Multi component foods	15	-0.236926	0.238518	-0.765273	0.291422
Raw meat and poultry	15	-0.280457	0.206353	-0.737554	0.176640
ready to eat foods	15	-0.170404	0.133439	-0.465988	0.125180
All Categories	75	-0.185591	0.258270	-0.703625	0.332443

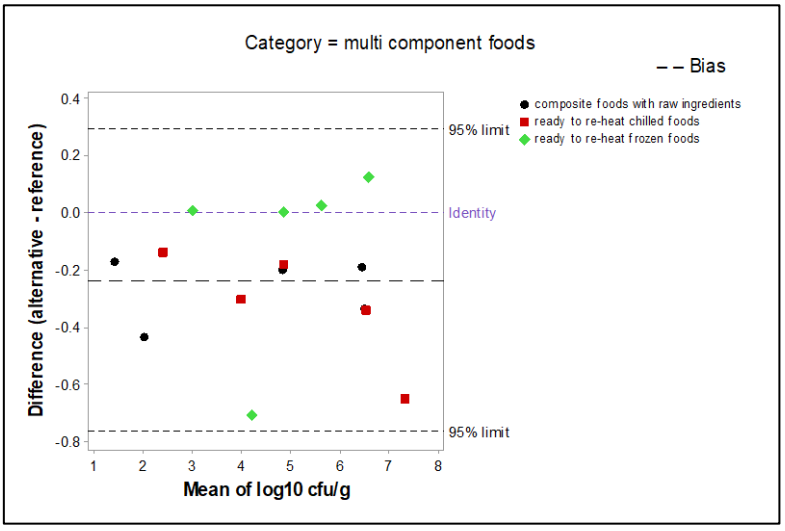
The individual sample differences were plotted against the mean values on a graph that shows the line of identity (zero difference), the line of bias, and the upper and lower 95% confidence limits of agreement of the bias. Although the text specifies four lines, the example in 16140 Figure 2 shows only three. We have plotted the “line of bias” at $\overline{D}$ as well as the line of identity and confidence limits.

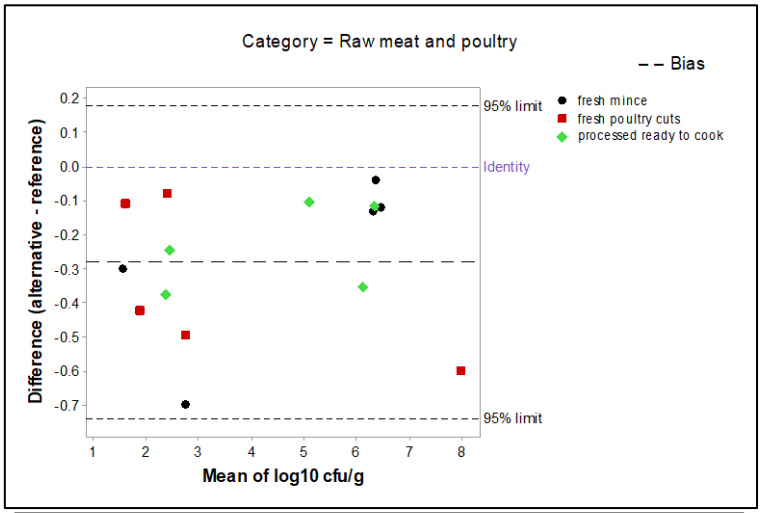
Figure 2: Bland-Altman plot for all categories

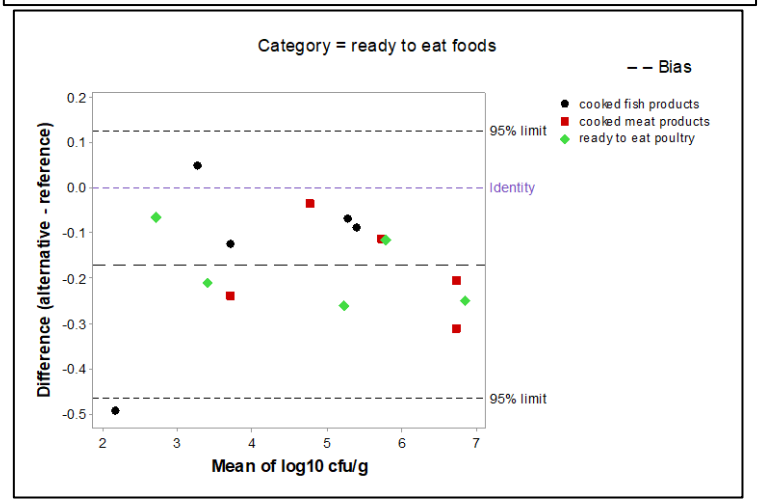

All Categories
– – Bias
Fresh produce and fruits
Milk and dairy
multi component foods
Raw meat and poultry
ready to eat foods
95% limit
Identity
95% limit
Difference (alternative - reference)
0.50
0.25
0.00
-0.25
-0.50
-0.75
1
2
3
4
5
6
7
8
Mean of log10 cfu/g


Category = Fresh produce and fruits
– – Bias
cut ready to eat fruits
cut ready to eat vegetables
Leafy greens/sprouts
95% limit
Identity
95% limit
Difference (alternative - reference)
0.5
0.0
-0.5
-1.0
2
3
4
5
6
7
Mean of log10 cfu/g


Category = Milk and dairy
– – Bias
95% limit
Identity
95% limit
dairy products
dry milk products
pasteurised milk products
Difference (alternative - reference)
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Mean of log10 cfu/g


Category = multi component foods
– – Bias
95% limit
Identity
95% limit
composite foods with raw ingredients
ready to re-heat chilled foods
ready to re-heat frozen foods
Difference (alternative - reference)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
1
2
3
4
5
6
7
8
Mean of log10 cfu/g





The results of the difference and scatter plot were interpreted based on a visual observation on the amount of bias and extreme results. 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 'All Categories' there are five in 75 values which lie outside the CLs. This is a little more than the expectation of less than one in 20.

The five points which were outside of the CLs are shown below in Table 3. The data covered 3 different food categories, and 4 different inoculated strains. Although there was a general slight negative bias to the data, only 1 data point was outside the lower CL and 4 were outside the upper CL.

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)
multi component foods	RTRH frozen foods	66	frozen fish cakes	*Hafnia alvei* 4009	frozen 2 weeks	-0.71
Fresh produce and fruits	cut RTE vegetables	25	cucumber, kale and mint juice	*Proteus mirabilis* 1588	chill 2-3 days	0.37
Milk and dairy	pasteurised milk products	15	strawberry milk	*Enterobacter cloacae* 6633	chill 2-3 days	0.38
Milk and dairy	pasteurised milk products	13	skimmed milk	*Enterobacter cloacae* 6633	chill 2-3 days	0.39
Fresh produce and fruits	cut RTE fruits	19	apple and mango juice	*E.adecarboxylata* 5503	chill 2-3 days	0.54

2.1.4 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 8 samples, 1 with a positive bias and 7 with negative bias.

The sample with the positive bias is only just discordant with a difference of +0.54logs. For the negative bias, 3 of the 7 results were for naturally contaminated samples and 4 were for inoculated sample. The inoculated samples covered three different categories and four different inoculated strains. The negative bias is potentially due to the reference method recovering non-target organisms as it has been shown to be less selective than the alternate method (see 2.4.2)

The results showing a HIGHER enumeration with the ALTERNATIVE method than with the REFERENCE method are shown below. (See Table 4).

Table 4 – Discordant results with a positive bias

Sample no.	**Product Category**	**Products**	**Bias log Alt - log Ref (log CFU/g)**	**Strain**	**Stress applied**
19	Fresh produce and fruits	apple and mango juice	0.54	E.adecarboxylata 5503	chill 2-3 days

The results showing a LOWER enumeration with the ALTERNATE method than with the REFERENCE method are shown below (See Table 5).

Table 5 – Discordant results with a negative bias

Sample no.	Product Category	Products	Bias log Alt - log Ref (log CFU/g)	Strain	Stress applied
66	multi component foods	frozen fish cakes	-0.711	Hafnia alvei 4009	frozen 2 weeks
40	Raw meat and poultry	turkey breast mince	-0.699	*natural*	N/A
8	Milk and dairy	halloumi with chilli	-0.675	Citrobacter braakii 16279	chill 2-3 days
65	multi component foods	pilau rice	-0.657	*Escherchia vulneris CRA 2005*	frozen 2 weeks
33	Raw meat and poultry	chicken breast fillets	-0.602	*natural*	N/A
27	Fresh produce and fruits	spinach	-0.561	*natural*	N/A
16	Fresh produce and fruits	melon, pineapple ,mango	-0.560	Escherchia fergusonii CRA 7522	chill 2-3 days

2.1.5 2.1.5 Conclusion
The relative trueness study of the ALTERNATIVE method is satisfied.

2.2 Accuracy profile study

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. Non –inoculated samples (5) of each product type were also tested. Each sample was bulk inoculated and separate replicate test portions examined

The tested categories, types, items and inoculated strains are provided in the Table 6.

Tbale 6: **

Category	Types	Strain	Item	Target Level*	Test portions
Dairy products	Pasteurised dairy products	*E. coli* CRA 1476 from dried milk	Pasteurised cream	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Enterobacter agglomerans CRA 5613 from milk powder	Cream cheese	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Fruits and vegetables	Fresh produce	*E.hermanii* CRA 7477 from sesame seeds	Ready to cook Vegetable preparation	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Citrobacter amalonaticus CRA 7458 from beansprouts	Vegetable juice	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Raw poultry	Fresh meat	*Salmonella Brandenberg* CRA	Pork mince	Low 10^2 cfu/g	5

Category	Types	Strain	Item	Target Level*	Test portions
and meats (Combined category raw/ RTC meats and poultry)		1070 from beef		Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Proteus mirabilis CRA 1588 from poultry	Raw bacon	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Ready to eat foods (Combined category RTE/RTRH meats and poultry)	Cooked fish products e.g. prawns	*E.coli* CRA 2003 from fish	Fresh prawns	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Klebsiella oxytoca ATCC 15926	Fish pate	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Multi component foods	Composite foods with raw ingredients	*Hafnia alvei* CRA 400 from sandwich	Sandwiches	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		E. adecarboxylata CRA 5501 from skimmed milk powder	Cooked chilled rice	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5

*these are target values only and actual values may be ± 1 log from the target dependent on microbial behaviour

Total number of samples = minimum 150

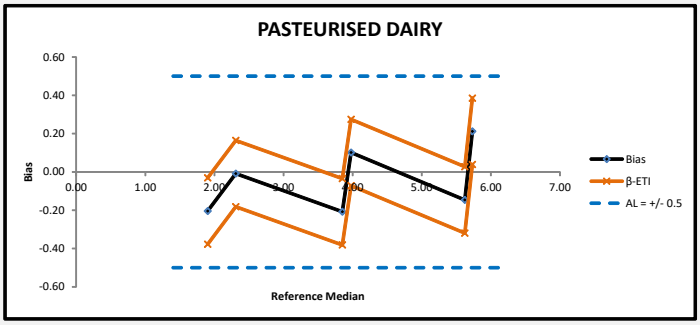
2.2.1 Calculations and interpretation of accuracy profile study

The raw data are provided Annex G and the summary tables (in log CFU/g) in Annex H**Fout! Verwijzingsbron niet gevonden.** The statistical results and the accuracy profiles are provided Figure 7.

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 3a – Accuracy profile –dairy products

(Food) Category	DAIRY
(Food) Type	PASTEURISED DAIRY

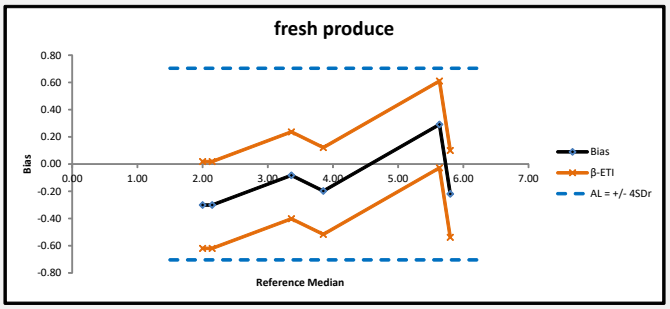


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
142 21 248 188 53	1.90	-0.204	-0.377	-0.031	YES	YES
107 B45B 75B 342B 322B	2.31	-0.009	-0.182	0.165	YES	YES
31 10 97 13 273	3.85	-0.208	-0.381	-0.035	YES	YES
314 346 25 63 328	3.98	0.101	-0.072	0.275	YES	YES
196 229 57 102 259	5.62	-0.146	-0.319	0.027	YES	YES
58 141 172 83 109	5.73	0.212	0.039	0.385	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.129	0.120	NO	+/- 0.500

Figure 3b – Accuracy profile – fruit and vegetable products

(Food) Category	fruit and veg
(Food) Type	fresh produce

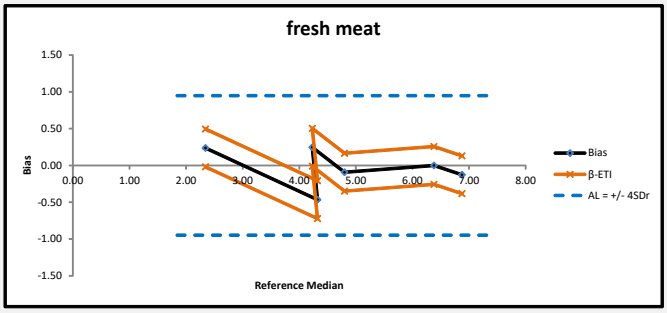


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
8 17 327 246 37	2.00	-0.301	-0.620	0.018	NO	YES
264 252 139 300 294	2.15	-0.301	-0.620	0.018	NO	YES
312 301 64 267 340	3.36	-0.083	-0.402	0.236	YES	YES
236 92 223 9 158	3.85	-0.198	-0.517	0.121	NO	YES
316 86 323 117 203	5.63	0.291	-0.028	0.610	NO	YES
74 234 272 34 144	5.80	-0.220	-0.539	0.099	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.176	0.221	NO	+/- 0.704

Figure 3c – Accuracy profile meat and poultry

(Food) Category	raw poultry and meat
(Food) Type	fresh meat

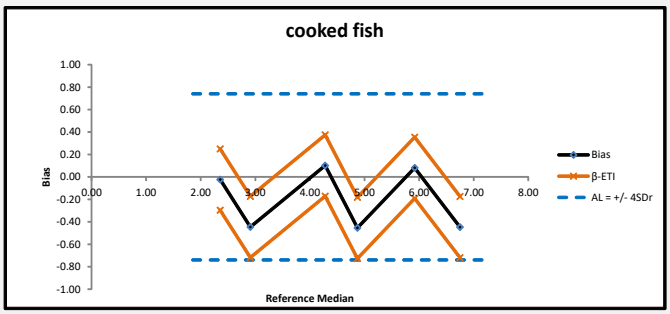


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
250 80 337 56 238	2.34	0.237	-0.020	0.494	YES	YES
194 72 177 12 270	4.32	-0.465	-0.722	-0.208	NO	YES
200 339 120 274 106	4.23	0.247	-0.010	0.504	NO	YES
243 124 297 268 347	4.80	-0.092	-0.349	0.165	YES	YES
183 197 2 40 5	6.38	0.000	-0.257	0.257	YES	YES
232 217 313 89 147	6.88	-0.127	-0.384	0.130	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.237	0.178	NO	+/- 0.948

Figure 3d – Accuracy profile

(Food) Category	RTE foods
(Food) Type	cooked fish

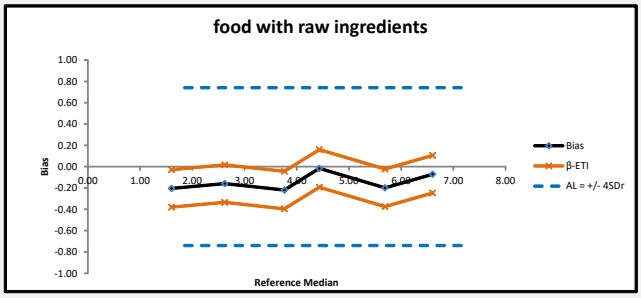


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
51 247 218 87 287	2.36	-0.024	-0.296	0.249	YES	YES
111 155 255 186 202	2.91	-0.446	-0.719	-0.173	NO	YES
289 68 23 309 226	4.28	0.101	-0.171	0.374	YES	YES
256 192 295 16 298	4.87	-0.454	-0.727	-0.181	NO	YES
195 261 42 61 320	5.92	0.081	-0.192	0.354	YES	YES
319 189 78 82 182	6.75	-0.447	-0.720	-0.174	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.185	0.189	NO	+/- 0.740

Figure 3e – Accuracy profile

(Food) Category	Mulit component
(Food) Type	food with raw ingredients



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
329 30 146 275 242	1.60	-0.204	-0.380	-0.028	YES	YES
307 290 157 288 32	2.62	-0.159	-0.335	0.017	YES	YES
242 184 211 334 331	3.76	-0.219	-0.395	-0.043	YES	YES
282 60 159 308 235	4.43	-0.016	-0.193	0.160	YES	YES
131 59 161 103 209	5.69	-0.199	-0.375	-0.023	YES	YES
239 179 29 251 128	6.60	-0.071	-0.247	0.106	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.115	0.122	YES	+/- 0.500

For some of the food categories the additional AL calculation was required. This was for the meat, fresh produce and ready to eat cooked fish products. For the meat category the lower level for the pork mince showed a negative bias and the medium level for the bacon showed a positive bias. For the ready to eat cooked fish category all three fish pate levels showed a negative bias. For the fresh produce category, the ready to cook produce and vegetable juice low levels and the vegetable juice medium level and high levels showed a negative bias, and the ready to cook produce high level a positive bias. Newly calculated AL's were 0.948 for the meat category and 0.704 for the fresh produce and 0.740 for the ready to eat category.

The observed profiles were witin the 0.5log AL or the recalcuated limit. All the accuracy profiles fufil the performance criteria after the permitted recaluation and the altenaitve mthi is accpted as bein equivalent to the reference method

2.3 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.

2.3.1 2.4.1 Protocol
After being grown according to appropriate conditions, decimal dilutions were made and the 20 target strains and 10 non-target strains were enumerated by the alternative method, the reference method and a non selective agar (PCA/MRSA).

2.3.2 2.4.2 Results
2.4.2.1 Original study

The inclusivity results (Appendix 8) revealed all 32 strains belonging to the family Enterobacteriaceae produced typical colonies in VRBGA and also appeared as typical colonies on the Compact Dry ETB plates.

The results from the 23 strains of non-target organisms used to determine the exclusivity of the ETB method (Appendix 8) showed that 21 strains did not grow on the ETB medium and 20 strains did not grow in VRBGA. The two strains that did grow in VRBGA included a strain of *Aeromonas hydrophila* (strain 4111) which appeared typical in this medium, a strain of *Vibrio parahaemolyticus* (strain 15737) which grew but was atypical in appearance on the ETB medium and which produced typical colonies in VRBGA, although growth was poor. One strain of *Pasteurella bettyae* yielded typical colonies by both methods whereas tests with other *Pasteurella* strains, including an additional *P. bettyae* strain showed inhibition of these bacteria by both media. *Pasteurella* spp belong to the family Pasteurellaceae and not the Enterobacteriaceae, and both members of these families are capable of fermenting glucose, and although their optimum growth temperature is 37°C most are fastidious in their growth requirements. However, unlike members of the Enterobacteriaceae *Pasteurella* spp. are oxidase-positive.

2.4.2.2 Current Study

Of the 23 inclusivity strains tested 18 strains were detected using the alternative and reference methods. Those not detected by either method were *Erwinia amylovora* 8037 and *Erwinia herbicola* 7057. Three strains were detected by the reference method but not by the alternative method- these were: *Serratia liquefaciens* 10670, *Rahnella aqualatis* NCIMB 13365 and *Yersinia intermedia* 380.

Of the 10 exclusivity strains tested, one strain was detected by both the alternate method and by the reference method, *A.sobria* CRA 8390.

2.4.2.3 Additional isolates tested September 2024

A gap analysis was carried out to check if all families named in the Enterobacterales were included in the inclusivity panel. The analysis showed that two families (Budviciae and Pectobacteriacae) had not been tested in the previous study. Two additional inclusivity isolates were tested to cover the missing genera from the Enterobacterales and the results are shown in Annex H for reference

Data revealed that the *Pectobacterium carotovorum* isolate (CRA 8036) did not grow on either the reference method or alternative method. The second isolate analysed *Leminorella richardii* (DSM 14849) gave typical colonies on the reference method, however did not grow within the 22h incubation time for the alternative method. No further isoates belonging to that genus was available from culture collections, however extending the incuabtion period to 48h enabled growth of *Leminorella richardii* on Compact Dry ETB.

Conclusion

The alternative Compact Dry ETB for enumeration of Enterobacteriacae is selective and specific.

2.4 Limit of quantification (LOQ)

The limit of Quantification (LOQ) is the lowest analyte concentration that can be quantified with an acceptable level of precision and trueness under the conditions of the test.

As the alternative method is based on counting visible colonies target microorganism, the LOQ was not required to be determined according to ISO 16140-2:2016.

2.4.1 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The alternative method Compact Dry ETB for enumeration of Enterobacteriacae shows satisfactory results for relative trueness;
- The alternative Compact Dry ETB for enumeration of Enterobacteriacae shows satisfactory results for accuracy profile;

- The alternative Compact Dry ETB for enumeration of Enterobacteriacae is selective and specific.

3 Interlaboratory study

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

3.1 Study **organisation**

There were 10 collaborative laboratories used in this study representing 5 different countries.

Pasteurised milk was used as the food matrix for the interlaboratory study and the samples of milk were artificially contaminated with a single strain of *E. coli* (CCFRA code 11017, NCTC 12241) and a single strain of *Enterobacter aerogenes* (CCFRA 15736). Each strain was cultured in 10 ml NB incubated overnight at 37 ± 1°C. Both cultures were serially diluted in MRD to give the desired levels of inoculum for the contamination of the samples and were mixed together in equal concentrations.

A set of 8 x 25 ml samples of pasteurised milk were prepared for each laboratory, including the organising laboratory. Two samples remained uninoculated, whereas the other six samples were inoculated at 3 different contamination levels (low, medium and high). Appropriate dilutions of the mixed culture cocktail were used to individually inoculate 2 x 25ml samples at the low (10^1 – 10^2 CFU/ml), medium (10^2 – 10^3 CFU/ml) and high (10^3 – 10^4 CFU/ml) contamination levels. The samples were blind coded and stored at 2 – 8°C prior to despatch to the collaborative laboratories. Before despatch, each set of eight samples was packed into a suitable container with cool packs. Also, an additional vial containing water was packed with each set of samples. This enabled the laboratories to take a temperature measurement upon receipt.

Upon receipt, each collaborative laboratory tested a 10 ml test portion from each of the eight milk samples by the ISO 4832:2006 method and the Compact Dry EC method. In addition, the organising laboratory tested a set of eight milk samples at the same time as the collaborative laboratories to confirm the presence of the target organism and the contamination levels. This data was not used in the analyses.

The study was done in November 2007

Table 7: Sample receipt data for ILS samples

Temperature of control sample upon receipt Laboratory	**Date received**	**Temperature of control sample upon receipt (°C)**
1	05/11/07	3.1
2	05/11/07	6

Temperature of control sample upon receipt Laboratory	Date received	Temperature of control sample upon receipt (°C)
3	05/11/07	7
4	05/11/07	2.65
5	05/11/07	5.2
6	05/11/07	6.5
7	05/11/07	2.9
8	05/11/07	7
9	05/11/07	2.1
10	05/11/07	2.7

3.2 Calculation and summary 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 8 below and the Accuracy profile graph is shown in Figure 4.

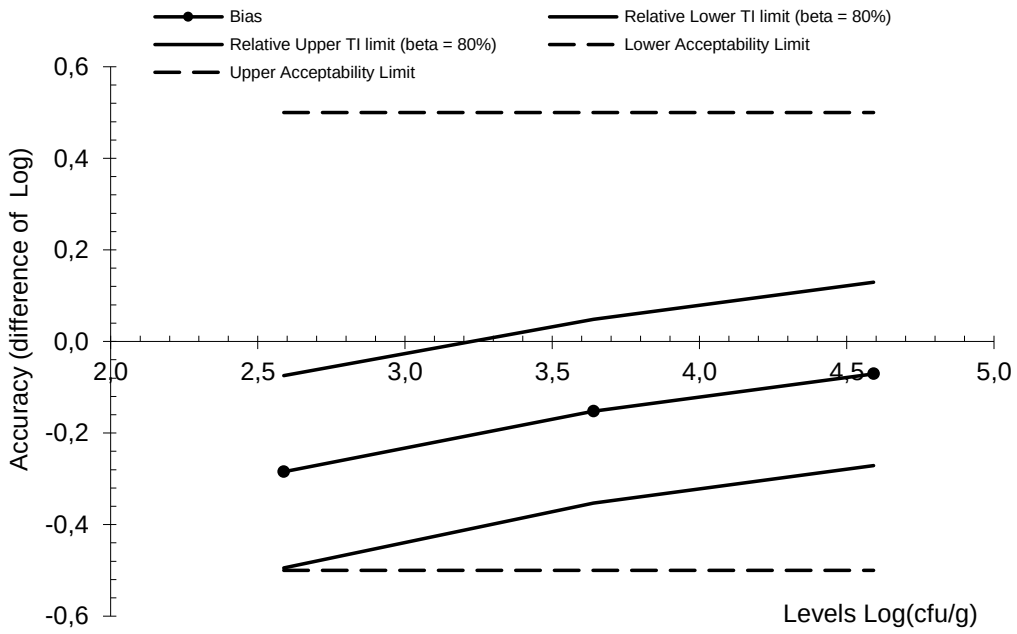
Table 8: Summary of the results of the interlaboratory study per analyte level (k)

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
1	Blank	<10	<10	<10	<10
2	Blank	<10	<10	<10	<10
3	Blank	<10	<10	<10	<10
4	Blank	<10	<10	<10	<10

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
5	Blank	<10	<10	<10	<10
6	Blank	<10	<10	<10	<10
7	Blank	<10	<10	<10	<10
8	Blank	<10	<10	<10	<10
9	Blank	<10	<10	<10	<10
10	Blank	<10	<10	<10	<10
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Low	2.695	2.626	2.621	2.639
2	Low	2.743	2.626	2.566	2.496
3	Low	2.508	2.571	2.597	2.549
4	Low	2.462	2.526	2.449	2.365
5	Low	2.462	2.260	2.396	2.338
6	Low	2.496	2.435	2.639	2.560
7	Low	2.483	2.571	2.413	2.449
8	Low	2.653	2.611	2.549	2.648
9	Low	2.477	2.555	2.520	2.490
10	Low	2.490	2.864	2.606	2.797
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Medium	3.754	3.670	3.373	3.310

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
2	Medium	3.740	3.803	3.754	3.686
3	Medium	3.653	3.611	3.538	3.695
4	Medium	3.520	3.538	3.373	3.280
5	Medium	3.566	3.581	3.329	3.347
6	Medium	3.526	3.718	3.496	3.602
7	Medium	3.815	3.576	3.526	3.576
8	Medium	3.691	3.470	3.611	3.358
9	Medium	3.544	3.648	3.538	3.356
10	Medium	3.747	3.621	3.496	3.502
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	High	4.644	4.508	4.508	4.320
2	High	4.872	4.806	4.803	4.856
3	High	4.648	4.571	4.469	4.581
4	High	4.597	4.538	4.428	4.490
5	High	4.666	4.280	4.397	4.310
6	High	4.581	4.635	4.635	4.571
7	High	4.678	4.653	4.442	4.597
8	High	4.635	4.670	4.630	4.508
9	High	4.456	4.456	4.496	4.397
10	High	4.389	4.538	4.463	4.502

Figure 6. Accuracy profile of Compact Dry ETB from the ILS



The statistical analysis of the existing ILS data is shown in Table 9 below. It can be seen that the repeatability standard deviation (S_r) was very similar for the alternate method and the reference method ranging from 0.080 to 0.132 for the compact dry CF and 0.084 to 0.150 for the reference method.

The between-labs standard deviation (S_L) was similar for the alternative method (0.076 to 0.115) and the reference method (0.096 to 0.119) as was the reproducibility standard deviation (S_R) with alternative method values of 0.142 to 0.152 and reference method values of (0.101 to 0.136).

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the ±0.5log AL then a further calculation is done to calculate the pooled average S_R of the reference method. This was not required as all values were within the required limits. The data are plotted in Figure 4 and it can be seen that no values lie outside of these AL_s values and therefore the alternative method is accepted as being equivalent to the reference method. It is worth noting that there was a slight negative bias for all levels which was larger at the lowest level (-0.285) and decreased as the levels got higher.

Table 9. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile	0.5						
Study Name	Compact Dry ETB						
Date	Study originally done 2007						
Coordinator	Re-calculated June 2017						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0.50	0.50	0.50				
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**2.588**	**3.640**	**4.591**				
Number of participants (K)	10	10	10		10	10	10
Average for alternative method	2.303	3.487	4.520		2.588	3.640	4.591
Repeatability standard deviation (sr)	0.115	0.087	0.076		0.119	0.096	0.102
Between-labs standard deviation (sL)	0.099	0.113	0.119		0.039	0.032	0.091
Reproducibility standard deviation (sR)	0.152	0.143	0.142		0.125	0.101	0.136
Corrected number of dof	15.436	12.994	11.999		18.593	18.577	15.274
Coverage factor	1.386	1.404	1.413				
Interpolated Student t	1.339	1.350	1.356				
Tolerance interval standard deviation	0.1569	0.1488	0.1477				
Lower TI limit	2.093	3.286	4.320				
Upper TI limit	2.513	3.688	4.720				
Bias	**-0.285**	**-0.152**	**-0.071**				
Relative Lower TI limit (beta = 80%)	**-0.495**	**-0.353**	**-0.271**	FALSE			
Relative Upper TI limit (beta = 80%)	**-0.075**	**0.049**	**0.129**	FALSE			
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**				
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0.122						

FALSE

Application of clause 6.2.3
Step 8: If any of the values for the β-ETI fall outside the acceptability limits, calculate the pooled average reproducibility standard deviation of the reference method.
Step 9: Calculate new acceptability limits as a function of this standard deviation.

Select ALL blue lines to draw the accuracy profile as illustrated in the worksheet "Graph Profile"

4 Overall conclusions of the validation study

- The alternative method Compact Dry ETB for enumeration of Enterobacteriaceae shows satisfactory results for relative trueness;
- The alternative Compact Dry EC ETB for enumeration of Enterobacteriaceae shows satisfactory results for accuracy profile;
- The ETB for enumeration of Enterobacteriaceae in foods method was shown to be specific and selective.
- The alternative method ETB for enumeration of Enterobacteriaceae shows satisfactory results for the ILS
- The alternative method ETB for enumeration of Enterobacteriaceae shows comparable performance to the reference method. ISO 21528-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony- count technique

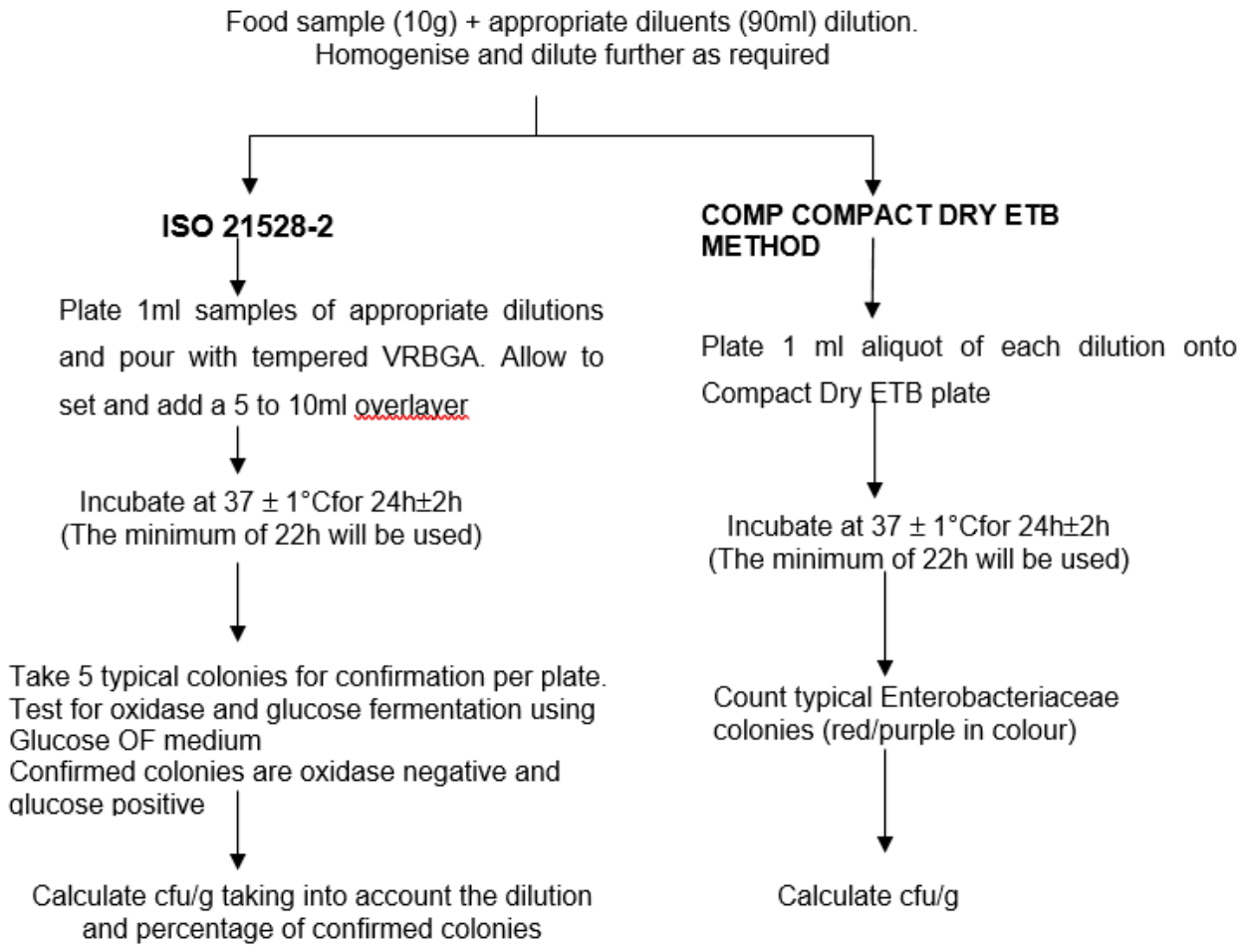
Date, 26 September 2024

Signature

Suzanne Jordan

ANNEX A: Flow diagram of the reference method

Figure 1: Comparison of Reference method (ISO 21528-2) and Alternative Method: HyServe Compact Dry ETB for enumeration of Enterobacteriaceae



ANNEX B: Kit insert(s)

Refer to separate files for details

ANNEX C: Raw data per category

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
Milk and dairy	dry milk products	1	dried skimmed milk	1000000	*Cronobacter sakazakii* NCIMB 8272	dried milk	ambient 2 weeks
Milk and dairy	dry milk products	2	strawberry whip	1000000	*Cronobacter sakazakii* NCIMB 8272	dried milk	ambient 2 weeks
Milk and dairy	dry milk products	3	blancmange	1000000	*Cronobacter sakazakii* NCIMB 8272	dried milk	ambient 2 weeks
Milk and dairy	dry milk products	4	instant custard mix	1000000	*Cronobacter sakazakii* NCIMB 8272	dried milk	ambient 2 weeks
Milk and dairy	dry milk products	5	baby mikk	1000000	*Cronobacter sakazakii* NCIMB 8272	dried milk	ambient 2 weeks
Milk and dairy	dairy products	6	strawberry yogurt	100000	*E.coli 108*	Fish cakes	chill 2-3 days
Milk and dairy	dairy products	7	single cream	1000	*E.coli* 108	Fish cakes	chill 2-3 days
Milk and dairy	dairy products	8	halloumi with chilli	10000	*Citrobacter braakii* 16279	Industrial isolate	chill 2-3 days
Milk and dairy	dairy products	9	full fat soft cheese	100000	*Citrobacter braakii* 16279	Industrial isolate	chill 2-3 days
Milk and dairy	dairy products	10	gran levato (raw cheese)	1000000	*Citrobacter braakii* 16279	Industrial isolate	chill 2-3 days
Milk and dairy	pasteurised milk products	11	full fat milk	100	*Enterobacter cloacae* 6633	Industrial isolate	chill 2-3 days
Milk and dairy	pasteurised milk products	12	semi skimmed milk	1000	*Enterobacter cloacae* 6633	Industrial isolate	chill 2-3 days
Milk and dairy	pasteurised milk products	13	skimmed milk	10000	*Enterobacter cloacae* 6633	Industrial isolate	chill 2-3 days
Milk and dairy	pasteurised milk products	14	chocolate milk	100000	*Enterobacter cloacae* 6633	Industrial isolate	chill 2-3 days

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
Milk and dairy	pasteurised milk products	15	strawberry milk	1000000	*Enterobacter cloacae* 6633	Industrial isolate	chill 2-3 days
Fresh produce and fruits	cut ready to eat fruits	16	melon,pineapple,mango	100	*Escherchia fergusonii* CRA 7522	sausages	chill 2-3 days
Fresh produce and fruits	cut ready to eat fruits	17	garpe, strawberry,blackberry	10000	*E.adecarboxylata* 5501	skimmed milk powder	chill 2-3 days
Fresh produce and fruits	cut ready to eat fruits	18	pineapple	10000	*E.adecarboxylata* 5501	skimmed milk powder	chill 2-3 days
Fresh produce and fruits	cut ready to eat fruits	19	apple and mango juice	100000	*E.adecarboxylata* 5501	skimmed milk powder	chill 2-3 days
Fresh produce and fruits	cut ready to eat fruits	20	orange juice	1000000	*E.adecarboxylata* 5501	skimmed milk powder	chill 2-3 days
Fresh produce and fruits	cut ready to eat vegetables	21	mixed leaf salad	none	none	N/A	natural
Fresh produce and fruits	cut ready to eat vegetables	22	carrot batons	100000	*Proteus mirabilis* 1588	poultry	chill 2-3 days
Fresh produce and fruits	cut ready to eat vegetables	23	brocoli	1000000	*Proteus mirabilis* 1588	poultry	chill 2-3 days
Fresh produce and fruits	cut ready to eat vegetables	24	casserole veg selection	100000	*Proteus mirabilis* 1588	poultry	chill 2-3 days
Fresh produce and fruits	cut ready to eat vegetables	25	cucumber,kale and mint juice	1000000	*Proteus mirabilis* 1588	poultry	chill 2-3 days
Fresh produce and fruits	Leafy greens/sprouts	26	beansprouts	none	none	N/A	natural
Fresh produce and fruits	Leafy greens/sprouts	27	spinach	1000000	*Serratia fonticola* 4613	chicken	chill 2-3 days

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
Fresh produce and fruits	Leafy greens/sprouts	28	pea shoots	none	none	N/A	natural
Fresh produce and fruits	Leafy greens/sprouts	29	watercress,spinach and rocket	none	none	N/A	natural
Fresh produce and fruits	Leafy greens/sprouts	30	flat leaf parsley	none	none	N/A	natural
Raw meat and poultry	fresh poultry cuts	31	chicken thighs	none	none	N/A	natural
Raw meat and poultry	fresh poultry cuts	32	chicken drumsticks	none	none	N/A	natural
Raw meat and poultry	fresh poultry cuts	33	chicken breast fillets	1000000	*Enterobacter amingenus* 4613	NCIMB 2118 sea water	chill 2-3 days
Raw meat and poultry	fresh poultry cuts	34	diced turkey thigh	none	none	N/A	natural
Raw meat and poultry	fresh poultry cuts	35	thin cut turkey breast steaks	none	none	N/A	natural
Raw meat and poultry	fresh mince	36	lean beef steak mince	none	none	N/A	natural
Raw meat and poultry	fresh mince	37	beef mince	100000	*Salmonella* Brandenburg 1070	beef	chill 2-3 days
Raw meat and poultry	fresh mince	38	lean pork mince	1000000	*Salmonella* Brandenburg 1070	beef	chill 2-3 days
Raw meat and poultry	fresh mince	39	lamb mince	1.00E+06	*E. coli* 4104	ATCC 25922 clinical isolate	chill 2-3 days
Raw meat and poultry	fresh mince	40	turkey breast mince	none	none	N/A	natural
Raw meat and poultry	processed ready to cook	41	frozen beef burger	none	none	N/A	natural

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
Raw meat and poultry	processed ready to cook	42	cheese and ham kievs	none	none	N/A	natural
Raw meat and poultry	processed ready to cook	43	southern fried chicken goujons	10000	*Salmonella* Brandenburg 1070	beef	chill 2-3 days
Raw meat and poultry	processed ready to cook	44	breaded mini chicken fillets	100000	*Salmonella* Brandenburg 1070	beef	chill 2-3 days
Raw meat and poultry	processed ready to cook	45	garlic chicken kievs	1000000	E.coli 4104	ATCC 25922 clinical isolate	chill 2-3 days
ready to eat foods	ready to eat poultry	46	roast turkey breast slices	100	*Salmonella* Hadar 1019	turkey	chill 2-3 days
ready to eat foods	ready to eat poultry	47	roast chicken breast pieces	1000	*Salmonella* Hadar 1019	turkey	chill 2-3 days
ready to eat foods	ready to eat poultry	48	chicken slices	10000	*Salmonella* Hadar 1019	turkey	chill 2-3 days
ready to eat foods	ready to eat poultry	49	chicken drumsticks	100000	*Salmonella* Hadar 1019	turkey	chill 2-3 days
ready to eat foods	ready to eat poultry	50	chicken thighs	1000000	*Salmonella* Hadar 1019	turkey	chill 2-3 days
ready to eat foods	cooked fish products	51	hot smoked mackerel	1000	*Enterobacter agglomerans* 5513	skimmed milk powder	chill 2-3 days
ready to eat foods	cooked fish products	52	prawn cocktail	1000	*Enterobacter agglomerans* 5513	skimmed milk powder	chill 2-3 days
ready to eat foods	cooked fish products	53	smoked slamon pate	10000	*Enterobacter agglomerans* 5513	skimmed milk powder	chill 2-3 days
ready to eat foods	cooked fish products	54	cold water prawns	100000	*Enterobacter agglomerans* 5513	skimmed milk powder	chill 2-3 days
ready to eat foods	cooked fish products	55	smoked salmon pieces	1000000	*Enterobacter agglomerans* 5513	skimmed milk powder	chill 2-3 days

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
ready to eat foods	cooked meat products	56	corned beef	1000	*Citrobacter amalonaticus* 7458	beansprouts	chill 2-3 days
ready to eat foods	cooked meat products	57	cooked ham	10000	*Citrobacter amalonaticus* 7458	beansprouts	chill 2-3 days
ready to eat foods	cooked meat products	58	pork luncheon meat	100000	*Citrobacter amalonaticus* 7458	beansprouts	chill 2-3 days
ready to eat foods	cooked meat products	59	german salami	1000000	*Citrobacter amalonaticus* 7458	beansprouts	chill 2-3 days
ready to eat foods	cooked meat products	60	brussels pate	1000000	*Citrobacter amalonaticus* 7458	beansprouts	chill 2-3 days
multi component foods	ready to re-heat chilled foods	61	tagliatelle	100	*Escherchia vulneris* CRA 2005	Industrial isolate	chill 2-3 days
multi component foods	ready to re-heat chilled foods	62	spaghetti	1000	*Escherchia vulneris* CRA 2005	Industrial isolate	chill 2-3 days
multi component foods	ready to re-heat chilled foods	63	chicken and bacon tortellini	10000	*Escherchia vulneris* CRA 2005	Industrial isolate	chill 2-3 days
multi component foods	ready to re-heat chilled foods	64	egg fried rice	100000	*E. coli* 4104	ATCC 25922 clinical isolate	chill 2-3 days
multi component foods	ready to re-heat chilled foods	65	pilau rice	1000000	Escherchia vulneris CRA 2005	Industrial isolate	chill 2-3 days
multi component foods	ready to re-heat frozen foods	66	frozen fish cakes	1000	*Hafnia alvei* 4009	sandwich	frozen 2 weeks

Category	Types	codes	item	inoculum level cfu per g	Culture	source	seeding
multi component foods	ready to re-heat frozen foods	67	frozen chicken nuggets	10000	*Hafnia alvei* 4009	sandwich	frozen 2 weeks
multi component foods	ready to re-heat frozen foods	68	frozen chips	10000	*Klebsiella rhinoscleromatis* 4272	Industrial isolate	frozen 2 weeks
multi component foods	ready to re-heat frozen foods	69	frozen cheese and tomato pizza	100000	*Hafnia alvei* 4009	sandwich	frozen 2 weeks
multi component foods	ready to re-heat frozen foods	70	frozen pepperoni pizza	1000000	*Klebsiella rhinoscleromatis* 4272	Industrial isolate	frozen 2 weeks
multi component foods	composite foods with raw ingredients	71	chicken salad mayo sandwich	none	none	N/A	natural
multi component foods	composite foods with raw ingredients	72	cheese and onion sandwich	10000	*Salmonella* Brandenburg 1070	beef	chill 2-3 days
multi component foods	composite foods with raw ingredients	73	bacon,lettuce, tomato sandwich	10000000	*E. coli* 4104	ATCC 25922 clinical isolate	chill 2-3 days
multi component foods	composite foods with raw ingredients	74	rainbow salad	none	none	N/A	chill 2-3 days
multi component foods	composite foods with raw ingredients	75	minted bean salad	none	none	N/A	natural

ANNEX D: Raw data per category

codes	item	Reference										Alternative									
	dilutions	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g	Log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g *	Log cfu/g
1	dried skimmed milk		T	T	37	3				3.60E+04	4.56		T	T	15	1				1.50E+04	4.18
2	strawberry whip				T	T	23			2.30E+06	6.36			T	78	69	14			1.40E+06	6.15
3	blancmange		T	T	83	5				8.00E+04	4.90		T	T	44	3				4.30E+04	4.63
4	instant custard mix			T	T	33	3			3.30E+05	5.52		T	T	T	17	1			1.60E+05	5.20
5	baby mikk				T	T	68	5		6.60E+05	5.82		T	T	T	27	1			2.60E+05	5.41
6	strawberry yogurt			T	T	18				1.80E+05	5.26			T	99	15				1.50E+05	5.18
7	single cream			T	14	5				1.40E+04	4.15			T	9	1				9.00E+03	3.95
8	halloumi with chilli			T	50	7				5.20E+04	4.72			109	11	4				1.10E+04	4.04
9	full fat soft cheese			T	T	22	7			2.60E+05	5.41			T	91	10				9.10E+04	4.96
10	gran levato (raw cheese)			T	109	12				1.10E+05	5.04			T	40	5				4.10E+04	4.61
11	full fat milk	99	95	5						9.27E+02	2.97	47	62	1						5.05E+02	2.70
12	semi skimmed milk		T	16	0					1.60E+03	3.20		T	32	1					3.00E+03	3.48
13	skimmed milk		T	30						3.00E+03	3.48			72	8					7.30E+03	3.86
14	chocolate milk		T	T	T	48	2			4.60E+05	5.66		T	T	T	34	6			3.60E+05	5.56
15	strawberry milk			T	T	26	12	1		1.20E+06	6.08		T	T	T	29	3			2.90E+06	6.46
16	melon,pineapple,mango	33	35	2						3.27E+02	2.51	8	10	3						9.00E+01	1.95
17	garpe,				T	20	1			1.90E+05	5.28				T	17	1			1.60E+05	5.20

codes	item	Reference										Alternative									
	dilutions	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g	Log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g *	Log cfu/g
	strawberry,blackberry																				
18	pineapple				T	17	1			1.60E+04	4.20			T	29	3				2.90E+04	4.46
19	apple and mango juice			T	150	16				1.60E+05	5.20			T	T	56				5.60E+05	5.75
20	orange juice				T	T	22			2.20E+06	6.34				T	T	24			2.40E+06	6.38
21	mixed leaf salad	T	T	19	4					2.10E+03	3.32	T	T	37	3					3.60E+03	3.56
22	carrot batons			T	T	57				5.70E+05	5.76			T	T	30				3.00E+05	5.48
23	brocoli				T	T	46			4.60E+06	6.66				T	T	28			2.80E+06	6.45
24	casserole veg selection			T	T	34				3.40E+05	5.53			T	T	35				3.50E+05	5.54
25	cucumber,kale and mint juice				T	T	17			1.70E+06	6.23				T	T	40			4.00E+06	6.60
26	beansprouts			T	T	38	3			3.70E+05	5.57			T	T	52	6			5.30E+05	5.72
27	spinach						40	2		4.00E+06	6.60					122	4			1.10E+06	6.04
28	pea shoots		T	44	3					4.30E+03	3.63		T	20	2					2.00E+03	3.30
29	watercress,spinach and rocket		T	24	1					2.30E+03	3.36		148	6						1.40E+03	3.15
30	flat leaf parsley			T	T	10	1			1.00E+05	5.00			98	61	3				5.80E+04	4.76
31	chicken thighs	4	5							4.50E+01	1.65	3	4							3.50E+01	1.54
32	chicken drumsticks	13	11	1						1.20E+02	2.08	5	4							4.50E+01	1.65
33	chicken breast fillets							178		1.80E+08	8.26							45		4.50E+07	7.65

codes	item	Reference										Alternative									
	dilutions	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g	Log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g *	Log cfu/g
34	diced turkey thigh	21	33							2.70E+02	2.43	27	18							2.25E+02	2.35
35	thin cut turkey breast steaks		95	9						9.45E+02	2.98		31	2						3.00E+02	2.48
36	lean beef steak mince	8	2							5.00E+01	1.70	3	2							2.50E+01	1.40
37	beef mince						23	2		2.30E+06	6.36						102	17		1.70E+06	6.23
38	lean pork mince						23			2.30E+06	6.36						21	2		2.10E+06	6.32
39	lamb mince				T	T	33			3.30E+06	6.52				T	T	25			2.50E+06	6.40
40	turkey breast mince		T	12	2					1.20E+03	3.08	22	4							2.40E+02	2.38
41	frozen beef burger	30	34	1						3.45E+02	2.54	24	5							1.45E+02	2.16
42	cheese and ham kievs		37	4						3.70E+02	2.57		21							2.10E+02	2.32
43	southern fried chicken goujons					14	0			1.40E+05	5.15				92	11	1			1.10E+05	5.04
44	breaded mini chicken fillets						20	1		1.90E+06	6.28					90	2			8.40E+05	5.92
45	garlic chicken kievs				T	T	25			2.50E+06	6.40				T	T	19			1.90E+06	6.28
46	roast turkey breast slices	48	61	4						5.32E+02	2.73	55	37	4						4.55E+02	2.66
47	roast chicken breast pieces		T	31	3					3.10E+03	3.49		T	20	1					1.90E+03	3.28
48	chicken slices					22	2			2.20E+05	5.34				102	12				1.20E+05	5.08
49	chicken drumsticks					71	4			6.80E+05	5.83					52	5			5.20E+05	5.72

codes	item	Reference										Alternative									
	dilutions	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g	Log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g *	Log cfu/g
50	chicken thighs						88	14		9.30E+06	6.97						49	8		5.20E+06	6.72
51	hot smoked mackerel	25	2							2.50E+02	2.40	8								8.00E+01	1.90
52	prawn cocktail		T	16	3					1.70E+03	3.23		T	17	4					1.90E+03	3.28
53	smoked slamon pate			57	5					5.60E+03	3.75			42	4					4.20E+03	3.62
54	cold water prawns			T	T	20				2.00E+05	5.30			T	T	17				1.70E+05	5.23
55	smoked salmon pieces			T	T	27				2.70E+05	5.43			T	T	22				2.20E+05	5.34
56	corned beef		T	65	7					6.60E+03	3.82		T	38	4					3.80E+03	3.58
57	cooked ham			T	*	6				6.00E+04	4.78			T	51	9				5.50E+04	4.74
58	pork luncheon meat			T	T	60				6.00E+05	5.78			T	T	46				4.60E+05	5.66
59	german salami				T	T	76			7.60E+06	6.88				T	T	37			3.70E+06	6.57
60	brussels pate				T	T	66			6.60E+06	6.82				T	T	41			4.10E+06	6.61
61	tagliatelle	35	24	2						2.86E+02	2.46	22	19	2						2.05E+02	2.31
62	spaghetti		T	143	9					1.40E+04	4.15		T	65	11					6.90E+03	3.84
63	chicken and bacon tortellini		T	T	84	12				8.70E+04	4.94				59	4				5.70E+04	4.76
64	egg fried rice				T	T	51			5.10E+06	6.71				T	T	23			2.30E+06	6.36
65	pilau rice							46	2	4.40E+07	7.64							92	15	9.70E+06	6.99
66	frozen fish cakes		T	T	36					3.60E+04	4.56		T	59	7					7.00E+03	3.85

codes	item	Reference										Alternative									
	dilutions	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g	Log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	count cfu/g *	Log cfu/g
67	frozen chicken nuggets			T	*	7				7.00E+04	4.85			T	66	11				7.00E+04	4.85
68	frozen chips		99	2						9.90E+02	3.00		108	5						1.00E+03	3.00
69	frozen cheese and tomato pizza			T	T	42				4.20E+05	5.62			T	T	44				4.40E+05	5.64
70	frozen pepperoni pizza				T	T	34			3.40E+06	6.53				T	T	45			4.50E+06	6.65
71	chicken salad mayo sandwich	18	15							1.65E+02	2.22	2	10							6.00E+01	1.78
72	cheese and onion sandwich				84	11				8.60E+04	4.93				52	7				5.40E+04	4.73
73	bacon,lettuce , tomato sandwich	3	3							3.00E+01	1.48	3	1							2.00E+01	1.30
74	rainbow salad				T	T	48			4.80E+06	6.68				T	T	22			2.20E+06	6.34
75	minted bean salad				T	T	36			3.60E+06	6.56				T	T	23			2.30E+06	6.36

T- Too many to count yellow shading indicates count used in calculation *5 colonies confirmed as typical per sample

ANNEX E: Calculation and interpretation of relative trueness

Category	Types	Sample ID	log(Ref)	log(Alt)	Mean	Difference
Fresh produce and fruits	cut ready to eat fruits	16	2.51	1.95	2.23	-0.56
Fresh produce and fruits	cut ready to eat fruits	17	5.28	5.2	5.24	-0.07
Fresh produce and fruits	cut ready to eat fruits	18	4.2	4.46	4.33	0.26
Fresh produce and fruits	cut ready to eat fruits	19	5.2	5.75	5.48	0.54
Fresh produce and fruits	cut ready to eat fruits	20	6.34	6.38	6.36	0.04
Fresh produce and fruits	cut ready to eat vegetables	21	3.32	3.56	3.44	0.23
Fresh produce and fruits	cut ready to eat vegetables	22	5.76	5.48	5.62	-0.28
Fresh produce and fruits	cut ready to eat vegetables	23	6.66	6.45	6.55	-0.22
Fresh produce and fruits	cut ready to eat vegetables	24	5.53	5.54	5.54	0.01
Fresh produce and fruits	cut ready to eat vegetables	25	6.23	6.6	6.42	0.37
Fresh produce and fruits	Leafy greens/sprouts	26	5.57	5.72	5.65	0.16
Fresh produce and fruits	Leafy greens/sprouts	27	6.6	6.04	6.32	-0.56
Fresh produce and fruits	Leafy greens/sprouts	28	3.63	3.3	3.47	-0.33
Fresh produce and fruits	Leafy greens/sprouts	29	3.36	3.15	3.25	-0.22
Fresh produce and fruits	Leafy greens/sprouts	30	5	4.76	4.88	-0.24
Milk and dairy	dairy products	6	5.26	5.18	5.22	-0.08
Milk and dairy	dairy products	7	4.15	3.95	4.05	-0.19
Milk and dairy	dairy products	8	4.72	4.04	4.38	-0.67
Milk and dairy	dairy products	9	5.41	4.96	5.19	-0.46

Category	Types	Sample ID	log(Ref)	log(Alt)	Mean	Difference
Milk and dairy	dairy products	10	5.04	4.61	4.83	-0.43
Milk and dairy	dry milk products	1	4.56	4.18	4.37	-0.38
Milk and dairy	dry milk products	2	6.36	6.15	6.25	-0.22
Milk and dairy	dry milk products	3	4.9	4.63	4.77	-0.27
Milk and dairy	dry milk products	4	5.52	5.2	5.36	-0.31
Milk and dairy	dry milk products	5	5.82	5.41	5.62	-0.4
Milk and dairy	pasteurised milk products	11	2.97	2.7	2.84	-0.26
Milk and dairy	pasteurised milk products	12	3.2	3.48	3.34	0.27
Milk and dairy	pasteurised milk products	13	3.48	3.86	3.67	0.39
Milk and dairy	pasteurised milk products	14	5.66	5.56	5.61	-0.11
Milk and dairy	pasteurised milk products	15	6.08	6.46	6.27	0.38
multi component foods	composite foods with raw ingredients	71	2.22	1.78	2	-0.44
multi component foods	composite foods with raw ingredients	72	4.93	4.73	4.83	-0.2
multi component foods	composite foods with raw ingredients	73	1.48	1.3	1.39	-0.18
multi component foods	composite foods with raw ingredients	74	6.68	6.34	6.51	-0.34
multi component foods	composite foods with raw ingredients	75	6.56	6.36	6.46	-0.19
multi component foods	ready to re-heat chilled foods	61	2.46	2.31	2.38	-0.14
multi component foods	ready to re-heat chilled foods	62	4.15	3.84	3.99	-0.31

Category	Types	Sample ID	log(Ref)	log(Alt)	Mean	Difference
multi component foods	ready to re-heat chilled foods	63	4.94	4.76	4.85	-0.18
multi component foods	ready to re-heat chilled foods	64	6.71	6.36	6.53	-0.35
multi component foods	ready to re-heat chilled foods	65	7.64	6.99	7.32	-0.66
multi component foods	ready to re-heat frozen foods	66	4.56	3.85	4.2	-0.71
multi component foods	ready to re-heat frozen foods	67	4.85	4.85	4.85	0
multi component foods	ready to re-heat frozen foods	68	3	3	3	0
multi component foods	ready to re-heat frozen foods	69	5.62	5.64	5.63	0.02
multi component foods	ready to re-heat frozen foods	70	6.53	6.65	6.59	0.12
Raw meat and poultry	fresh mince	36	1.7	1.4	1.55	-0.3
Raw meat and poultry	fresh mince	37	6.36	6.23	6.3	-0.13
Raw meat and poultry	fresh mince	38	6.36	6.32	6.34	-0.04
Raw meat and poultry	fresh mince	39	6.52	6.4	6.46	-0.12
Raw meat and poultry	fresh mince	40	3.08	2.38	2.73	-0.7
Raw meat and poultry	fresh poultry cuts	31	1.65	1.54	1.6	-0.11
Raw meat and poultry	fresh poultry cuts	32	2.08	1.65	1.87	-0.43
Raw meat and poultry	fresh poultry cuts	33	8.26	7.65	7.95	-0.6
Raw meat and poultry	fresh poultry cuts	34	2.43	2.35	2.39	-0.08
Raw meat and poultry	fresh poultry cuts	35	2.98	2.48	2.73	-0.5
Raw meat and poultry	processed ready to cook	41	2.54	2.16	2.35	-0.38
Raw meat and poultry	processed ready to cook	42	2.57	2.32	2.45	-0.25

Category	Types	Sample ID	log(Ref)	log(Alt)	Mean	Difference
Raw meat and poultry	processed ready to cook	43	5.15	5.04	5.09	-0.1
Raw meat and poultry	processed ready to cook	44	6.28	5.92	6.1	-0.35
Raw meat and poultry	processed ready to cook	45	6.4	6.28	6.34	-0.12
ready to eat foods	cooked fish products	51	2.4	1.9	2.15	-0.49
ready to eat foods	cooked fish products	52	3.23	3.28	3.25	0.05
ready to eat foods	cooked fish products	53	3.75	3.62	3.69	-0.12
ready to eat foods	cooked fish products	54	5.3	5.23	5.27	-0.07
ready to eat foods	cooked fish products	55	5.43	5.34	5.39	-0.09
ready to eat foods	cooked meat products	56	3.82	3.58	3.7	-0.24
ready to eat foods	cooked meat products	57	4.78	4.74	4.76	-0.04
ready to eat foods	cooked meat products	58	5.78	5.66	5.72	-0.12
ready to eat foods	cooked meat products	59	6.88	6.57	6.72	-0.31
ready to eat foods	cooked meat products	60	6.82	6.61	6.72	-0.21
ready to eat foods	ready to eat poultry	46	2.73	2.66	2.69	-0.07
ready to eat foods	ready to eat poultry	47	3.49	3.28	3.39	-0.21
ready to eat foods	ready to eat poultry	48	5.34	5.08	5.21	-0.26
ready to eat foods	ready to eat poultry	49	5.83	5.72	5.77	-0.12
ready to eat foods	ready to eat poultry	50	6.97	6.72	6.84	-0.25

ANNEX F: Raw data accuracy profile study

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
pasteurised cream	low	107B	31	18	3	0				2.50E+02	2.40	22	18	1	0				2.00E+02	2.30
		45B	22	17	3	0				2.04E+02	2.31	21	19	2	0				2.00E+02	2.30
		75B	23	24	4	0				2.50E+02	2.40	27	21	1	1				2.27E+02	2.36
		342B	17	16	1	0				1.59E+02	2.20	28	17	7	0				2.95E+02	2.47
		322B	24	12	4	0				2.00E+02	2.30	25	13	0	0				1.90E+02	2.28
pasteurised cream	medium	314	T		124	14	4			1.30E+04	4.11		T	97	8				9.54E+02	2.98
		346	T		104	11	0			1.00E+04	4.00		T	62	8				6.30E+03	3.80
		25	T		103	16	1			1.10E+04	4.04		T	114	4				1.10E+04	4.04
		63	T		124	10	0			1.20E+04	4.08		T	104	7				1.00E+04	4.00
		328			126	12	1			1.30E+04	4.11		T	81	4				7.70E+03	3.89
pasteurised cream	high	58			T	T	90	7		8.80E+05	5.94			T	T	54	5		5.40E+05	5.73
		141			T	T	73	12		7.70E+05	5.89			T	T	37	3		3.60E+05	5.56
		172			T	T	83	11		8.60E+05	5.93			T	T	37	6		3.90E+05	5.59
		83			T	T	136	5		1.30E+06	6.11			T	T	T	10		1.00E+06	6.00

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
		109			T	T	98	14		1.00E+06	6.00					54	11		5.90E+05	5.77
cream cheese	low	142	8		0	0	0	0		8.00E+01	1.90		3	1	0				3.00E+01	1.48
		21	6		0	0	0	0		6.00E+01	1.78		8	1	0				8.00E+01	1.90
		248	2		0	0	0	0		2.00E+01	1.30		11	0	0				1.10E+02	2.04
		188	3		0	0	0	0		3.00E+01	1.48		8	1	0				8.00E+01	1.90
		53	5		0	0	0	0		5.00E+01	1.70		6	0	0				6.00E+01	1.78
cream cheese	medium	31	T		46	2	0			4.40E+03	3.64		T	67	7				6.70E+03	3.83
		10	T		43	4	0			4.30E+03	3.63		T	71	7	1			7.10E+03	3.85
		97	T		45	3	0			4.40E+03	3.64		T	59	6	1			5.90E+03	3.77
		13	T		50	1	0			4.60E+03	3.66		T	72	9	1			7.40E+03	3.87
		273	T		57	6	0			5.70E+03	3.76		T	73	8	2			7.40E+03	3.87
cream cheese	high	196			T	T	33	1		3.10E+05	5.49			T	T	56	7		5.70E+05	5.76
		229			T	T	27	2		2.70E+05	5.43			T	T	54	5		5.40E+05	5.73
		57			T	T	44	5		4.50E+05	5.65			T	T	38	4		3.80E+05	5.58
		102			T	T	25	0		2.50E+05	5.40			T	T	43	3		4.20E+05	5.62
		259			T	T	30	0		3.00E+05	5.48			T	T	43	2		4.10E+05	5.61
RTC veg	low	264	6		0	0	0	0		60	1.78		12	2	1				1.20E+	2.08

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
																			02	
		252	7		0	0	0	0		70	1.85		6	4	0				6.00E+01	1.78
		139	8		0	0	0	0		80	1.90		14	3	0				1.40E+02	2.15
		300	7		0	0	0	0		70	1.85		17	3	0				1.80E+02	2.26
		294	3		0	0	0	0		30	1.48		19	2	0				1.90E+02	2.28
RTC veg	medium	312	192		1	0	0	0		1.90E+03	3.28		T	24	1				2.30E+03	3.36
		301	246		1	0	0	0		2.50E+03	3.40		T	36	1				3.40E+03	3.53
		64	220		3	0	0	0		2.20E+03	3.34		T	44	7				4.60E+03	3.66
		267	162		0	0	0	0		1.60E+03	3.20		T	18	1				1.70E+03	3.23
		340	170		1	0	0	0		1.70E+03	3.23		T	16	3				1.70E+03	3.23
RTC veg	high	316			15	5	2	0		1.80E+03	3.26			T	T	46	5		4.60E+05	5.66
		86			84	8	0	0		8.40E+03	3.92			T	T	43	4		4.30E+05	5.63
		323			150	1	0	0		1.40E+04	4.15			T	T	66	4		6.40E+05	5.81
		117			59	9	0	0		6.20E+03	3.79			T	T	26	6		2.90E+05	5.46
		203			17	3	0	0		1.80E+03	3.26			T	T	33	2		3.20E+05	5.51
Vegetable juice	low	8	4		0	0	0	0		4.00E+01	1.60		8	0	0				8.00E+01	1.90
		17	7		0	0	0	0		7.00E+01	1.85		12	1	0				1.20E+02	2.08

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
		327	5		0	0	0	0		5.00E+01	1.70		10	0	0				1.00E+02	2.00
		246	2		0	0	0	0		2.00E+01	1.30		10	2	0				1.00E+02	2.00
		37	3		0	0	0	0		3.00E+01	1.48		5	4	0				5.00E+01	1.70
Vegetable juice	medium	236	T		36	3	0			3.60E+03	3.56		T	85	12	1			8.80E+03	3.94
		92	T		47	9	0			5.10E+03	3.71		T	68	10	1			7.10E+03	3.85
		223	T		43	6	0			4.50E+03	3.65		T	75	5	2			7.20E+03	3.86
		9	T		55	12	0			6.10E+03	3.79		T	60	4	1			5.80E+03	3.76
		158	T		36	6	0			3.80E+03	3.58		T	59	10	0			6.30E+03	3.80
Vegetable juice	high	74			T	T	28	5		3.00E+05	5.48			T	T	62	4		6.00E+05	5.78
		234			T	T	30	3		3.00E+05	5.48			T	T	65	11		6.90E+05	5.84
		272			T	T	41	7		4.40E+05	5.64			T	T	62	8		6.30E+05	5.80
		34			T	T	38	0		3.80E+05	5.58			T	T	56	7		5.70E+05	5.76
		144			T	T	47	2		4.50E+05	5.65			T	T	74	6		7.40E+05	5.87
pork mince	low	194	T		75	8				7.60E+03	3.88		T	T	19				1.90E+04	4.28
		72	T		54	4				5.20E+03	3.72		T	T	31				3.10E+04	4.49
		177	T		72	2				6.70E+03	3.83		T	T	21				2.10E+04	4.32
		12	T		75	4				7.20E+	3.86		T	T	1				1.90E+	4.28

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
										03					9				04	
		270	T		74	9				7.60E+03	3.88		T	T	31				3.10E+04	4.49
pork mince	medium	243	T		T	52	3			5.00E+04	4.70		T	T	51	4			5.00E+04	4.70
		124	T		T	51	5			5.10E+04	4.71		T	T	60	9			6.30E+04	4.80
		297	T		T	41	5			4.20E+04	4.62		T	T	52	6			5.30E+04	4.72
		268	T		T	67	4			6.50E+04	4.81		T	T	59	10			6.30E+04	4.80
		347	T		T	71	7			7.10E+04	4.85		T	T	79	7			7.80E+04	4.89
pork mince	high	232	T		T	T	T	56	6	5.60E+06	6.75			T	T	T	82	7	8.10E+06	6.91
		217	T		T	T	T	55	7	5.60E+06	6.75			T	T	T	69	5	6.70E+06	6.83
		313	T		T	T	T	59	13	6.60E+06	6.82			T	T	T	61	3	5.80E+06	6.76
		89	T		T	T	T	53	2	5.00E+06	6.70			T	T	T	76	7	7.60E+06	6.88
		147	T		T	T	T	57	4	5.60E+06	6.75			T	T	T	71	11	7.50E+06	6.88
raw bacon	low	250	T	T	12	1				1.20E+03	3.08	T	T	22	4				2.40E+03	3.38
		80	*	*	9	0				9.00E+02	2.95	16	20	4	0				2.00E+02	2.30
		337	50	24	5	0				3.80E+02	2.58	25	18	3	0				2.20E+02	2.34
		56	*	35	3	0				3.80E+02	2.58	16	21	2	0				1.90E+02	2.28
		238	27	23	2	0				2.50E+02	2.40	26	23	4	0				2.60E+02	2.41

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
raw bacon	medium	200	T		T	49	5			4.90E+04	4.69		T	T	50	4			4.90E+04	4.69
		339		T	T	27	1			2.60E+04	4.41		T	T	18	1			1.70E+04	4.23
		120		T	T	38	4			3.80E+04	4.58		T	T	24	4			2.60E+04	4.41
		274		T	T	31	2			3.00E+04	4.48		T	T	17	0			1.70E+04	4.23
		106		T	T	11	2			1.10E+04	4.04		T	T	14	0			1.40E+04	4.15
raw bacon	high	183			T	T	T	32	1	3.00E+06	6.48			T	T	T	31	2	3.00E+06	6.48
		197			T	T	T	23	3	2.40E+06	6.38			T	T	T	25	1	2.40E+06	6.38
		2			T	T	T	27	3	2.70E+06	6.43			T	T	T	36		3.60E+06	6.56
		40			T	T	T	17	1	1.60E+06	6.20			T	T	121	21	0	2.10E+06	6.32
		5			T	T	110	10	1	1.10E+06	6.04			T	T	67	10	1	1.00E+06	6.00
fresh prawns	low	51	12	15	2	0				1.40E+02	2.15	10	15	1	0				1.22E+02	2.09
		247	30	13	0					2.15E+02	2.33	22	26	3	0				2.45E+02	2.39
		218	46	37	1					3.86E+02	2.59	29	27	4					2.90E+02	2.46
		87	23	33	2					2.73E+02	2.44	19	27	2					2.27E+02	2.36
		287	19	18	1					1.77E+02	2.25	13	28	2					2.05E+02	2.31
fresh prawns	medium	289		T	T	20	2			2.00E+04	4.30			T	T	26	5		2.80E+04	4.45
		68		T	71	7				7.10E+	3.85		T	88	7				8.60E+	3.93

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
										03									03	
		23		T	T	29	7			3.30E+04	4.52			T	T	34	5		3.60E+04	4.56
		309		T	T	55	5			5.50E+04	4.74			T	T	51	2		4.80E+03	3.68
		226		T	T	25	1			2.40E+04	4.38			T	T	20	1		1.90E+04	4.28
fresh prawns	high	195			T	T	57	6		5.70E+05	5.76			T	T	68	5		6.60E+05	5.82
		261			T	114	13	0		1.20E+06	6.08			T	T	102	13		1.00E+06	6.00
		42			T	T	83	7		8.20E+05	5.91			T	T	84	7		8.30E+05	5.92
		61			T	T	104	9		1.00E+06	6.00			T	T	81	7		8.10E+05	5.91
		320			T	T	127	11		1.30E+06	6.11			T	T	110	10		1.10E+06	6.04
fish pate	low	111	38	39	3	1				3.80E+02	2.58	112	106	12	2				1.10E+03	3.04
		155	25	29	5	0				2.90E+02	2.46	88	80	9	0				8.00E+02	2.90
		255	42	12	5	0				2.90E+02	2.46	96	60	8	0				8.10E+02	2.91
		186	28	32	2	2				2.90E+02	2.46	71	61	9	1				6.80E+02	2.83
		202	14	35	2	0				2.40E+02	2.38	67	108	6	3				8.50E+02	2.93
fish pate	medium	256		T	T	24	4			2.60E+04	4.41	T	T	119	7				1.10E+05	5.04
		192		T	T	21	1			2.00E+04	4.30	T	T	67	6				6.60E+04	4.82
		295		T	T	20	1			1.90E+04	4.28	T	T	74	13				7.40E+04	4.87

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
		16		T	T	38	4			3.80E+04	4.58	T	T	78	5				5.00E+04	4.70
		298		T	T	36	3			3.60E+04	4.56	T	T	96	11				1.10E+05	5.04
fish pate	high	319			T	T	T	14	2	2.00E+06	6.30			T	T	T	57	5	5.60E+06	6.75
		189			T	T	T	22	1	2.20E+06	6.34			T	T	T	63	4	6.10E+06	6.79
		78			T	T	T	16	2	1.60E+06	6.20			T	T	T	45	3	4.40E+06	6.64
		82			T	T	T	10	1	1.00E+06	6.00			T	T	T	39	4	3.90E+06	6.59
		182			T	T	T	31	0	3.10E+06	6.49			T	T	T	80	12	8.40E+06	6.92
sandwiches	low	307	20	24	1	0				2.10E+02	2.32	31	32	3	0				3.14E+02	2.50
		290	28	34	1	0				2.90E+02	2.46	56	57	10	2				6.04E+02	2.78
		157	21	27	1	0				2.90E+02	2.46	51	47	4	1				4.82E+02	2.68
		288	24	25	1	0				2.27E+02	2.36	37	47	4	0				4.18E+02	2.62
		32	30	28	5	0				3.09E+02	2.49	28	38	3	0				3.27E+02	2.51
sandwiches	medium	282		T	T	24	2			2.40E+04	4.38		T	T	26	4			2.70E+04	4.43
		60		T	T	30	2			2.90E+04	4.46		T	T	32	2			3.10E+04	4.49
		159		T	T	27	1			2.60E+04	4.41		T	169	14	0			1.40E+04	4.15
		308		T	T	21	3			2.20E+04	4.34				23	0			2.30E+04	4.36
		235		T	T	31	3			3.10E+	4.49		T	T	3	6			3.30E+	4.52

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
										04					0				04	
sandwiches	high	239			T	T	T	38	3	3.70E+06	6.57			T	T	T	38	0	3.80E+06	6.58
		179			T	T	T	37	0	3.70E+06	6.57			T	T	T	62	3	5.90E+06	6.77
		29			T	T	T	27	4	2.80E+06	6.45			T	T	T	34	3	3.40E+06	6.53
		251			T	T	T	34	3	3.40E+06	6.53			T	T	T	41	3	4.00E+06	6.60
		128			T	T	T	32	4	3.30E+06	6.52			T	T	T	45	5	4.60E+06	6.66
cooked chilled rice	low	329	3	2	0					2.50E+01	1.40	4	11						7.50E+01	1.88
		30	2	2						2.00E+01	1.30	*	4						4.00E+01	1.60
		146	2	4						3.00E+01	1.48	5	7						6.00E+01	1.78
		275	4	3						3.50E+01	1.54	5	3						4.00E+01	1.60
		242	1	0						1.00E+01	1.00	5	3						4.00E+01	1.60
cooked chilled rice	medium	242		T	30	8				3.50E+03	3.54		T	46	3				4.50E+03	3.65
		184		T	40	2				3.80E+03	3.58		T	38	8				4.20E+03	3.62
		211		T	43	6				4.50E+03	3.65		T	58	6				5.80E+03	3.76
		334		T	27	4				2.80E+03	3.45		T	60	7				6.10E+03	3.79
		331		T	30	3				3.00E+03	3.48		T	62	9				6.50E+03	3.81
cooked chilled rice	high	131			T	T	26	2		2.60E+05	5.41			T	T	49	2		4.60E+05	5.66

Item	Target Level*	sample number	Alternative							cfu/g	log cfu/g		Reference						cfu/g **	log cfu/g
			-1	-1	-2	-3	-4	-5	-6			-1	-1	-2	-3	-4	-5	-6		
		59			T	T	34	4		3.50E+05	5.54			T	T	52	4		5.10E+05	5.71
		161			T	T	15	3		1.60E+05	5.20			T	T	50	0		5.00E+05	5.70
		103			T	T	28	6		3.10E+05	5.49			T	T	49	5		4.90E+05	5.69
		209			T	T	43	3		4.20E+05	5.62			T	T	28	0		2.80E+05	5.45

T= Too many to count
*= dilution not plated
Yellow shading indicates count used in calculation

** 5 colonies confirmed as typical per sample

ANNEX G: Summary tables accuracy profile study.

(Food) Category 1	DAIRY
(Food) Type 1	PASTEURISED

			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
142 21 248 188 53	CREAM CHEESE	Low	30	80	110	80	60	80	60	20	30	50
107 B45B 75B 342B 322B	CREAM	Low	250	204	250	159	200	191	200	227	295	190
31 10 97 13 273	CREAM CHEESE	Med	6700	7100	5900	7400	7400	4400	4300	4400	4600	5700
314 346 25 63 328	CREAM	Med	9500	6300	11000	10000	7700	13000	10000	11000	12000	13000
196 229 57 102 259	CREAM CHEESE	High	570000	540000	380000	420000	410000	310000	270000	450000	250000	300000
58 141 172 83 109	CREAM	High	540000	360000	390000	1000000	590000	880000	770000	860000	1300000	1000000

(Food) Category 2	fruit and veg
(Food) Type 2	fresh produce

			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
8 17 327 246 37	veg juice	Low	80	100	100	200	400	100	70	50	20	30
264 252 139 300 294	RTC	Low	120	60	140	180	190	60	70	80	70	30
312 301 64 267 340	RTC	Med	2300	3400	4600	1700	1700	1900	2500	2200	1600	1700
236 92 223 9 158	veg juice	Med	8800	7100	7200	5800	6300	3600	5100	4500	6100	3800
316 86 323 117 203	RTC	High	460000	430000	640000	290000	320000	180000	840000	1400000	620000	1800000
74 234 272 34 144	veg juice	High	600000	690000	630000	570000	740000	300000	300000	620000	380000	450000

(Food) Category 3	raw poultry and
(Food) Type 3	fresh meat

			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
250 80 337 56 238	raw bacon	Low	2400	200	220	190	260	1200	900	380	380	250
194 72 177 12 270	pork mince	Low	19000	31000	21000	19000	31000	7600	5200	6700	7200	7600
200 339 120 274 106	raw bacon	Med	49000	17000	26000	17000	14000	49000	26000	38000	30000	11000
243 124 297 268 347	pork mince	Med	50000	63000	53000	63000	78000	50000	51000	42000	65000	71000
183 197 2 40 5	raw bacon	High	3000000	2400000	3600000	2100000	1000000	3000000	2400000	2700000	1600000	1100000
232 217 313 89 147	pork mince	High	8100000	6700000	5800000	7600000	7500000	5600000	5600000	6600000	5000000	5600000

(Food) Category 4	RTE foods
(Food) Type 4	cooked fish

			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
51 247 218 87 287	fresh prawns	Low	122	245	290	227	205	140	215	386	273	177
111 155 255 186 202	fish pate	Low	1100	800	810	680	850	380	290	290	290	240
289 68 23 309 226	fresh prawns	Med	28000	8600	36000	4800	19000	20000	7100	33000	55000	24000
256 192 295 16 298	fish pate	Med	110000	66000	74000	50000	110000	26000	20000	19000	38000	36000
195 261 42 61 320	fresh prawns	High	660000	1000000	830000	810000	1100000	570000	1200000	820000	1000000	1300000
319 189 78 82 182	fish pate	High	5600000	6100000	4400000	3900000	8400000	2000000	2200000	1600000	1000000	3100000

(Food) Category 6	Mulit component
(Food) Type 6	food with raw

			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
329 30 146 275 242	cooked chilled rice	Low	75	40	60	40	40	25	20	30	35	10
307 290 157 288 32	sandwiches	Low	314	600	482	418	327	210	290	290	227	309
242 184 211 334 331	cooked chilled rice	Med	4500	4200	5800	6100	6500	3500	3800	4500	2800	3000
282 60 159 308 235	sandwiches	Med	27000	31000	14000	23000	33000	24000	29000	26000	22000	31000
131 59 161 103 209	cooked chilled rice	High	460000	510000	500000	490000	280000	260000	350000	160000	310000	420000
239 179 29 251 128	sandwiches	High	3800000	5900000	3400000	4000000	4600000	3700000	3700000	2800000	3400000	3300000

ANNEX H: Raw data inclusivity and exclusivity study

				ALTERNATE				REFERENCE				NON SELECTIVE			
No	Organism	Source (if known)	code	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g
1	*Citrobacter amalonaticus*	Beansprouts	7458	T	T	55	5.50E+08	T	T	48	4.80E+08	T	T	75	7.50E+08
2	*Citrobacter braakii*	Industrial isolate	16279	T	55	7	6.20E+07	T	T	42	4.20E+08	T	T	58	5.80E+08
4	*Enterobacter agglomerans*	Skimmed milk powder	5513	T	T	27	2.70E+08	T	T	30	3.00E+08	T	T	44	4.40E+08
6	*Enterobacter amingenus*	Sea water	NCIMB 2118	T	T	30	3.00E+08	T	T	61	6.10E+08	T	T	44	4.40E+08
8	*Enterobacter cloace*	Industrial isolate	6633	T	T	17	1.70E+08	T	T	40	4.00E+08	T	T	39	3.90E+08
14	*Escherichia fergusonii*	Sausages	7522	T	T	21	2.10E+08	T	T	109	1.10E+09	T	T	103	1.00E+09
15	*Escherichia hermanii*	Sesame seeds	7477	T	17	0	1.70E+08	T	T	14	1.40E+08	T	T	28	2.80E+08
16	*Escherichia*	Industrial isolate	16260	T	118	11	1.20E+08	T	T	26	2.60E+08	T	T	27	2.70E+08

				ALTERNATE				REFERENCE				NON SELECTIVE			
No	Organism	Source (if known)	code	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g
	vulneris														
19	*Klebsiella rhinoscleromatis*	Industrial isolate	4272	T	19	3	2.00E+07	T	30	1	2.80E+07	T	114	4	1.10E+08
27	*Serratia fonticola*	Chicken	4613	T	T	22	2.20E+08	T	T	40	4.00E+08	T	T	37	3.70E+08
28	*Serratia liquefaciens*	Industrial isolate	10670	0	0	0	<1.0E+05	T	T	62	6.20E+08	T	T	42	4.20E+08
29	*Serratia proteamaculans*	Mushroom	1643	T	T	67	6.70E+08	T	T	66	6.60E+08	T	T	78	7.80E+08
31	*Escherichia coli*	Salmon fish cakes	108	T	T	27	2.70E+08	T	T	55	5.50E+08	T	T	42	4.20E+08
53	*Cronobacter sakazakii*	Dried milk	NA	T	T	116	1.20E+09	T	T	135	1.40E+09	T	T	161	1.60E+09
54	*Erwinia amylovora*	Industrial isolate	8037	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	38	3.80E+08
55	*Erwinia herbicola*	process water	7057	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	14	1.40E+08
56	*Escherichia*	Skimmed milk powder	5501	T	T	16	1.60E+08	T	T	21	2.10E+08	T	T	31	3.10E+08

				ALTERNATE				REFERENCE				NON SELECTIVE			
No	Organism	Source (if known)	code	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g
	adecarboxylata														
57	*Providencia alcalifaciens*	Chicken	7469	T	T	16	1.60E+08	T	T	48	4.80E+08	T	T	47	4.70E+08
58	*Rahnella aqualatis*	Drinking water	NCIMB 13365	0	0	0	<1.0E+05	T	T	87	8.70E+08	T	T	11	1.10E+08
59	*Salmonella bongori*	Industrial isolate	16379	T	T	67	6.70E+08	T	T	87	8.70E+08	T	T	181	1.80E+09
60	*Salmonella brandenburg*	beef	1070	T	T	89	8.90E+08	T	T	74	7.40E+08	T	T	101	1.00E+09
61	*Salmonella hadar*	turkey	1019	T	T	79	7.90E+08	T	T	87	8.70E+08	T	T	92	9.20E+08
62	*Yersinia intermedia*	Industrial isolate	380	0	0	0	<1.0E+05	T	T	55	5.50E+08	T	T	59	5.90E+08
63	*Pectobacterium carotovorum*	Industrial isolate	8036	0	0	0	<1.0E+05	0	0	0	<1.0E+05	224	22	2	2.20E+07

				ALTERNATE				REFERENCE				NON SELECTIVE			
No	Organism	Source (if known)	code	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g
64	*Leminorella richardii*	clincial	DSM 148949	0	0	0	<1.0E+05	82	6	0	8.00+06	T	25	3	2.20E+07
64	*Leminorella richardii 48 incubation*	clincial	DSM 148949	T	23	3	2.20E+07	nt							

5 Raw data Exclusivity strains

				ALTERNATE				REFERENCE				NON SELECTIVE			
Number	Organism	Source (if known)	code	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g	-5	-6	-7	cfu/g
22	Aeromonas salmonicida	Not known	8388	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	39	3	3.80E+07
23	Aeromonas sobria	sewage	8390	T	T	40	4.00E+08	T	T	83	8.30E+07	T	T	105	1.10E+09
24	Flavobacterium resinovorum	soil	9000	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	48	4.80E+08
25	Lactobacillus acidophilus	rats	7570	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	41	0	4.10E+07
63	Pseudomonas	blood	16479	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	44	4.40E+08

	aeroginosa														
64	Listeria inocua	cattle	6602	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	185	1.90E+09
65	Listeria ivanovii	Not known	6599	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	225	2.30E+09
66	Staphylococcus delphinii	dolphins	NCIMB 13206	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	T	20	2.00E+08
67	Staphylococcus epidermidis	Not known	NCIMB 8853	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	50	0	5.00E+07
68	Shewneall putrefaciens	Not known	NCTC 13457	0	0	0	<1.0E+05	0	0	0	<1.0E+05	T	84	7	8.30E+07

T= Too many too count

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

Ett Inclusivity results obtained with 32 strains using the Compact Dry ETB coliform method and ISO 21528-2:2004 (data from old study)

ORGANISM	CCFRA CODE	COMPACT DRY ETB	ISO 21528-2 (2004)
Citrobacter freundii	40	T	T
Citrobacter freundii	3163	T	T
Edwardsiella tarda	8392	T	T
Enterobacter aerogenes	15736	T	T
Enterobacter cloacae	1472	T	T
Enterobacter cloacae	6633	T	T
Escherichia coli	1476	T	T
Escherichia coli	1871	T	T
Escherichia coli	2003	T	T
Escherichia coli	2091	T	T
Escherichia coli	2092	T	T
Escherichia coli	11017	T	T
Escherichia coli	11626	T	T
Escherichia coli	15943	T	T
Escherichia coli	16041	T	T
Hafnia alvei	4009	T	T
Klebsiella oxytoca	8387	T	T
Klebsiella oxytoca	15926	T	T

ORGANISM	CCFRA CODE	COMPACT DRY ETB	ISO 21528-2 (2004)
Pantoea agglomerans	15947	T	T
Proteus mirabilis	1588	T	T
Proteus vulgaris	1581	T	T
Providencia rettgeri	8386	T	T
Salmonella Dublin	1356	T	T
Salmonella Enteritidis	1004	T	T
Salmonella Poona	725	T	T
Salmonella Typhimurium	11634	T	T
Serratia marcescens	1521	T	T
Shigella boydii	324	T	T
Shigella flexneri	325	T	T
Shigella sonnei	326	T	T
Shigella sonnei	4107	T	T
Yersinia enterocolitica	4103	T	T

Exclusivity obtained with 23 strains using the Compact Dry ETB method and ISO 21528-2 (2004)

ORGANISM	CCFRA CODE	COMPACT DRY ETB	ISO 21528-2 (2004)
Aeromonas hydrophila	4111	NG	T
Avibacterium avium	8389	NG	NG
Bacillus cereus	1761	NG	NG
Bacillus cereus	4110	NG	NG
Bacillus subtilis	4112	NG	NG
Brochothrix thermospacta	16019	NG	NG
Enterococcus faecalis	4113	NG	NG
Enterococcus faecalis	16049	NG	NG
Lactobacillus gasseri	6804	NG	NG
Pasteurella bettyae	8391	T	T
Pasteurella bettyae	16395	NG	NG
Pasteurella multocida subsp multocida	16396	NG	NG
Pasteurella multocida subsp multocida	16397	NG	NG
Pediococcus pentosaceus	16030	NG	NG
Pseudomonas aeruginosa	8299	NG	NG
Pseudomonas fluorescens	15937	NG	NG
Pseudomonas fragi	16050	NG	NG
Staphylococcus aureus	1216	NG	NG

ORGANISM	CCFRA CODE	COMPACT DRY ETB	ISO 21528-2 (2004)
Staphylococcus aureus	1224	NG	NG
Staphylococcus aureus	1227	NG	NG
Staphylococcus aureus	4105	NG	NG
Vibrio mimicus	6351	NG	NG
Vibrio parahaemolyticus	15737	A	T*

NG = No Growth

T = Typical Colonies

A = Atypical Growth

* = Very little or poor growth