ISO 16140-2:2016 validation of Kikkoman Biochemifa Company Easy Plate CC, for the enumeration of coliforms in a broad range of foods

MicroVal study number: 2021LR104

Method/Kit name: Easy Plate CC

Report version: Summary report

MicroVal Expert Laboratory: Campden BRI

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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.

Company: Kikkoman Biochemifa Company

Expert Laboratory: Campden BRI

Method/Kit name: Easy Plate CC

Validation standard: Microbiology of the food chain— Method validation

- Part 1: Vocabulary (ISO 16140-1:2016)
- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)

Reference method: ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

Scope of validation: Broad range of food

Certification organization: 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
- $\overline{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Incl/Excl Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- 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

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1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of coliforms in 5 different categories was carried out by Campden BRI as the MicroVal Expert Laboratory.

Alternative method

The alternative method used was:

Easy Plate CC is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate CC method is intended to indicate the level of coliform bacteria in food and beverage products. After incubation, coliform colonies grow as blue colonies on the growth medium of the Easy plate CC plate.

Reference methods was:

ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

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

Categories included:

- Milk and dairy products (combined category raw and heat processed Milk and dairy products)
- Fresh produce and fruits
- Fishery products
- Poultry and meats
- Multi-component foods or meal components

Criteria evaluated during the MCS study:

- Relative Trueness study
- Accuracy profile study
- Limit of Quantification study(LOQ)
- Inclusivity and exclusivity study

The final conclusions on the Method Comparison and Interlaboraotory studies are summarised below:

- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for relative trueness.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for accuracy profile.

- The alternative method Easy Plate CC for enumeration of coliforms is selective and specific.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory performance in the ILS.
- The alternative method Easy Plate CC for enumeration of coliforms. shows comparable performance to the reference method ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method.

2 Method protocols

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

According to ISO 16140-2 the reference method and alternative methods were performed with, as far as possible, the same sample and were therefore treated as paired data.

2.1 Reference method

A flow diagram of the reference methods are shown in Annex A for reference.

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. Two plates per dilution were also be used to improve reliability. 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 the calculations. Duplicate plates were used for the -1 dilution and single plates for the subsequent dilutions in the series for all samples tested.

2.2 Alternative method

See the flow diagram in Annex A

The kit insert for the alternative method (Easy Plate CC) is given in Annex B.

The alternative method principle is based on chromogenic media. Easy Plate CC is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate CC method is intended to indicate the level of coliform bacteria in food and beverage products. After incubation at 37°C for 24h ±2h, coliform

colonies grow as blue colonies on the growth medium of the Easy plate CC plate. In this validation study, the minimum incubation time of 22h was used for incubation of Easy Plate CC.

2.3 Study design

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

3 Methods Comparison Study

3.1 Sample preparation

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.

See Table 1 for specific preparations used in the validation study.

3.2 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 a combination of naturally and 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.

3.2.1 Number of samples

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

Table 1. List of Categories, Types, number of samples analysed and results obtained from testing within the relative trueness study.

Category	Types	Items	No of samples	ISO 6887
Milk and dairy products (combined category raw and heat processed Milk	Raw milk and dairy products	Raw milk, raw milk cheese	5	6887-5
	Pasteurised milk and milk based products	Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk)	5	6887-5

Category	Types	Items	No of samples	ISO 6887
and dairy products)	Dry milk products	Milk powders and powder for milk based desserts	5	6887-5
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon filet, tuna, bonito	5	6887-3
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods, semi-dried fish	5	6887-3
	Crustaceans	Shrimp, crab	5	6887-3
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot, radish sprouts, alfalfa	5	6887-4
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	6887-4
	Heat treated fruit and vegetables	Past smoothies/juice, blanched frozen vegetables	5	6887-4
Multi-component foods or meal components	Chilled pasta salad, egg and cress sandwich	Chilled pasta salad, egg and cress sandwich	5	6887-1, 6887-4
	Cooked chilled pasta, frozen fries, rice products, quiche	Cooked chilled pasta, frozen fries, rice products, quiche	5	6887-1, 6887-4
	Vegetable salad, egg mayonnaise	Vegetable salad, egg mayonnaise	5	6887-1, 6887-4
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	5	6887-2
	Raw processed meat	Frozen burger patties, pork meat balls,	5	6887-2
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	6887-2

75 samples were analysed, leading to 75 explotiable results

All results were calculated and interpreted according to ISO 16140-2.

3.2.2 Test sample preparation

All samples were screened for naturally contamination to ensure suitable levels of contamination for the study. During the study 26.7% of the samples tested were naturally contaminated. The remaining samples were artificially contaminated using a range of seeding protocols and strains in order to examine a wide range of different conditions.

Samples were inoculated with coliform strains before storage of the inoculated samples, e.g. frozen foods were stored for at least 2 weeks at -20 °C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature.

In addition, 5 pasteurised milk and dairy samples were spiked with a heat treated coliform isolate. The injury level achieved for the isolate was at least 0.5 log.

Eleven colifom islates were used for the artificial contamination in a mixture of seeding and spiking protocols. Each isolate was used to contaminate no more than 5 items during the study.

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

3.2.3 Protocols applied during the validation study

A single protocol was applied for the study.
Reference method plates were incubated at 37±1ºC for 24±2h.

Alternative method was incubated at 37±1ºC for 24±2h.

In all cases the minimum incubation times were used.

Where required atypical colonies were confirmed in the reference method following the protocol described in ISO 4832. Up to five atypical colonies were inoculated into tubes of brilliant green lactose bile broth which was then incubated at 37°C for 24 ±2h. Lactose fermentation was confirmed if gas formation has occurred in the Durham tube and the colour of the broth changed from green to yellow.

3.2.4 Test results

All raw data per category are given in Excel file 2021LR103: tab Relative Trueness.

The samples were analysed by the reference and the alternative methods in order to have at least 15 interpretable results per category, and at least 5 interpretable results per tested type by the two methods.

3.2.5 Calculation and interpretation of relative trueness study

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

Figures 1 to 5 shows the scatter plots for the individual categories and Figure 6 for all categories.

Figure 1 - Scatter plot of the reference method versus alternative method results for Milk and dairy products (combined category raw and heat processed Milk and dairy products) for coliforms

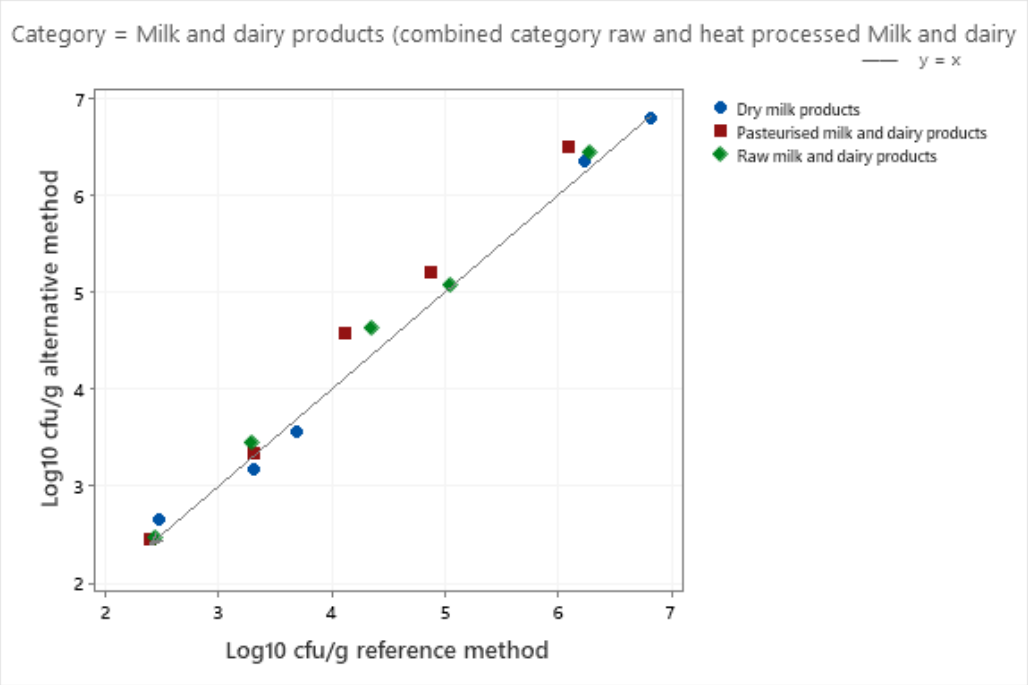


Figure 2 - Scatter plot of the reference method versus alternative method results for Fishery products, Combined category: raw, RTE, RTRH, RTC for coliforms

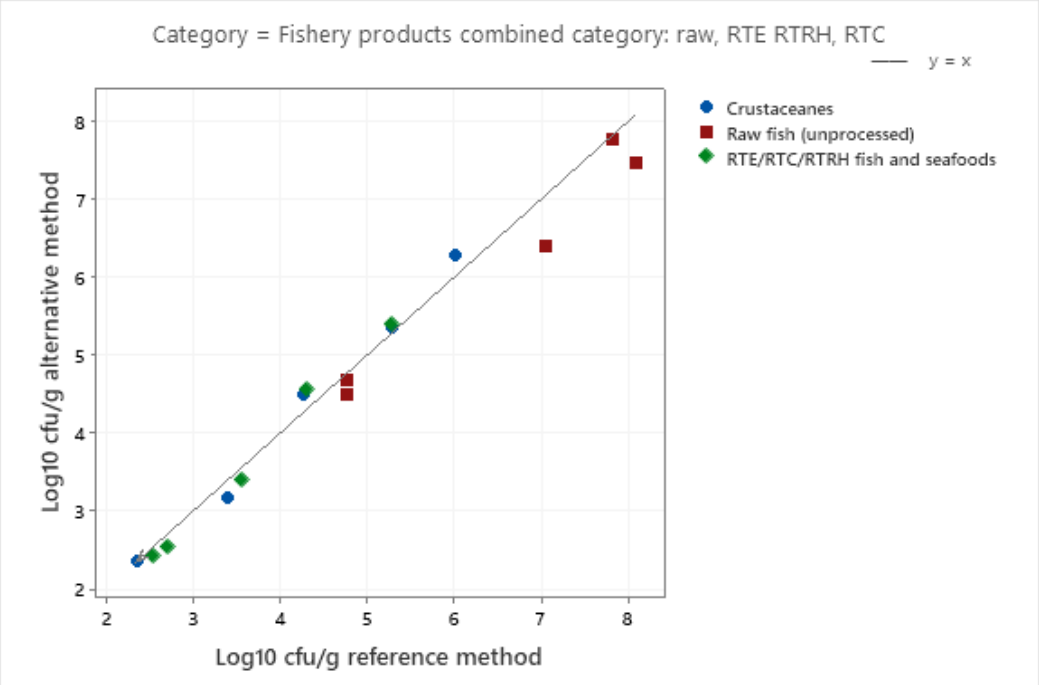


Figure 3 - Scatter plot of the reference method versus alternative method results for Produce and fruits (combined category fresh and processed) for coliforms

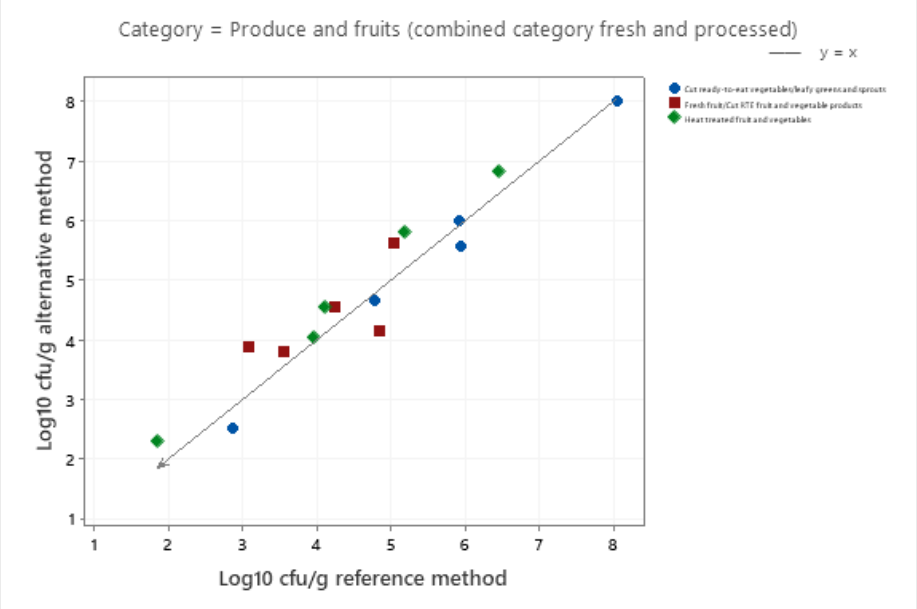


Figure 4 - Scatter plot of the reference method versus alternative method results for Raw and RTC Meat and poultry (Combined category) for coliforms

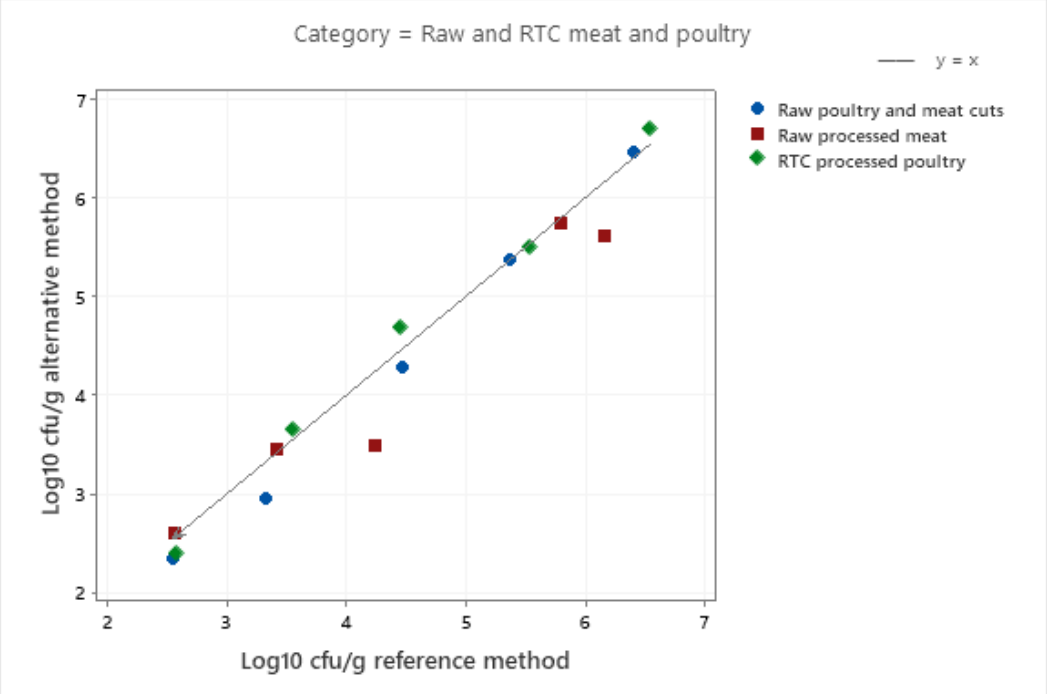


Figure 5 - Scatter plot of the reference method versus alternative method results for Multicomponent foods for coliforms

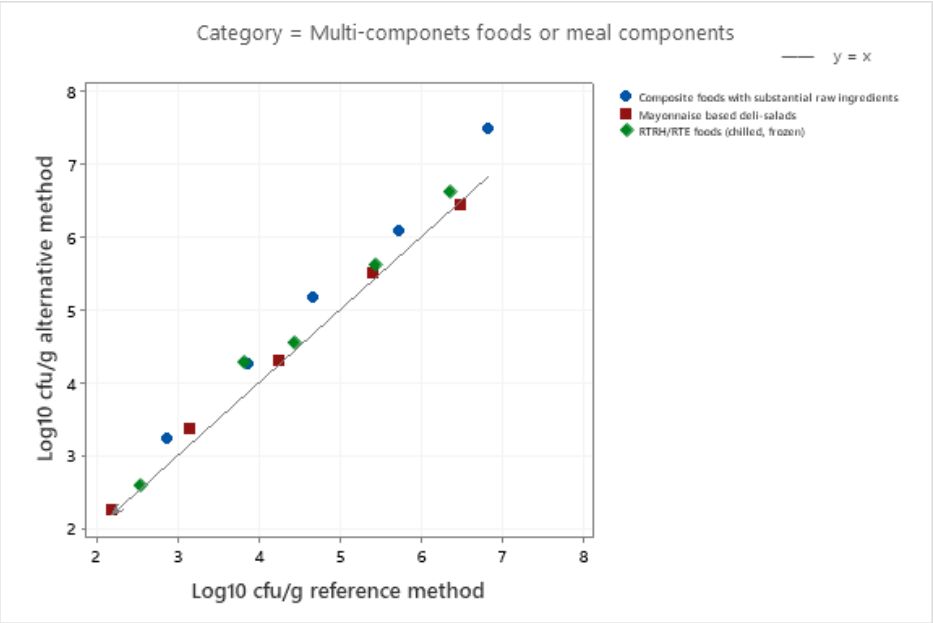
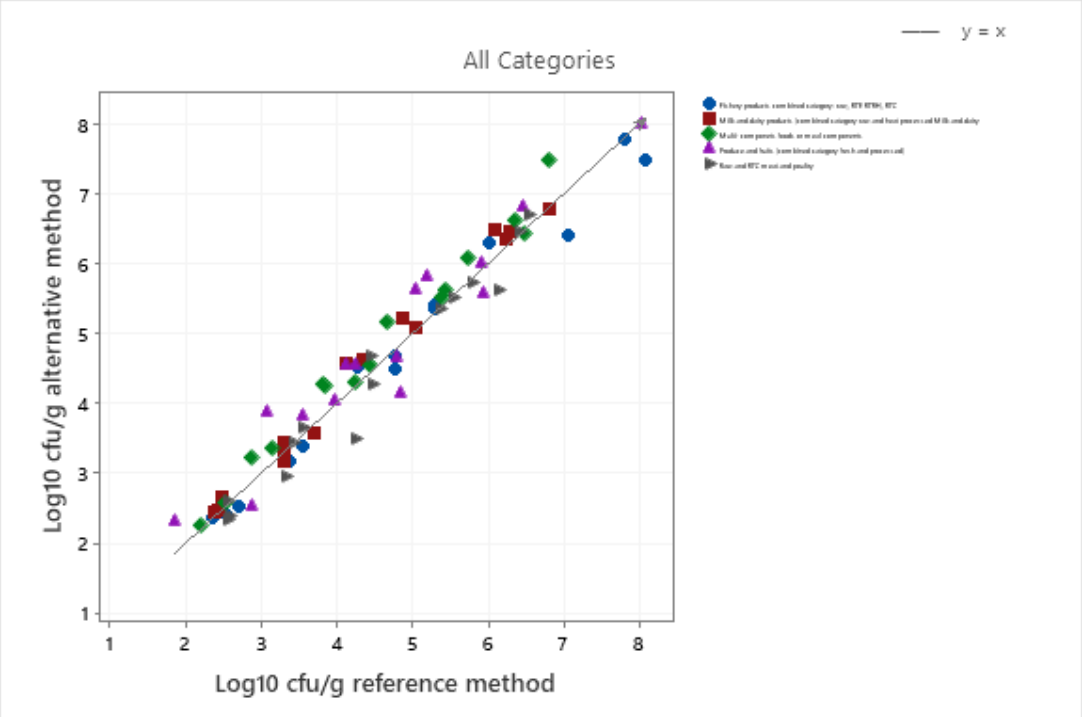


Figure 6 - Scatter plot of the reference method versus alternative method results for all categories for coliforms



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 in the scatter plots show no obvious disagreement across all the samples.

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

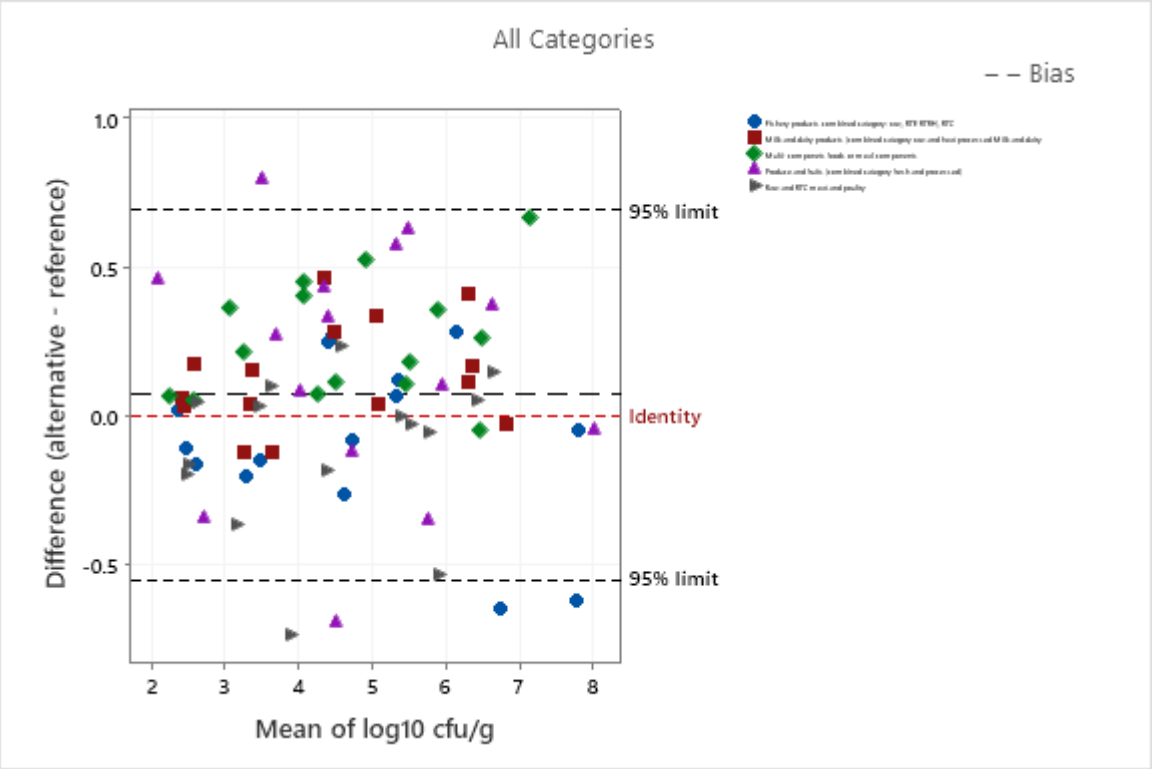
Table 2 - Summary of the calculated values per category

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Fishery products combined category: raw, RTE RTRH, RTC	15	-0.097	0.286	-0.705	0.534
Milk and dairy products (combined category raw and heat processed Milk and dairy	15	0.133	0.178	-0.262	0.528
Multi-components foods or meal components	15	0.253	0.202	-0.195	0.700
Produce and fruits (combined category fresh and processed)	15	0.163	0.416	-0.759	1.085
Raw and RTC meat and poultry	15	-0.110	0.264	-0.696	0.475
All Categories	75	0.071	0.309	-0.549	0.690

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

The Bland-Altman difference plot for all the samples is given Figure 7.

Figure 7- The Bland-Altman difference plot for all the samples



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in the Table 3.

Table 3 – data which are outside of the Accepted limits

Category	item	Code	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference (Alternative-reference)	Lower/ upper limits
Produce and fruits (combined category fresh and processed)	Fresh fruit/Cut RTE fruit and vegetable products	J8	3.079	3.875	3.477	0.796	0.690
Raw and Ready to cook RTC Meat and poultry	mini pork meatballs	L7	4.230	3.491	3.861	-0.739	-0.549
Produce and fruits (combined category fresh and processed)	Fresh fruit/Cut RTE fruit and vegetable products	J7	4.845	4.146	4.496	-0.699	-0.549
Fishery products combined category: raw, RTE RTRH, RTC	Diced salmon fillets	I1	8.079	7.462	7.771	-0.617	-0.549
	Wild Salmon	I4	7.041	6.398	6.720	-0.643	-0.549

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 5 in 75 data values which lie outside the CLs (Table 3). This is outside the expectation however, there are no trends to the outlying data which represented five of the seven categories.

One point was higher than the upper limit of 0.691 and four points were slightly lower than the lower limit of -0.557. The samples covered a diverse range of foods items with different groups of organisms present as both natural and artificial contamination present and therefore these results show good agreement between the two methods for enumeration of coliform count with a slight negative bias for the alternate method with an overall bias from all the categories of 0.067.

Two of the samples that were outside the CL on the Bland Altman blot were salmon products. A root cause analysis on the outlying results revealed that in date media was used and the correct incubation time and temperatures were used. Negative bias was not observed during the accuracy profile and a positive bias was noted on the ILS. The results of the root cause analysis indicate that the outliers on the Bland Altman plot are random differences in results and are not caused by the matrix itself.

3.2.6 RT conclusions

The relative trueness of the Alternative method is satisfied as there was a good agreement between the reference method and alternative method in the scatterplots and Bland Altman analyses for Coliforms.

3.3 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. As per ISO 16140-2:2016 guidelines, this study was conducted using artificially contaminated samples.

3.3.1 Categories, sample types and strains

Five categories were tested with a single batch of two different food 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 analysed per food type. The following food type/strain pairs were studied (See Table 4)

Each sample was bulk inoculated and five replicate test portions examined from the bulk sample/ individually inoculated as a separate test portion, with the exception of salad where single test portions were inoculated.
The tested categories, types and items are provided in Table 4

Table 4. Categories, types, items, strains and inoculation levels for Accuracy profile study

Category	Types	Strain	Item	Target Level* cfu/g	Test portions
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Pasteurised dairy products	*Leclercia adecarboxylata* (previously known as *Escherichia adecarboxylata*) CRA 5501 Isolated from skimmed milk powder	Pasteurised cream	100-250	5
				10000-20000	5
				1000000-3000000	5
			Cream cheese	80-250	5
				10000-20000	5
				800000-2000000	5
Produce and fruits (combined category fresh and processed)	Fresh produce	*Enterobacter taylorae* CRA 7530 isolated from seeds	Ready to cook Vegetable preparation	200-300	5
				20000-30000	5
				2000000-5500000	5
			Vegetable juice	200-500	5
				30000-50000	5
				3000000-5500000	5
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh meat	*Escherichia fergusonii* CRA 7522 Isolated from sausages	Pork mince	100-200	5
				9500-36000	5
				600000-2500000	5
			Raw bacon	150-250	5
				1000-13000	5
				820000-1500000	5
			Fresh prawns	200-400	5

Fishery products Combined category: raw, RTE, RTRH, RTC	Cooked fish products e.g. prawns	*Leliottia amingena* , previously known as *(Enterobacter amingenus)* NCIMB 2118 Isolated from seawater		10000-60000	5
				1500000-4500000	5
			Fish pate	250-400	5
				25000-40000	5
				1900000-4000000	5
Multi-component foods or meal components	Composite foods with raw ingredients	*Escherichia hermanii* CRA 7477 Isolated from sesame seeds	Sandwiches	400-700	5
				10000-60000	5
				7000000-1800000	5
			Cooked chilled rice	200-400	5
				15000-55000	5
				300000-700000	5

For all matrices used in the study, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis.

3.3.2 Calculations and interpretation of Accuracy profile study

All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in Figures 8 to 12.

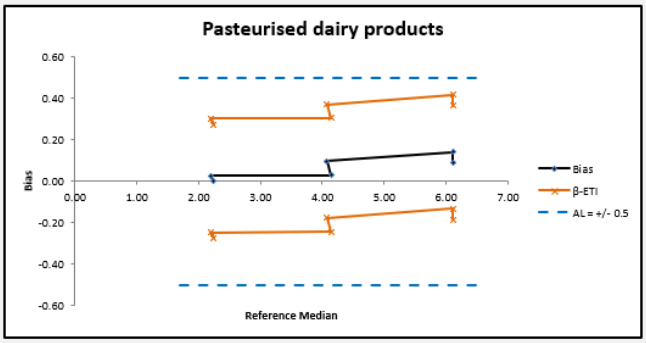
All raw data per category are given in Excel file 2021LR103: tab Accuracy Profile

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 8: Accuracy profile of dairy products (combined category; raw milk and heat processed) for Easy Plate CC method

Matrices used pasteurised cream = (1-3) and cream cheese (4-6)

(Food) Category	Dairy products
(Food) Type	Pasteurised dairy products



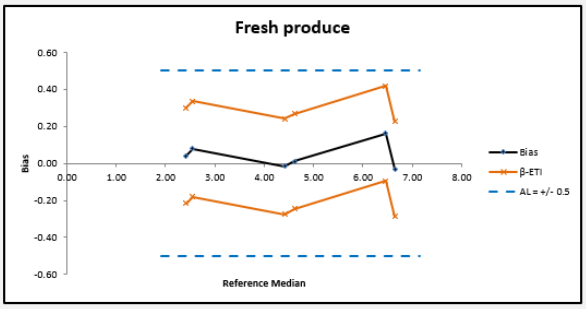
Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Pasteurised cream	2.24	0.000	-0.274	0.274	YES	YES
Cream cheese	2.19	0.027	-0.247	0.301	YES	YES
Pasteurised cream	4.15	0.030	-0.244	0.304	YES	YES
Cream cheese	4.08	0.097	-0.177	0.371	YES	YES
Pasteurised cream	6.11	0.141	-0.133	0.416	YES	YES
Cream cheese	6.11	0.090	-0.184	0.364	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.188	0.190	NO	+/- 0.500

Figure 9: Accuracy profile for Fruit and vegetables for Easy Plate CC method

Matrices used = Ready to cook vegetable preparation (7-9) and vegetable juice (10-12)

(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce

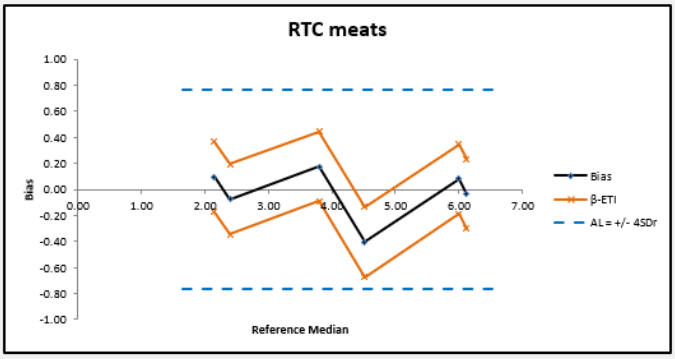


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7 a-e	2.41	0.042	-0.215	0.299	YES	YES
10 a-e	2.54	0.077	-0.180	0.334	YES	YES
8 a-e	4.41	-0.017	-0.274	0.240	YES	YES
11 a-e	4.61	0.010	-0.246	0.267	YES	YES
9 a-e	6.46	0.161	-0.096	0.418	YES	YES
12 a-e	6.65	-0.030	-0.287	0.227	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.072	0.178	YES	+/- 0.500

Figure 10: Accuracy profile for Meat and poultry for Easy Plate CC method

Matrices used = pork mince (13-15) and raw bacon (16-18)



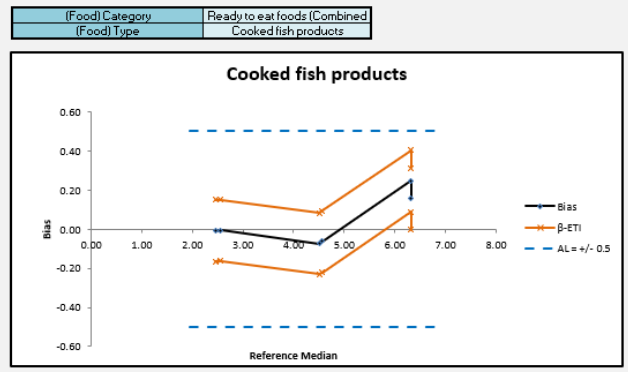
(Food) Category	Raw poultry and meats
(Food) Type	RTC meats

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
16 a-e	2.15	0.097	-0.172	0.365	YES	YES
13 a-e	2.40	-0.076	-0.344	0.193	YES	YES
17 a-e	3.81	0.176	-0.092	0.445	YES	YES
14 a-e	4.52	-0.405	-0.673	-0.136	NO	YES
18 a-e	6.00	0.079	-0.189	0.348	YES	YES
15 a-e	6.11	-0.035	-0.303	0.234	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.192	0.186	NO	+/- 0.768

Figure 11: Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate CC method

Matrices used = fresh prawns (19-21) and fish pate (22-24)

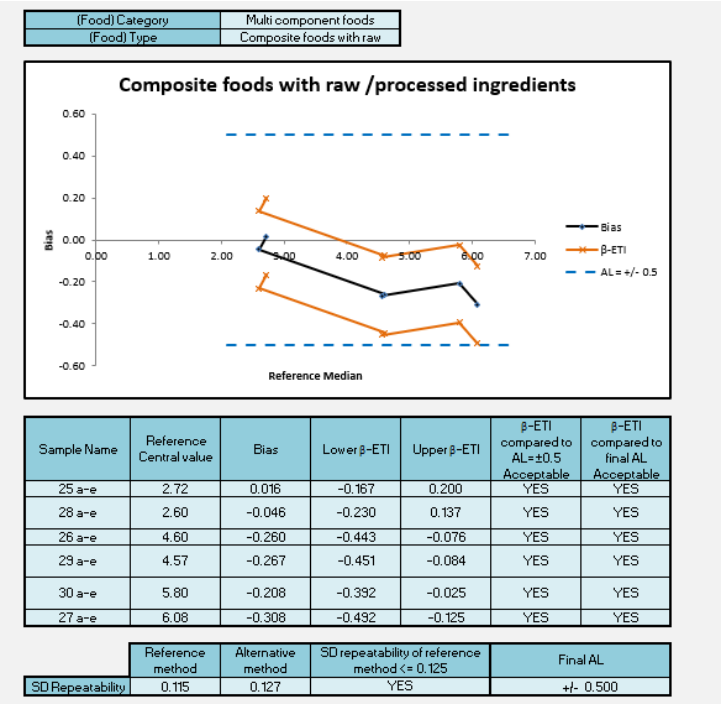


(Food) Category	Ready to eat foods (Combined
(Food) Type	Cooked fish products

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
19 a-e	2.45	-0.008	-0.165	0.150	YES	YES
22 a-e	2.56	-0.006	-0.163	0.151	YES	YES
20- a-e	4.51	-0.074	-0.231	0.084	YES	YES
23 a-e	4.57	-0.063	-0.220	0.094	YES	YES
21 a-e	6.32	0.246	0.089	0.403	YES	YES
24 ae	6.32	0.155	-0.002	0.312	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.131	0.109	NO	+/- 0.500

Figure 12: Accuracy profile for Multicomponent foods for Easy Plate CC method
Matrices used = Sandwich (25-27) and cooked chilled rice (28-30)



(Food) Category	Multi component foods
(Food) Type	Composite foods with raw

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
25 a-e	2.72	0.016	-0.167	0.200	YES	YES
28 a-e	2.60	-0.046	-0.230	0.137	YES	YES
26 a-e	4.60	-0.260	-0.443	-0.076	YES	YES
29 a-e	4.57	-0.267	-0.451	-0.084	YES	YES
30 a-e	5.80	-0.208	-0.392	-0.025	YES	YES
27 a-e	6.08	-0.308	-0.492	-0.125	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.115	0.127	YES	+/- 0.500

Four of the five categories included in the study met the AL of 0.5log (dairy, fresh produce, fish and seafood, multicomponent foods). One category (raw meat and poultry) required the new AL to be calculated. All data met the new AL value of 0.768.

In the meat and poultry category only one data point fell outside the lower β-ETI and this was pork mince contaminated at the intermediate level. Following calculation of the AL due to the higher repeatability of the reference method the lower β-ETI limit for this group of samples fell within the recalculated AL of 0.768.

3.3.3 Conclusion Accuracy profile study

The Accuracy of the Alternative method (Easy Plate CC) is satisfied as all categories met the 0.5log AL or the re-calculated AL.

3.4 Inclusivity and exclusivity study

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

3.4.1 Protocols

Inclusivity:

50 pure cultures of coliforms were tested in this section of the study. Each strain was grown overnight in a non-selective broth and diluted so that the inoculum level was at least 100 times greater than the minimum level for quantification of the alternative method being validated.

Each test was performed once with the alternative method, the reference method and a non-selective agar.

Exclusivity:

31 pure cultures of (non-target) microorganisms were tested. The inoculum level used was similar to the greatest level of contamination expected to occur in any of the categories being used. The pure culture was grown in a suitable non-selective broth under optimal conditions of growth for at least 24 h and diluted to an appropriate level before testing begins.

Each test was performed once and with the alternative, the reference method and a non-selective agar.

3.4.2 Results inclusivity and exclusivity study

Raw data is given in Excel file 2020LR91 Quantitative data file: tabs Inclusivity and Exclusivity.

Inclusivity

For the inclusivity study,46 out of the 50 coliform strains gave typical colonies on the alternate and reference method. Four isolates were detected by the reference method and not the alternative method. These were *Citrobacter amalonaticus* (CRA7458), *Citrobacter amalonaticus* (CRA7056), *Shimwellia blattae* (NCTC 12127), *Serratia proteamaculans ssp. quinovora* (CRA 16463). Three out of these four isolates giving discrepant results were negative when run through the BGLBB confirmation test and therefore finally gave a negative result. The remaining isolate *Citrobacter amalonaticus* (CRA7458) tested positive when confirmed in BGLBB.

Four additional typical coliforms were included in the panel and these gave the expected results for the reference and alternative methods. These were *Siccibacter turicensis* (CRA 17681), *Franconibacter pulveris* (CRA 17679), *Franconibacter helveticus* (CRA 17678) and *Cronobacter condimentii* (CRA 17021).

The level enumerated on the reference method and alternative method were similar with no negative or positive bias shown.

Exclusivity

The number of isolates that gave the expected results for the reference and alternative method are shown in the table below for reference.

Method	No of colonies that gave expected presumptive results	No of colonies that gave expected confirmed results
Reference	24/31	30/31
Alternate	28/31	n/a

Further analysis of the 7 isolates that gave presumptive positive results on either the reference or alternative methods was carried out to resolve the discrepancies.

Two strains *Shigella boydii* (NCTC 11312) and *Shigella sonneii* (ATCC 25931) gave positive results for the alternate and reference agars. Further analysis of both isolates showed that on confirmation they gave a negative result on BGLBB, indicating that they were not coliforms.

One isolate *Shigella sonneii* (was gave positive results on both reference and alternative methods). This strain was positive when subcultured into BGLBB.

Four isolates *Shigella flexneri* (NCTC 9950), *Vibrio mimicus (*NCIMB 12702*), Vibrio parahaemolyticus* (NCTC 11435) and *Yersinia enterocolitica (*NCTC 11344*)* gave typical colonies on the reference method, however on confirmation they did not produce gas when grown in BGLBB verifying that they were not coliforms.

After confirmation, 30 out of the 31 isolates gave the expected results with the reference method.

Conclusion

50 out of the 54 isolates were correctly identified following the alternative method. Three out of the four isolates giving unexpected results in the inclusivity panel were confirmed to be negative for BGLBB, indicating that they should be considered to be atypical coliforms. Taking this into account, 49 out of the 50 typical coliforms were correctly identified following the alternative method.

28 out of the 31 isolates in the exclusivity panel gave the expected results with the alternative method.

The alternative method gave comparable performance to the reference method and is therefore selective and specific to the coliform group.

3.5 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.

The LOQ did not have to be calculated for the alternative method in this study.

3.6 Conclusion (MCS)

- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for Accuracy profile.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for relative trueness
- Easy Plate CC shows comparable performance to the reference method for enumeration of coliforms in a broad range of foods.

4 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.

4.1 Study organization

Collaborators

Samples were sent to 10 laboratories.

Matrix and strain used

Smoked salmon was inoculated with *Escherichia coli* CRA 108 (isolated from salmon fish cakes) and *Citrobacter diversus* CRA 7119 (an industrial isolate)

Sample preparation

Samples were prepared and inoculated on 21 February 2023 as described below:

For each collaborator, a set of samples was prepared containing 2 samples at a low level, two samples at a medium level, two samples at a high level and a single uninoculated blank sample. The samples were blind-coded so that the collaborators did not know the intended contamination level. A set of samples was also prepared for the EL although the data from these was not used in the data analysis.

The target levels and codes are shown below

Table 5 : Contamination levels

Contamination level	Sample code
Uninoculated	4
Low (10^2 cfu/g)	1
Low (10^2 cfu/g)	5
Medium (10^4 cfu/g)	2

Medium (10^4 cfu/g)	6
High (10^6 cfu/g)	3
High (10^6 cfu/g)	7

Labelling and shipping

Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

Samples were shipped in a frozen condition on 22 Februaury 2023 and were received within 24 h to 72 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs. On receipt at the laboratories, the samples were stored frozen at ≤-18°C and defrosted prior to analysis as recommended in ISO 6887-1. The analyses was started on Monday 27 February 2023. Stability studies had been conducted to show that the required level of target organisms would be present after 7 and 8 days frozen storage. The expert lab analysed a set of samples on Monday 27 February 2023.

Analysis of Samples

Collaborative study laboratories and the expert laboratory carried out the analyses on Monday 27 February 2023. The analyses by the reference method and the alternative method were performed on the same day.

4.2 Experimental parameters controls

Detection of coliforms in the matrix before inoculation

In order to ensure absence of *coliforms* in the matrix, the reference method was performed on five portions (10 g) before the inoculation. All the results were negative.

Strain stability during transport

Duplicate samples inoculated at low, medium and high levels were tested for enumeration of coliforms after 6 and 7 days storage at -18°C. Samples were thawed under controlled conditions prior to analysis. The data shows good stability under the storage regime tested (Table 6).

Table 6 - Coliform stability in the matrix

	Alternative method cfu/g						Reference method cfu/g					
	Low level		Medium level		*High level*		Low level		Medium level		*High level*	
Day	a	b	a	b	a	b	a	b	a	b	a	b
0	2.30E+03	1.90 E+03	2.40 E+05	2.10 E+05	2.40 E+06	1.40 E+06	1.10 E+03	1.20 E+03	1.20 E+05	1.10 E+05	1.20 E+06	6.70 E+05
6	1.40E+03	1.40 E+03	1.30 E+05	1.30 E+05	1.50 E+06	1.40 E+06	809	982	5.60 E+04	6.50 E+04	9.90 E+05	9.60 E+05
7	1.40E+03	1.40 E+03	1.70 E+05	1.10 E+05	1.90 E+06	1.10 E+06	473	645	6.70 E+04	6.50 E+04	7.40 E+05	5.10 E+05

Logistic conditions

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, the receipt dates are given in Table 7.

Table 7 - Sample temperatures at receipt

Collaborator	Temperature measured by probe (°C)	Receipt date and time	State of the package and samples at receipt	Analysis date
1	3.9	22/2/23, 11:55	Very Good	27/02/23
2	2.1	22/2/23, 11:55	Very Good	27/02/23
4	4.6	22/2/23, 14:00	Acceptable	27/02/23
5	8.8	22/2/23, 14:00	Acceptable	27/02/23
6	5.0	24/02/23, 11:00	Good	27/02/23
7	12.9	23/2/23, 09:15	Non frozen samples	27/02/23
9	5.95	22/2/23; 10:55	Good, no damage	27/02/23
10	1.2	22/2/23, 13:30	Intact	27/02/23
11	5.2	22/2/23, 13:30	Intact	27/02/23
12	14.2	23/2/23, 09:15	Non frozen samples	27/02/23

No issues were encountered during the transport or at receipt for 10 out of 10 collaborators. All the samples were delivered on time and in appropriate conditions to 10 laboratories.

4.3 Calculation and summary of data

The raw data are given in Annex E.

MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 8.

Table 8 – Results obtained by the expert lab.

Level	Reference method cfu per g	Alternative method cfu per g
Blank	<10	<10
Low	6.90E+02	8.30E+02
Low	1.90E+02	3.20E+02
Medium	2.00E+04	4.60E+04
Medium	1.40E+04	2.40E+04
High	7.50E+05	1.30E+06
High	4.10E+05	8.10E+05

Results obtained by the collaborative laboratories

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). Version 14-03-2016 was used for these calculations.

The results obtained by the collaborators are shown in Table 9.

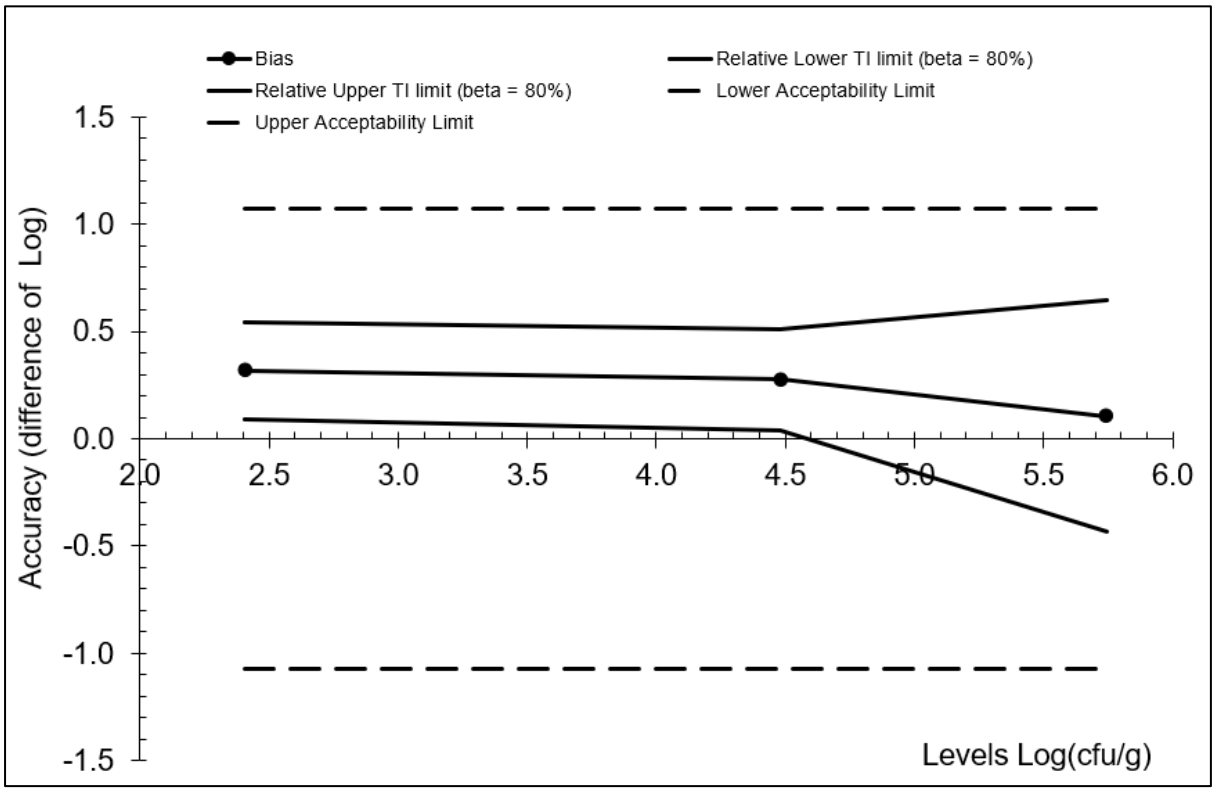
The accuracy profile plot is shown in Figure 13 and the statistical analysis of the data shown in Table 10.

Table 9: Summary of the results of the interlaboratory study per analyte level

Collaborator	**Level**	**Reference method (Log cfu/g)**		**Alternative method (Log cfu/g)**	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	2.5	2.0	2.7	2.4
2	low	2.6	2.0	2.7	2.6
4	low	2.6	2.4	2.8	2.9
5	low	2.4	2.4	2.9	2.6
6	low	2.7	2.6	2.9	2.7
7	low	3.4	2.4	2.8	2.7
9	low	2.3	2.0	2.7	2.5
10	low	2.2	1.5	2.6	2.4
11	low	2.4	2.6	2.8	2.9
12	low	2.5	2.0	2.7	2.4

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
1	medium	4.5	4.4	4.7	4.6
2	medium	4.3	4.4	4.4	4.7
4	medium	4.5	4.4	4.8	4.9
5	medium	4.5	4.7	4.8	5.0
6	medium	4.7	4.7	4.9	4.9
7	medium	4.4	4.5	4.8	4.9
9	medium	4.3	4.1	4.6	4.5
10	medium	4.0	4.3	4.6	4.6
11	medium	4.6	4.8	4.9	4.8
12	medium	4.5	4.4	4.7	4.6
1	high	5.6	5.6	5.7	5.6
2	high	5.8	5.6	5.6	5.7
4	high	5.9	5.9	6.1	6.1
5	high	6.0	6.5	6.5	6.5
6	high	6.4	6.3	6.2	6.2
7	high	5.7	5.6	5.9	5.8
9	high	5.3	5.3	5.7	5.8
10	high	5.4	5.3	5.7	5.7
11	high	5.5	5.8	5.9	5.0
12	high	5.6	5.6	5.7	5.6
1	blank	<1		<1	
2	blank	<1		<1	
3	blank	<1		<1	
4	blank	<1		<1	
5	blank	<1		<1	
6	blank	<1		<1	
7	blank	<1		<1	
9	blank	<1		<1	
10	blank	<1		<1	
11	blank	<1		<1	
12	blank	<1		<1	

Figure 13. Accuracy profile for Easy Plate CC from the ILS



A review of the accuracy profile and statistical analysis revealed that there was a positive bias of 0.3 observed for the low and medium levels of contamination and a bias of 0.1 with the high level of contamination in the ILS samples. A root cause analysis was carried out to determine possible reasons for this.

The same batches of media were used for all participants in the study and the time and temperature used for the incubation of the plates was correct. Each participant in the study prepared their own agar for the reference method which could have introduced differences in how the media was handled. Other potential causes could be the type of plate used for the analysis. The alternative method used a surface plating, whereas the reference method involved a pour plate where colonies growing in the agar could be exposed to more inhibitory compound with the agar.

A positive bias was also noted in the high level samples plated in the accuracy profile part of the study using cooked fish. In the relative trueness a slight negative bias of 0.086 was recorded for the fishery products category.

To investigate possible reasons for the high AL seen in the ILS, a further root cause analysis was carried out. A relatively high SD variability was recorded for the low level samples for the reference method which resulted in the recalculated AL of 1.07log.

Table 10. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile	0.5						
Study Name	Free text						
Date	Free text						
Coordinator	Free text						
Tolerance probability (beta)	80%	80%	80%		TRUE		
Acceptability limit in log (lambda)	1.07	1.07	1.07				
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	2.408	4.481	5.745				
Number of participants (K)	10	10	10		10	10	10
Average for alternative method	2.727	4.756	5.850		2.408	4.481	5.745
Repeatability standard deviation (sr)	0.110	0.092	0.310		0.328	0.117	0.145
Between-labs standard deviation (sL)	0.119	0.138	0.236		0.183	0.189	0.322
Reproducibility standard deviation (sR)	0.162	0.166	0.389		0.376	0.222	0.354
Corrected number of dof	14.103	12.211	16.152		17.522	11.852	10.655
Coverage factor	1.395	1.411	1.381				
Interpolated Student t	1.345	1.355	1.336				
Tolerance interval standard deviation	0.1681	0.1727	0.4025				
Lower TI limit	2.501	4.521	5.312				
Upper TI limit	2.953	4.990	6.388				
Bias	**0.319**	**0.275**	**0.105**				
Relative Lower TI limit (beta = 80%)	**0.093**	**0.040**	**-0.432**	FALSE			
Relative Upper TI limit (beta = 80%)	**0.545**	**0.509**	**0.643**	TRUE			
Lower Acceptability Limit	**-1.07**	**-1.07**	**-1.07**				
Upper Acceptability Limit	**1.07**	**1.07**	**1.07**				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0.324						

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"

5 Overall conclusions of the MCS/ILS study

- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for relative trueness.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for accuracy profile.
- The alternative method Easy Plate CC for enumeration of coliforms is selective and specific.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory performance in the ILS.

6 Overall conclusions of the MCS/ILS study

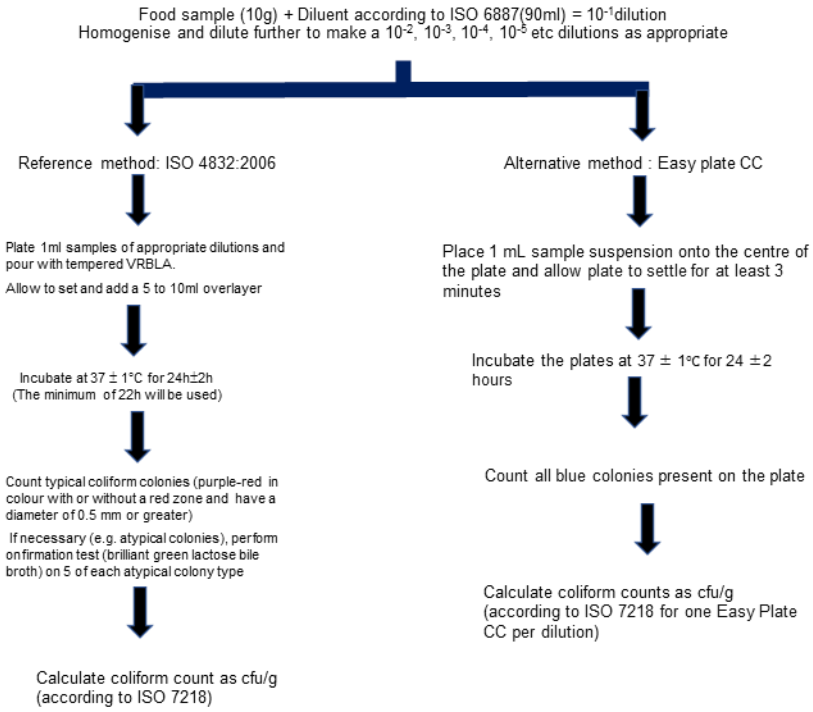
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for relative trueness.
- The alternative method Easy Plate CC for enumeration of coliforms shows satisfactory results for accuracy profile.

- The alternative method Easy Plate CC for enumeration of Coliforms is selective and specific.
- The alternative method Easy Plate CC for enumeration of *coliforms* shows satisfactory performance in the ILS.
- The alternative method Easy Plate CC for enumeration of coliforms. shows comparable performance to the reference method ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method.

Date 08/09/23

Signature Suzanne Jordan

7 ANNEX A: flow diagram of the reference method and alternative methods



8 ANNEX B: Kit insert

Please refer to separate pdf document

ANNEX C: Raw data per category – screening of samples

9 Annex C: Calculations and interpretation of relative trueness

Type	Code	log(Ref)	log(Alt)	Mean	Difference
Crustaceans					
prawn cocktail	I11	2.342	2.362	2.352	0.019
brown shrimp	I12	3.380	3.176	3.278	-0.204
Lemon & Garlic King Prawns	I13	4.255	4.505	4.380	0.250
Big & Juicy Crayfish Tails	I14	5.279	5.342	5.311	0.064
Chilli & Lime King Prawns	I15	6.000	6.279	6.139	0.279
Raw fish (unprocessed)					
Diced salmon fillets	I1	8.079	7.462	7.771	-0.617
Cod loin	I2	4.756	4.491	4.624	-0.265
skinless basa fillets	I3	7.944	7.756	7.850	-0.189
Wild Salmon	I4	7.041	6.398	6.720	-0.643
British Coley fillet	I5	4.756	4.672	4.714	-0.084
RTE/RTC/RTRH fish and seafoods					
Frozen Tuna steaks	I10	5.279	5.398	5.338	0.119
Smoked Salmon	I6	2.695	2.531	2.613	-0.163
Honey Roast Salmon Flakes	I7	2.526	2.415	2.471	-0.111
Ready to eat smoked mackerel fillets	I8	3.544	3.398	3.471	-0.146
Herring Creamy Sauce	I9	4.301	4.568	4.435	0.267
Dry milk products					
Milk powder	H11	3.301	3.176	3.239	-0.125
Pancake mix	H12	3.690	3.568	3.629	-0.122
Dessert powder	H13	2.477	2.653	2.565	0.176
Scone powder	H14	6.230	6.342	6.286	0.112
Instant Custard Powder	H15	6.813	6.785	6.799	-0.028
Pasteurised milk and dairy products					
Skimmed milk	H10	6.079	6.491	6.285	0.412
Cream cheese 'n' prawns	H6	2.389	2.447	2.418	0.058
Protein shake strawberry	H7	3.301	3.342	3.322	0.041
Double cream	H8	4.114	4.580	4.347	0.466
Vanilla ice cream	H9	4.869	5.204	5.037	0.335
Raw milk and dairy products					
Raw milk (AMP)	H1	2.431	2.462	2.447	0.031
Raw milk (straightfromthecow)	H2	3.297	3.447	3.372	0.150
Raw milk cheese 1	H3	4.342	4.623	4.483	0.281
Raw milk cheese 2	H4	5.041	5.079	5.060	0.038

Raw milk cheese 3 (No.1 Mountain Comté AOP)	H5	6.362	6.447	6.404	0.085
Composite foods with substantial raw ingredients					
triple grain salad	K1	2.865	3.230	3.047	0.366
prawn layered salad	K2	3.851	4.255	4.053	0.404
cheese spring onion sandwich	K3	4.653	5.176	4.915	0.523
egg mayo sandwich	K4	5.724	6.079	5.902	0.355
egg and cress sandwich	K5	6.813	7.477	7.145	0.664
Mayonnaise based deli-salads					
Coleslaw with real mayo	K11	2.190	2.255	2.223	0.065
Potato salad with mayo	K12	3.146	3.362	3.254	0.216
cheese colesaw with mayo	K13	4.230	4.301	4.266	0.071
Egg mayonnaise	K14	5.398	5.505	5.452	0.107
Chicken, tomato and basil pasta with egg yolk and pasturized egg mayo	K15	6.477	6.431	6.454	-0.046
RTRH/RTE foods (chilled, frozen)					
Spinach and tomato quiche	K10	6.362	6.623	6.492	0.262
Chicken tomato & basil pasta	K6	2.525	2.580	2.552	0.055
Cod fillet & chips	K7	3.826	4.279	4.052	0.453
Roasted Vegetable Couscous	K8	4.431	4.544	4.488	0.113
Savers Chicken Curry	K9	5.431	5.613	5.522	0.181
Cut ready-to-eat vegetables/leafy greens and sprouts					
Sweet salad, mix of iceberg, romaine, green frilly lettuce	J1	5.903	6.000	5.952	0.097
Sliced Carrot	J2	2.863	2.519	2.691	-0.345
Sweet beets and little leaves	J3	5.934	5.580	5.757	-0.355
Sprouted salad topper	J4	8.041	7.996	8.019	-0.046
Spinach, watercress & rocket salad	J5	4.778	4.653	4.716	-0.125
Fresh fruit/Cut RTE fruit and vegetable products					
Super Blue (non heat treated, cold pressed) smoothie	J10	4.230	4.556	4.393	0.326
Mango	J6	3.544	3.813	3.678	0.269
Watermelon chunks	J7	4.845	4.146	4.496	-0.699
smooth freshly squeezed orange juice	J8	3.079	3.875	3.477	0.796
super green (non heat treated, cold pressed) smoothie	J9	5.041	5.613	5.327	0.571
Heat treated fruit and vegetables					
Wonder Green Juice	J11	1.845	2.301	2.073	0.456
Cranberry juice drink (heat treated) juice	J12	3.964	4.041	4.003	0.078
Juice burst orange (heat treated) juice	J13	4.114	4.544	4.329	0.430

V8 original vegetable juice (heat treated) smoothie	J14	5.176	5.799	5.488	0.623
Brilliant Beetroot Juice (heat treated) smoothie	J15	6.447	6.820	6.633	0.372
Raw processed meat					
chicken meat	L1	6.398	6.447	6.423	0.049
diced beef	L2	2.538	2.342	2.440	-0.195
pork loin chops,	L3	3.322	2.954	3.138	-0.368
Turkey minifillet,	L4	6.041	4.431	5.236	-1.610
Swab taken from chicken	L5	5.362	5.362	5.362	0.000
Raw processed meat					
Raw diced beef 500g	L10	5.785	5.732	5.759	-0.053
Fire Pit 6 Beef Burgers	L6	2.555	2.602	2.579	0.047
mini pork meatballs	L7	4.230	3.491	3.861	-0.739
seasoned diced pork	L8	6.146	5.613	5.879	-0.533
Bag of mince beef	L9	3.415	3.447	3.431	0.032
RTC processed poultry					
Fire pit piri piri chicken steaks	L11	2.562	2.398	2.480	-0.164
Southern chicken goujons	L12	3.544	3.643	3.594	0.099
Spiced poultry	L13	4.447	4.681	4.564	0.234
Turkey burgers	L14	5.531	5.505	5.518	-0.026
Turkey escalopes ham and cheese	L15	6.544	6.690	6.617	0.146

11 Annex D: Summary tables Accuracy profile study

Dairy products

(Food) Category 1	Dairy products
(Food) Type 1	Pasteurised dairy products

			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
Pasteurised cream	1a-e	low	175	236	140	180	130	175	155	175	185	140
Cream cheese	4 a-e	low	175	125	170	80	155	180	165	125	140	215
Pasteurised cream	2 a-e	intermediate	1.40E+04	1.80E+04	1.30E+04	1.40E+04	1.50E+04	1.60E+04	1.50E+04	1.40E+04	1.50E+04	1.80E+04
Cream cheese	5 a-e	intermediate	1.20E+04	1.20E+04	1.40E+04	1.00E+04	1.30E+04	1.50E+04	1.50E+04	1.60E+04	1.40E+04	1.60E+04
Pasteurised cream	3 a-e	high	2.30E+06	2.60E+05	1.30E+06	2.30E+06	6.50E+05	2.20E+06	1.90E+05	1.80E+06	2.20E+06	1.00E+06
Cream cheese	6 a-e	high	1.30E+06	8.60E+05	1.40E+06	1.30E+06	7.70E+05	1.70E+06	1.90E+06	1.60E+06	1.50E+06	1.60E+06

Fruits and Vegetables

(Food) Category 2	Fruits and vegetables
(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
7 a-e	Veg prep	low	232	227	259	295	268	285	255	245	305	345
10 a-e	Veg juice	low	318	277	350	373	373	445	414	400	464	418
8 a-e	Veg prep	intermediate	2.50E+04	2.60E+04	2.90E+04	2.20E+04	2.70E+04	2.10E+04	2.50E+04	2.40E+04	2.50E+04	2.70E+04
11 a-e	Veg juice	intermediate	3.30E+04	3.60E+04	4.10E+04	4.30E+04	4.30E+04	5.60E+04	4.60E+04	4.20E+04	3.70E+04	4.20E+04
9 a-e	Veg prep	high	2.20E+06	4.20E+06	3.60E+06	2.90E+06	2.90E+06	4.20E+06	5.30E+06	3.60E+06	4.20E+06	4.70E+06
12 a-e	Veg juice	high	3.00E+06	4.50E+06	5.10E+06	4.50E+06	5.50E+06	4.20E+06	4.20E+06	4.90E+06	5.50E+05	5.50E+06

Cooked fish products

(Food) Category 4	Ready to eat foods (Combined
(Food) Type 4	Cooked fish products

			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
19 a-e	Fresh prawns	low	230	300	250	385	285	225	245	280	325	340
22 a-e	Fish pate	low	360	395	365	340	285	355	420	315	405	310
20- a-e	Fresh prawns	intermediate	1.10E+04	3.70E+04	3.20E+04	3.10E+04	4.40E+04	1.70E+04	2.70E+04	2.50E+04	3.10E+04	5.90E+04
23 a-e	Fish pate	intermediate	4.30E+04	3.80E+04	3.00E+04	3.70E+04	3.50E+04	3.50E+04	3.00E+04	3.20E+04	3.40E+04	2.50E+04
21 a-e	Fresh prawns	high	1.40E+06	1.90E+06	3.30E+06	2.60E+06	2.10E+06	3.80E+06	2.90E+06	3.50E+06	4.40E+06	3.70E+06
24 ae	Fish pate	high	1.90E+06	1.80E+06	2.10E+06	3.20E+06	3.00E+06	3.70E+06	1.90E+06	2.40E+06	3.00E+06	3.60E+06

RTC meat and poultry

(Food) Category 7	Raw poultry and meats										
(Food) Type 7	RTC meats										
			Reference method result					Alternative method result				
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
16 a-e	Raw Bacon	low	1.95E+02	9.50E+01	1.65E+02	1.40E+02	1.00E+02	1.85E+02	1.20E+02	1.95E+02	1.75E+02	1.27E+02
13 a-e	Pork	low	2.60E+02	1.95E+02	2.55E+02	2.30E+02	2.50E+02	2.35E+02	2.10E+02	1.55E+02	2.35E+02	2.05E+02
17 a-e	Raw Bacon	intermediate	1.20E+03	6.70E+03	5.70E+03	8.10E+03	6.40E+03	2.40E+03	9.50E+03	9.60E+03	1.30E+04	1.00E+04
14 a-e	Pork	intermediate	4.30E+04	3.60E+04	1.30E+04	3.30E+04	3.10E+04	1.70E+04	1.30E+04	1.00E+04	2.70E+04	9.50E+03
18 a-e	Raw Bacon	high	8.20E+05	1.40E+06	1.00E+06	9.00E+05	1.00E+06	7.30E+05	1.70E+06	1.30E+06	1.20E+06	1.10E+06
15 a-e	Pork	high	8.80E+05	7.10E+05	1.30E+06	1.50E+06	2.20E+06	9.00E+05	6.10E+05	1.20E+06	1.60E+06	2.50E+06

Multicomponent foods

(Food) Category 5	Multi component foods											
(Food) Type 5	Composite foods with raw											
			Reference method result					Alternative method result				
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
25 a-e	Sandwich	low	525	890	415	845	455	545	635	665	455	480
28 a-e	Cooked chilled rice	low	345	395	435	395	455	355	355	415	210	220
26 a-e	Sandwich	intermediate	4.20E+04	3.20E+04	4.00E+04	2.70E+04	5.50E+04	1.80E+04	2.20E+04	4.00E+04	2.00E+04	2.90E+04
29 a-e	Cooked chilled rice	intermediate	6.00E+04	4.50E+04	3.30E+04	3.30E+04	3.70E+04	2.00E+04	2.10E+04	1.50E+04	1.00E+04	3.50E+04
30 a-e	Cooked chilled rice	high	5.60E+05	6.70E+05	4.90E+05	6.30E+05	6.60E+05	3.90E+05	4.10E+05	3.90E+05	3.10E+05	4.50E+05
27 a-e	Sandwich	high	1.50E+06	1.20E+06	7.00E+05	1.10E+06	1.80E+06	5.90E+05	6.40E+05	8.00E+05	4.40E+05	5.20E+05

12 Annex E: Raw data from the ILS

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
1	1	Low	2.5	2.7	27/02/23
	2	Medium	4.5	4.7	27/02/23
	3	High	5.6	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.0	2.4	27/02/23
	6	Medium	4.4	4.6	27/02/23
	7	High	5.6	5.6	27/02/23
2	1	Low	2.6	2.7	27/02/23
	2	Medium	4.3	4.4	27/02/23
	3	High	5.8	5.6	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.0	2.6	27/02/23
	6	Medium	4.4	4.7	27/02/23
	7	High	5.6	5.7	27/02/23
4	1	Low	2.6	2.8	27/02/23
	2	Medium	4.5	4.8	27/02/23
	3	High	5.9	6.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.9	27/02/23
	6	Medium	4.4	4.9	27/02/23
	7	High	5.9	6.1	27/02/23
5	1	Low	2.4	2.9	27/02/23
	2	Medium	4.5	4.8	27/02/23
	3	High	6.0	6.5	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.6	27/02/23
	6	Medium	4.7	5.0	27/02/23
	7	High	6.5	6.5	27/02/23
6	1	Low	2.7	2.9	27/02/23
	2	Medium	4.7	4.9	27/02/23
	3	High	6.4	6.2	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.7	27/02/23
	6	Medium	4.7	4.9	27/02/23
	7	High	6.3	6.2	27/02/23
7	1	Low	3.4	2.8	27/02/23
	2	Medium	4.4	4.8	27/02/23
	3	High	5.7	5.6	27/02/23
	4	Blank	<1	<1	27/02/23

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
	5	Low	2.4	2.7	27/02/23
	6	Medium	4.5	4.9	27/02/23
	7	High	5.9	5.8	27/02/23
9	1	Low	2.7	3.0	27/02/23
	2	Medium	4.6	4.9	27/02/23
	3	High	5.6	5.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.9	27/02/23
	6	Medium	4.8	4.9	27/02/23
	7	High	5.8	6.1	27/02/23
10	1	Low	2.3	2.7	27/02/23
	2	Medium	4.3	4.6	27/02/23
	3	High	5.3	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.0	2.5	27/02/23
	6	Medium	4.1	4.5	27/02/23
	7	High	5.3	5.8	27/02/23
11	1	Low	2.2	2.6	27/02/23
	2	Medium	4.0	4.6	27/02/23
	3	High	5.4	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	1.5	2.4	27/02/23
	6	Medium	4.3	4.6	27/02/23
	7	High	5.3	5.7	27/02/23
12	1	Low	2.4	2.9	27/02/23
	2	Medium	4.6	4.9	27/02/23
	3	High	5.5	5.9	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.9	27/02/23
	6	Medium	4.8	4.8	27/02/23
	7	High	5.8	5.0	27/02/23
Expert lab	1	Low	2.8	2.9	27/02/23
	2	Medium	4.3	4.7	27/02/23
	3	High	5.9	6.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.3	2.5	27/02/23
	6	Medium	4.1	4.4	27/02/23
	7	High	5.6	5.9	27/02/23