ISO 16140-2:2016 validation of Kikkoman Biochemifa Company Easy Plate EC, for the enumeration of *E. coli* and coliforms in a broad range of foods plus pet food and animal feed and environmental samples

MicroVal study number: 2021LR103

Method/Kit name: Easy Plate EC

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 EC

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 methods: ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

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 plus pet food and environmental samples

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

Table of Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of *E. coli* and coliforms in a broad range of foods in combination with pet food and animal feed as well as environmental samples was carried out by Campden BRI as the MicroVal Expert Laboratory.

Alternative method

The alternative method used is:

Easy Plate EC is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate EC method is intended to indicate the level of *Escherichia coli* and coliform bacteria in selected food and beverage products. After incubation, *E. coli* colonies appear as navy blue/blue purple colonies and non E. coli coliforms as pink/red colonies on the Easy Plate EC growth medium. In this study, the minimum incubation time of 22h was used for the Easy plate EC. A total coliform count includes both *E. coli* and non-*E. coli* coliform colonies.

Reference methods are:

ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

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 is: A broad range of foods, pet food and ainmal feed, and environmental samples

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
- Pet food and animal feed
- Environmental samples

Criteria evaluated during the MCS study:

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

The final conclusion on the Method Comparison and Interlaboratory studies are summarised below:

- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms shows satisfactory results for relative trueness.
- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms shows satisfactory results for accuracy profile.
- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms is selective and specific.
- The alternative method Easy Plate EC for enumeration of *E. coli*, and coliforms shows satisfactory performance in the ILS.
- The alternative method Easy Plate EC for enumeration of *E. coli* shows comparable performance to the reference method ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide
- The alternative method Easy Plate EC 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 was 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 were used with at least two successive dilutions. Two plates per dilution may 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 calculations

2.2 Alternative method

See the flow diagram in Annex A

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

Easy Plate EC is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate EC method is intended to indicate the level of *Escherichia coli* and coliform bacteria in selected food and beverage products. After incubation for 24h ±2h at 37°C, *E.coli* colonies appear as navy blue/blue purple colonies and coliforms as pink/red colonies on the Easy Plate EC growth medium. In this study, the minimum incubation time of 22h was used for the Easy plate EC.

A total coliform count was used for the coliform study, which included both *E. coli* and non *E. coli* coliforms (non Ec Coliforms) colonies as a combined count.

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

A total of 7 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 for *E.coli*	No of samples for non *E.c* coliforms	ISO 6887
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Raw milk and dairy products	Raw milk, raw milk cheese	5	5	6887-5
	Pasteurised milk and milk based products	Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk)	5	5	6887-5
	Dry milk products	Milk powders and powder for milk based desserts	5	5	6887-5
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon filet, tuna, bonito	5	5	6887-3
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods, semi-dried fish	5	5	6887-3
	Crustaceans	Shrimp, crab	5	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	5	6887-4
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	5	6887-4
	Heat treated fruit and vegetables	Pasteurised smoothies/juice, blanched frozen vegetables	5	5	6887-4
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, egg and cress sandwich	5	5	6887-1, 6887-4
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products, quiche	5	5	6887-1, 6887-4
	Mayonnaise based deli-salads	Vegetable salad, egg mayonnaise	5	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	5	6887-2
	Raw processed meat	Frozen burger patties, pork meat balls,	5	5	6887-2
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	5	6887-2
Pet food and animal feed	Dry Food	Pellets, kibbles, treats	5	5	6887-4
	Wet food (raw and canned)	Pates, sausages	5	5	6887-2
	Animal feeds (poultry and fish)	Cereals and flours	5	5	6887-4
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	Equipment, floors, walls	5	5	6887-1 ISO 18593:2018
	Process water	Wash water, cooling water	5	5	6887-1
	Dusts	Bakery and food manufacturing environment	5	5	6887-1 ISO 18593:2018

105 samples were analysed, leading to 105 explotiable results

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

3.2.2 Test sample preparation- E. coli

Samples were screened for natural contamination to ensure suitable levels of contamination for the study. None of the samples tested during the screening phase were naturally contaminated with *E. coli*. The 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 *E. coli* 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 Heat treated fruit and vegetables samples were spiked with a heat-treated *E. coli* isolate. The injury level achieved for the isolate was at least 0.5 log.

Twenty *E. coli* isolates 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 . Test sample preparation Coliforms

All samples were screened for natural contamination to ensure suitable levels of contamination of coliforms for the study. During the study 23.8% 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 products and 5 samples of wet pet food samples were spiked with a heat treated coliform isolate. The injury level achieved for the isolate was at least 0.5 log.

Sixteen colifom isolates 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

3.2.4 Protocols applied during the validation study

A single protocol was applied for the study for the alternative method and two reference methods, one for each of the target organisms being tested.

Reference method plates were incubated at 37±1ºC for 24±2h for coliforms and 44±1ºC for 18-24h for *E. coli*. For samples that were thought to contain heat acid or osmotically stressed cells, plates were per incubated at 37°C for 4h prior to incubation at 44°C.

Where required presumptive coliform 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.

The Alternative method was incubated at 37±1ºC for 24±2h. A total coliform count was used for the coliform study, which included both *E. coli* and non *E. coli* coliforms (non Ec Coliforms) colonies as a combined count.

In all cases the minimum incubation times were used.

3.2.5 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.

3.2.6 Calculation and interpretation of relative trueness study E. coli

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x).
Figures 1 to 7 shows the scatter plots for the individual categories and Figure 8 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 E. coli

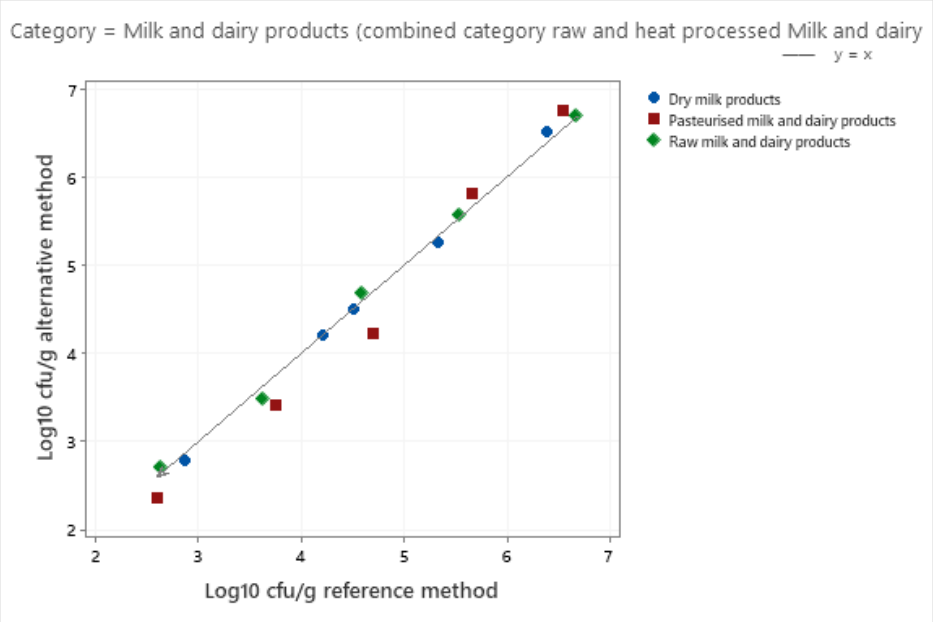


Figure 2 - Scatter plot of the reference method versus alternative method results for Fishery products, Combined category: raw, RTE, RTRH, RTC for E. coli

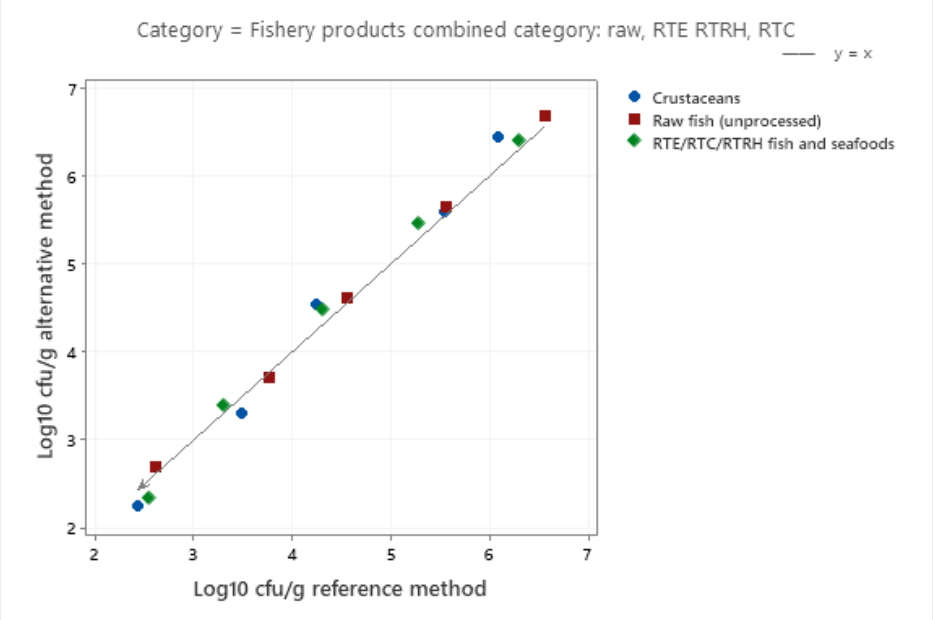


Figure 3 - Scatter plot of the reference method versus alternative method results for Produce and fruits (combined category fresh and processed) for E. coli

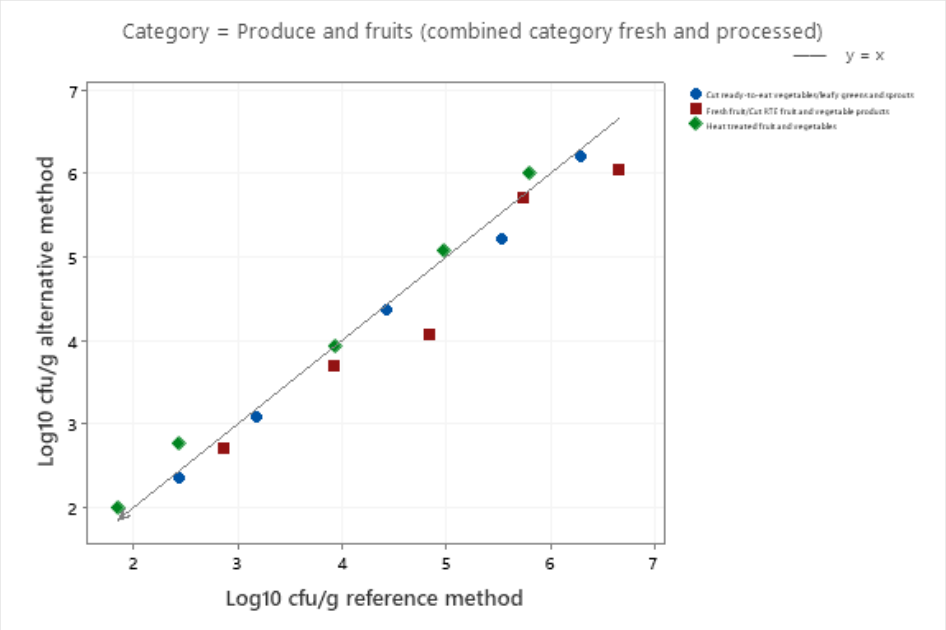


Figure 4 - Scatter plot of the reference method versus alternative method results for Raw and RTC Meat and poultry (Combined category) for E. coli

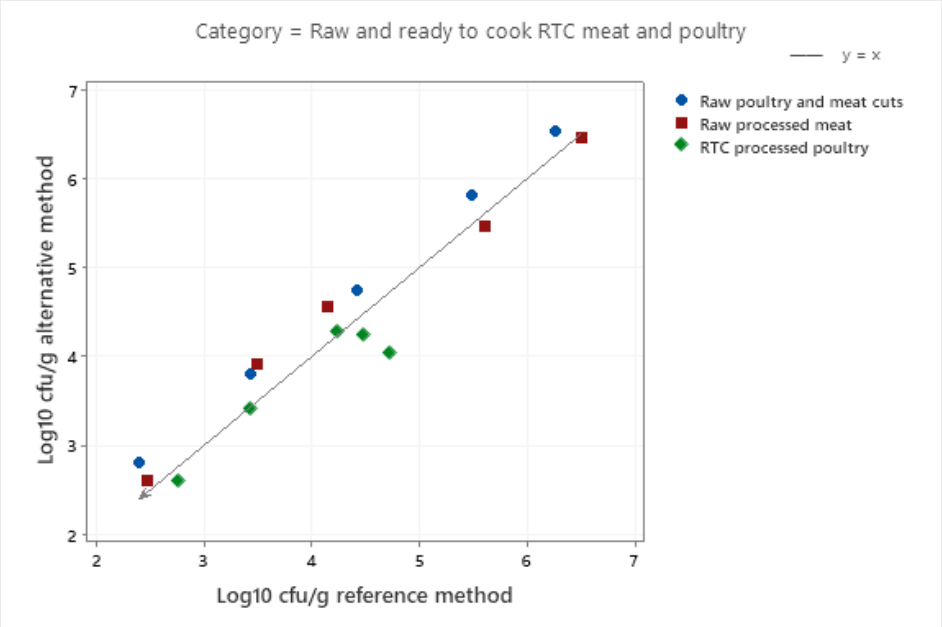


Figure 5 - Scatter plot of the reference method versus alternative method results for Multicomponent foods for E. coli

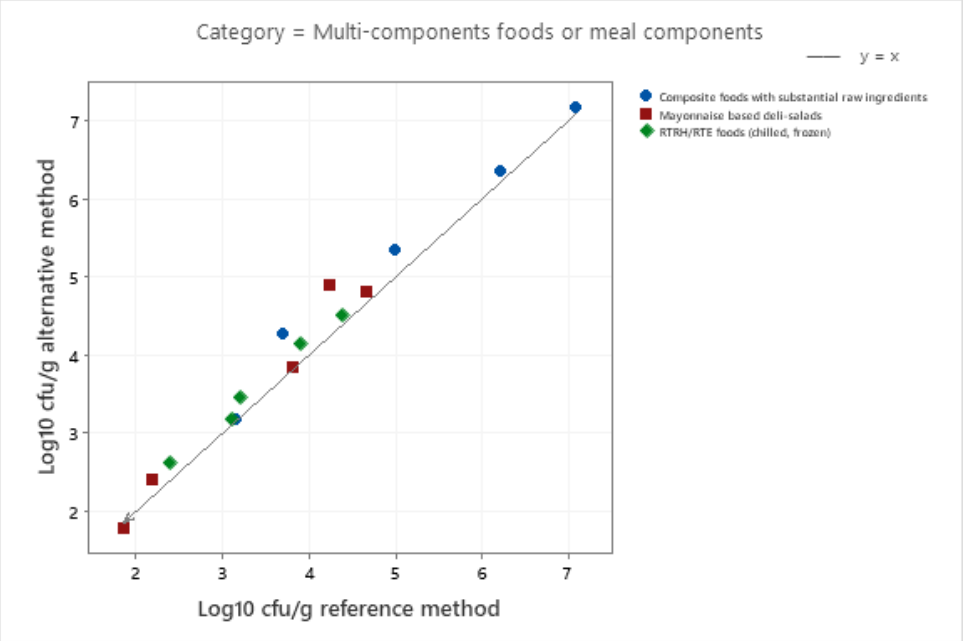


Figure 6 - Scatter plot of the reference method versus alternative method results for Pet food and animal feed for E. coli

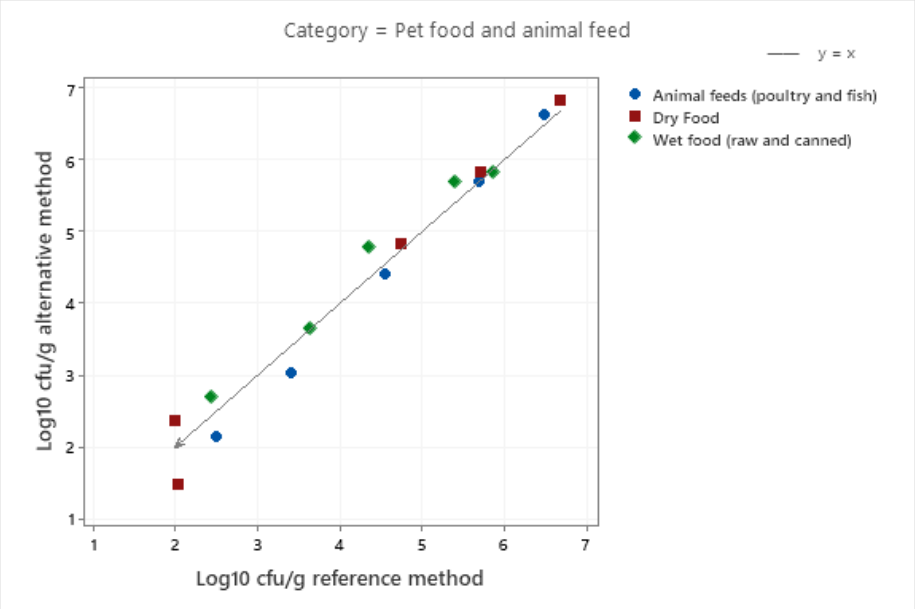


Figure 7- Scatter plot of the reference method versus alternative method results for Environmental samples for E. coli

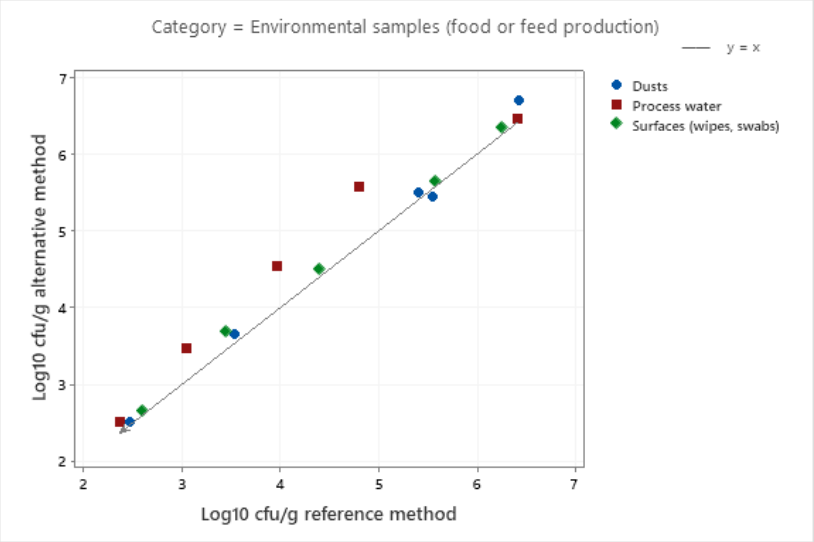
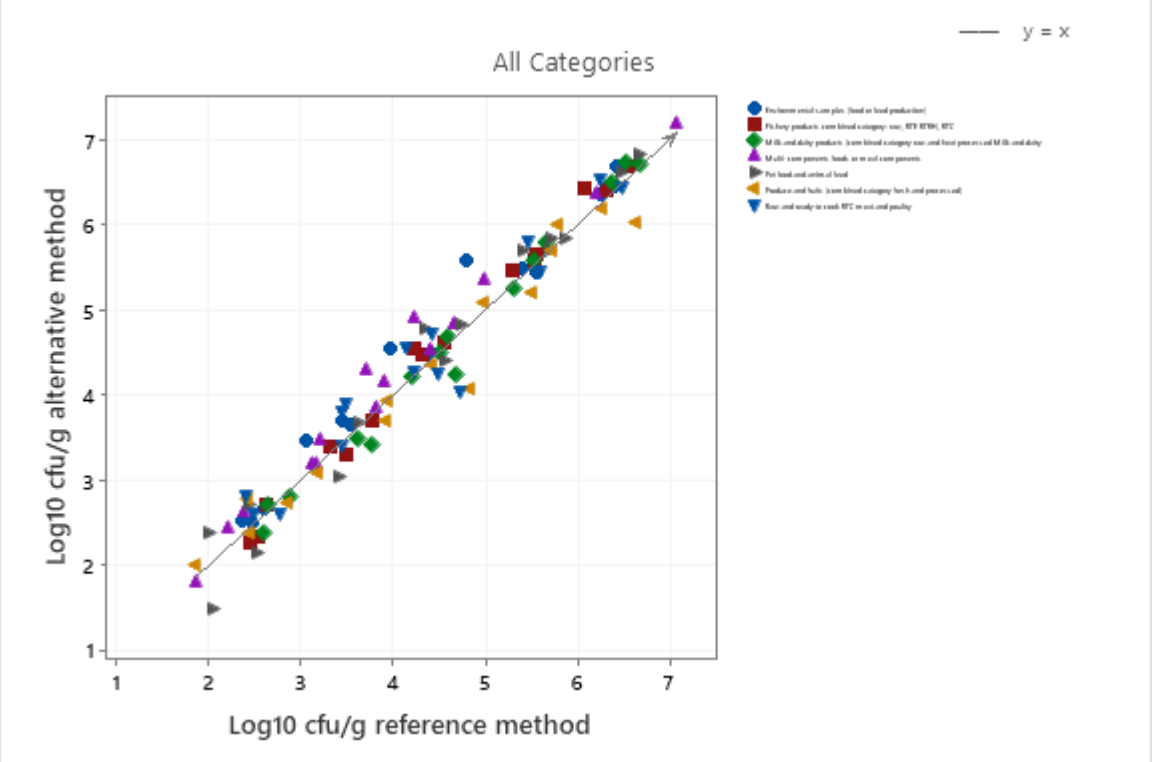


Figure 8 - Scatter plot of the reference method versus alternative method results for all categories for E. coli



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.

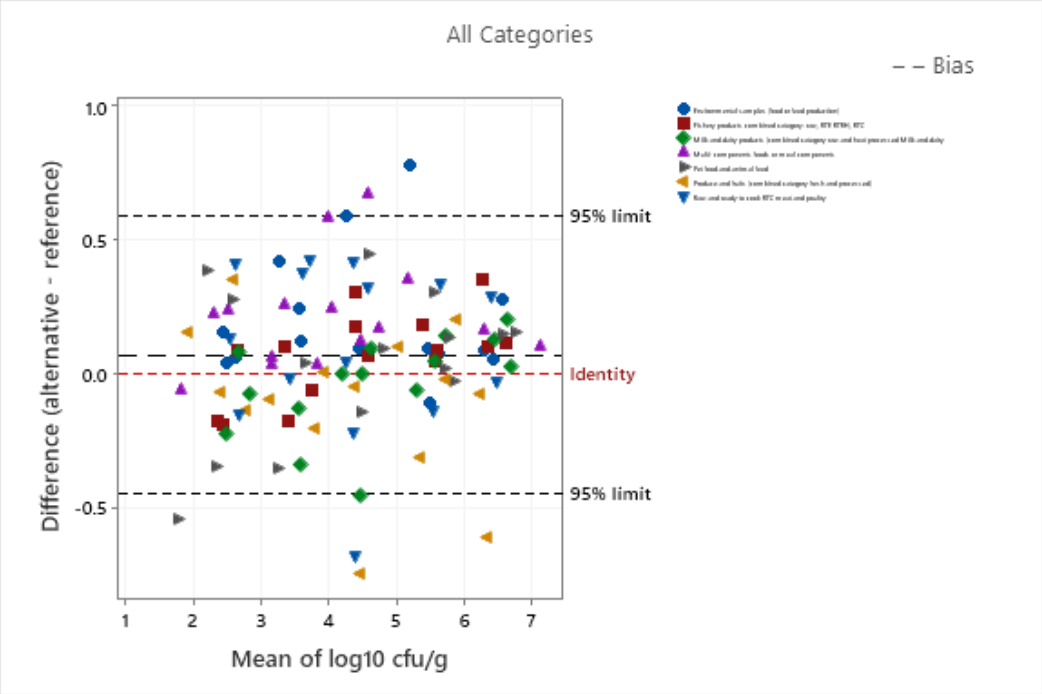
A summary of the calculated values per category for *E. coli* is provided in Table 2

Table 2 - Summary of the calculated values per category for E. coli

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Environmental samples (food or feed production)	15	0.198	0.233	-0.319	0.714
Fishery products combined category: raw, RTE RTRH, RTC	15	0.067	0.162	-0.292	0.426
Milk and dairy products (combined category raw and heat processed Milk and dairy	15	-0.039	0.185	-0.450	0.371
Multi-components foods or meal components	15	0.211	0.201	-0.233	0.655
Pet food and animal feed	15	0.037	0.284	-0.593	0.667
Produce and fruits (combined category fresh and processed)	15	-0.103	0.288	-0.742	0.536
Raw and ready to cook RTC meat and poultry	15	0.102	0.316	-0.598	0.801
All Categories	105	0.067	0.261	-0.452	0.587

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

Figure 9 - The Bland-Altman difference plot for all the samples for E. coli



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 for E. coli

Category	item	Code	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference (Alternative-reference)	Lower/ upper limits
Multi-components foods or meal components	Chicken, tomato and basil pasta with egg yolk and pasteurized egg mayo	**D15**	4.230	4.903	4.567	0.673	0.587
Environmental samples (food or feed production)	Surface run off - industrial kitchen	**G9**	4.792	5.568	5.180	0.776	0.587
Pet food and animal feed	Grain free Salmon with sweet potato minibites	**F2**	2.021	1.477	1.749	-0.544	-0.452
Raw and ready to cook RTC meat and poultry	Turkey escalopes ham and cheese	**E15**	4.724	4.041	4.383	-0.683	-0.452
Produce and fruits (combined category fresh and processed)	smooth freshly squeezed orange juice	**C8**	4.833	4.079	4.456	-0.753	-0.452
	Super Blue (non heat treated, cold pressed) smoothie	**C10**	6.653	6.041	6.347	-0.612	-0.452
Milk and dairy products (combined category raw and heat processed Milk and dairy	Vanilla ice cream	A9	4.690	4.230	4.460	-0.460	-0.452

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 7 in 105 data values which lie outside the CLs (Table 3). This is higher than the expectation however, there are no trends to the outlying data which represented six of the seven categories. Two points were slightly higher than the upper limit of 0.587 and four points were slightly lower than the lower limit of -0.452. The samples covered a diverse range of foods items with different groups organisms present and therefore these results show good agreement between the two methods for enumeration of *E. coli* with a slight positive bias for the alternate method with an overall bias from all the categories of 0.067.

Two of the categories included in the study (multicomponent foods and environmental) showed a slight positive bias. One possible explanation could be that for the alternative method the sample is incubated at 37°C for the full incubation time, rather than the reference method where the majority of the incubation time is at 44°C which could slow the growth of the *E. coli.*

3.2.1 Calculation and interpretation of relative trueness study coliforms

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x).
Figures 10 to 16 shows the scatter plots for the individual categories and Figure 17 for all categories.

Figure 10 - 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

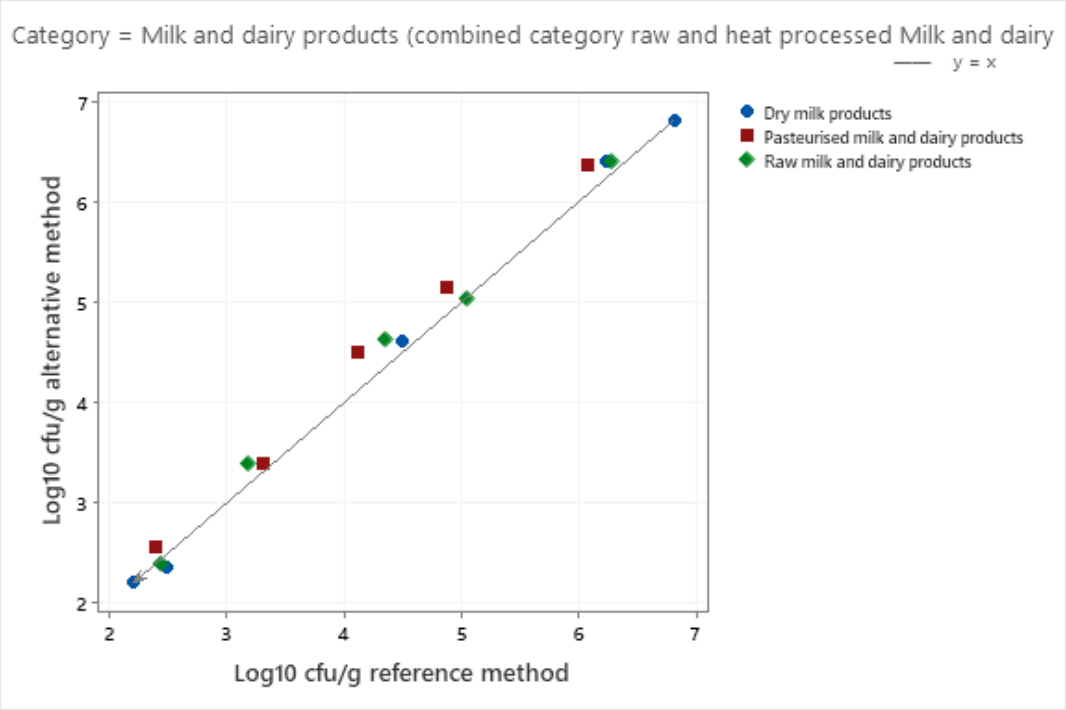


Figure 11 - Scatter plot of the reference method versus alternative method results for Fishery products, Combined category: raw, RTE, RTRH, RTC for coliforms

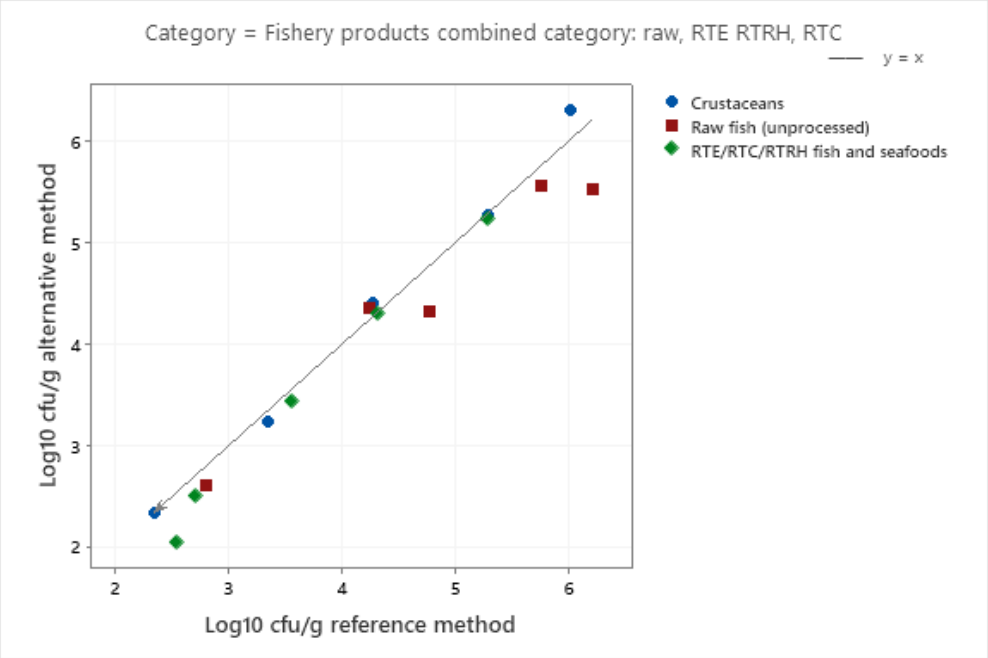


Figure 12 - Scatter plot of the reference method versus alternative method results for Produce and fruits (combined category fresh and processed) for coliforms

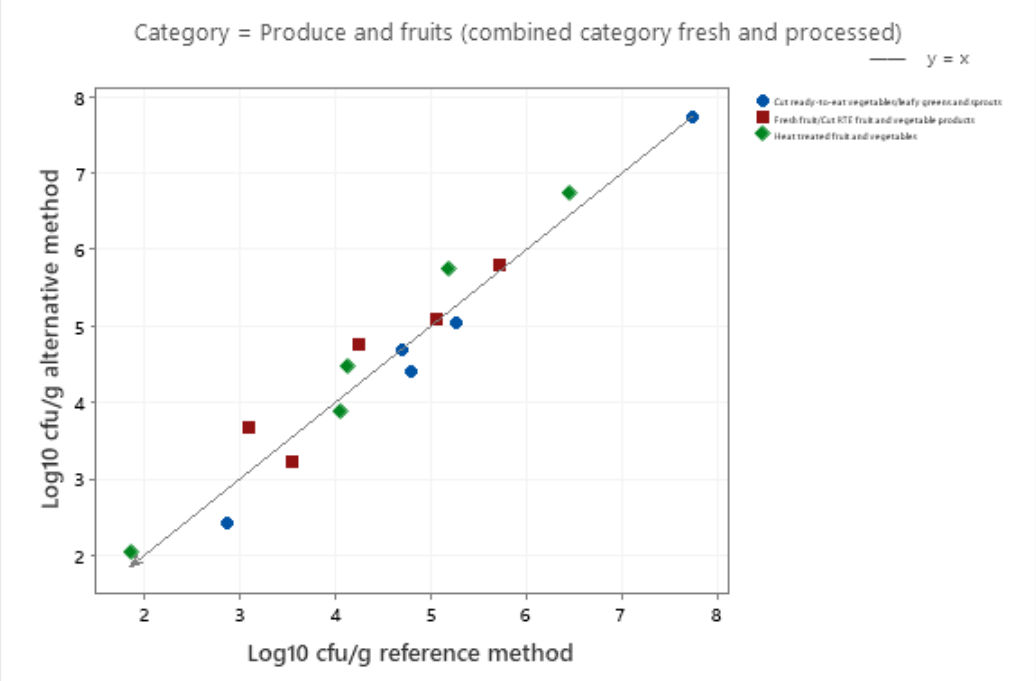


Figure 13 - Scatter plot of the reference method versus alternative method results for Raw and RTC Meat and poultry (Combined category) for coliforms

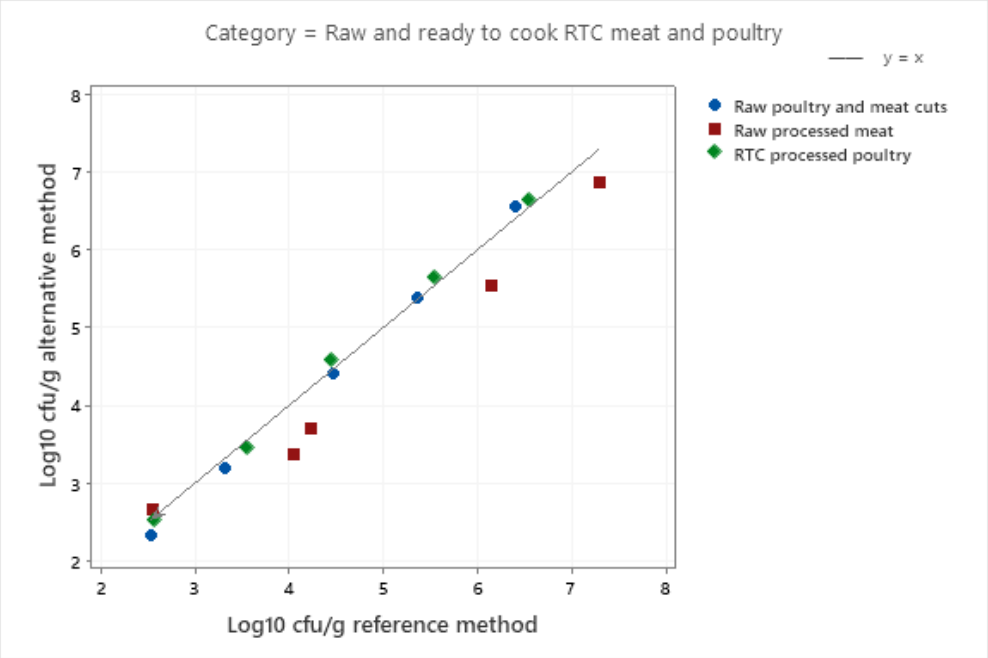


Figure 14 - Scatter plot of the reference method versus alternative method results for Multicomponent foods for coliforms

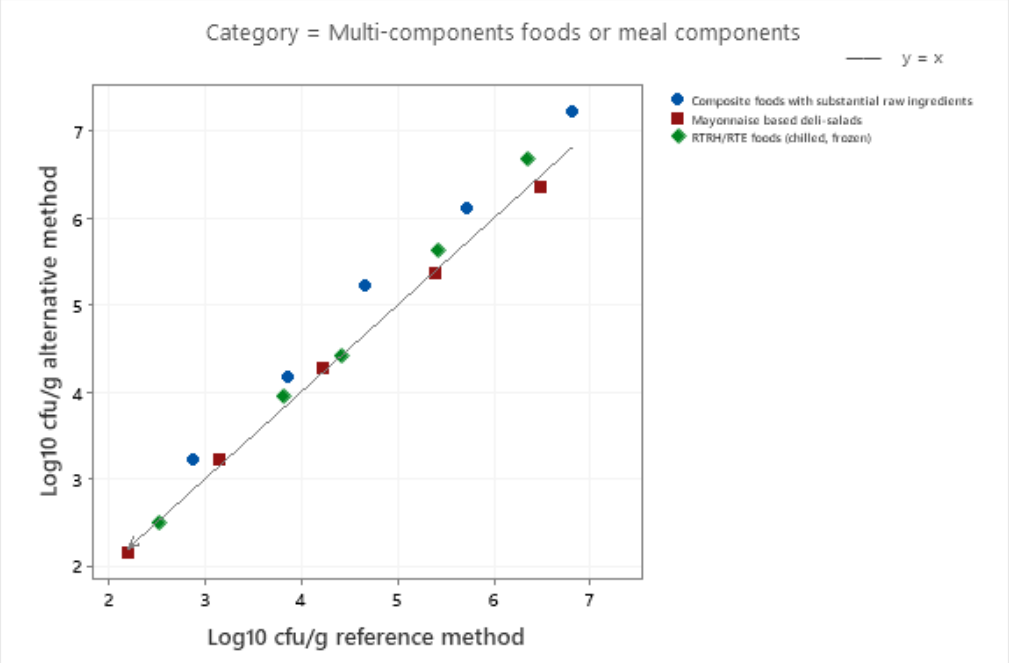


Figure 15- Scatter plot of the reference method versus alternative method results for Pet food and animal feed for coliforms

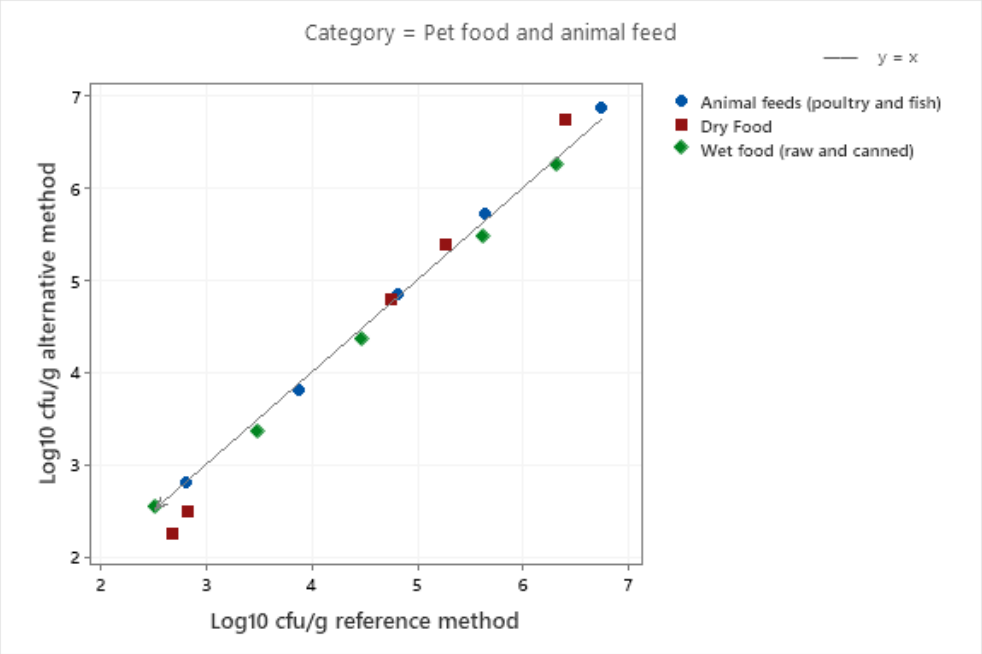


Figure 16- Scatter plot of the reference method versus alternative method results for Environmental samples for coliforms

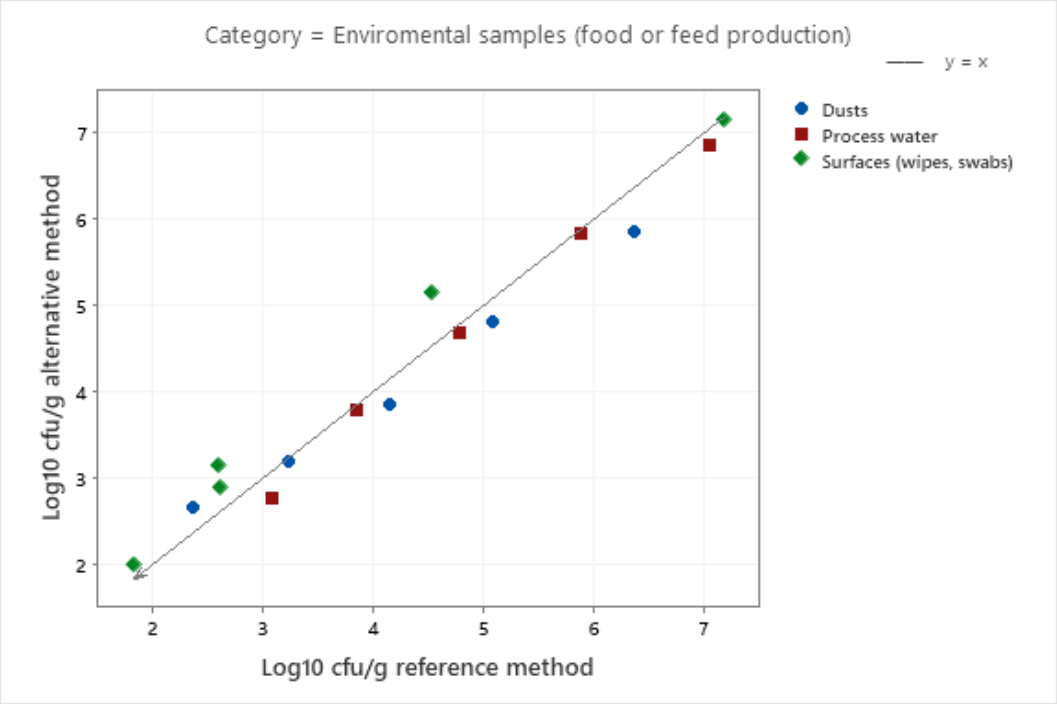
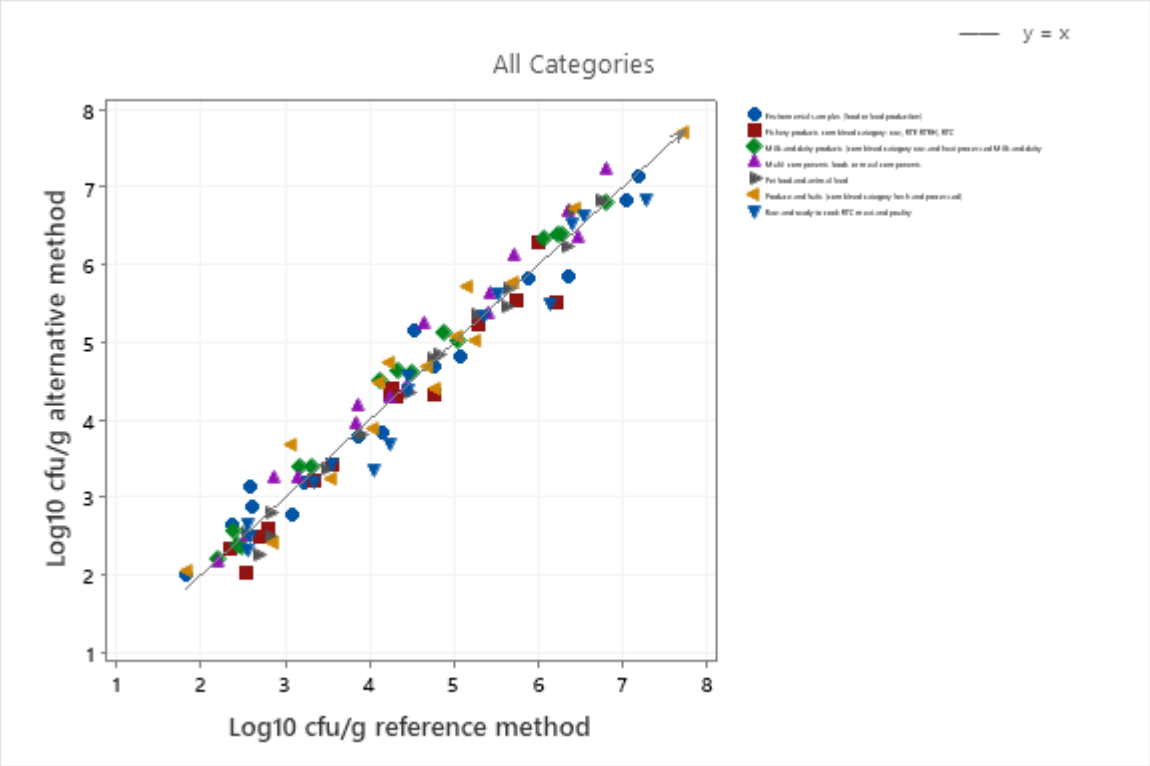


Figure 17 - Scatter plot of the reference method versus alternative method results for all categories for coliforms



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 4

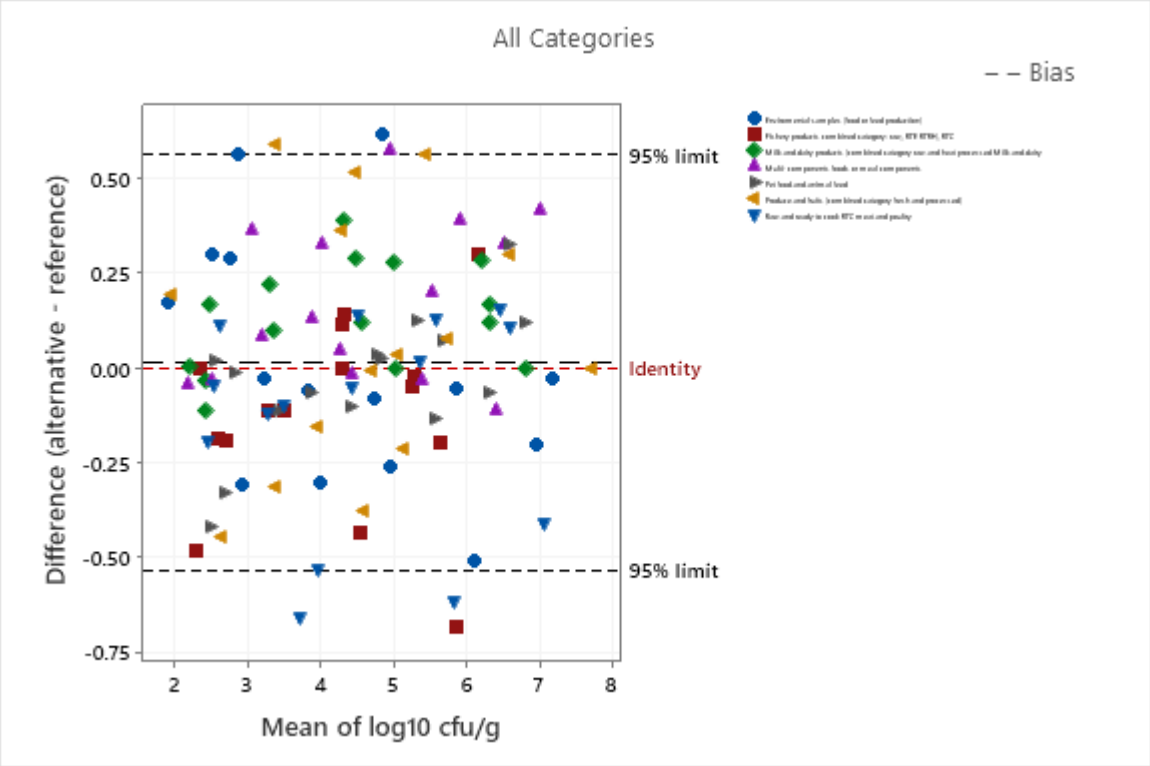
Table 4 - Summary of the calculated values per category for coliforms

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Environmental samples (food or feed production)	15	0.007	0.324	-0.710	0.724
Fishery products combined category: raw, RTE RTRH, RTC	15	-0.128	0.255	-0.693	0.436
Milk and dairy products (combined category raw and heat processed Milk and dairy	15	0.133	0.144	-0.185	0.451

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Multi-components foods or meal components	15	0.175	0.213	-0.297	0.646
Pet food and animal feed	15	-0.035	0.182	-0.437	0.368
Produce and fruits (combined category fresh and processed)	15	0.075	0.342	-0.682	0.832
Raw and ready to cook RTC meat and poultry	15	-0.136	0.286	-0.769	0.497
All Categories	105	0.013	0.275	-0.535	0.561

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

Figure 18 - The Bland-Altman difference plot for all the samples for coliforms



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 5.

Table 5 – data which are outside of the Accepted limits for coliforms

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)	Smooth freshly squeezed orange juice	**J8**	3.079	3.672	3.376	0.593	0.561
	V8 original vegetable juice (heat treated) smoothie	**J14**	5.176	5.740	5.458	0.564	0.561
Environmental samples (food or feed production)	Plastic - equipment surface	**N4**	4.531	5.146	4.839	0.615	0.561
	Ceramic - sink splashback	**N2**	2.582	3.146	2.864	0.564	0.561
Multi-components foods or meal components	cheese spring onion sandwich	**K3**	4.653	5.230	4.942	0.577	0.561
Fishery products combined category: raw, RTE RTRH, RTC	skinless basa fillets	**I3**	6.204	5.519	5.861	-0.686	-0.535
Raw and ready to cook RTC meat and poultry	Seasoned diced pork	**L8**	6.146	5.531	5.839	-0.615	-0.535
	Bag of mince beef	**L9**	4.041	3.380	3.711	-0.661	-0.535

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 8 in 105 data values which lie outside the CLs (Table 3). This is higher than the expectation however, there are no trends to the outlying data which represented five of the seven categories. Five points were slightly higher than the upper limit of 0.561 and three points were slightly lower than the lower limit of -0.535. The samples covered a diverse range of foods items with

different groups of naturally present organisms present and therefore these results show good agreement.

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

Seven 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 5a and b)

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 6a and b.

Table 6a. Categories, types, items, strains and inoculation levels for Accuracy profile study for E. coli

Category	Types	Strain *E.coli*	Item	Level (cfu per g)
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	*E. coli* CRA 1476 Isolated from dried milk	Milk powder	1500-3000
				20000-70000
				1500000-250000000
			Dessert powder	1000-8000
				30000-75000
				2000000-90000000
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	*E.coli* CRA 2003 isolated from fish	Frozen white fish	100-900
				1000-3000
				80000-500000
			Chilled tuna steak	200-500
				2000-5000
				100000-800000
Produce and fruits (combined category fresh and processed)	Cut ready to eat	*E.coli* CRA3379 isolated from spinach	Lettuce	150-300
				15000-30000
				3000000-6000000
			Spinach	150-400
				40000-80000
				2000000-4500000
Meat and poultry (Combined category)	Fresh meats	*E. coli* CRA 3384	Raw ground beef	30-100
				1000-6000
				90000-300000

Category	Types	Strain *E.coli*	Item	Level (cfu per g)
		isolated from pork	Chicken breast fillets	60-100
				900-3000
				100000-400000
Multi-components foods or meal components	Composite foods with raw /processed ingredients	*E.coli* CRA 1265 isolated from dried foods	Sandwich	150-350
				15000-40000
				1500000-3500000
			Pasta salad	150-300
				20000-45000
				2000000-4500000
Pet food and animal feed	Wet food (cooked)	*E. coli* CRA 4739 isolated from pork mince	Dog pate	100-350
				10000-40000
				200000-900000
			Cat food with gravy	150-550
				10000-65000
				540000-1500000
Environmental samples	Process water	*E. coli* CRA 6121 isolated from Horlicks drink	Wash water	200-400
				20000-400000
				1000000-4000000
			Cooling water	150-400
				200000-40000
				1500000-4500000

For all matrices used in the study except pet food animal feed and dry milk samples, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis. For the wet pet food ,the samples were spiked with heat treated *E. coli*. Samples of dry milk were inoculated with freeze dried culture and stored at ambient for 2 weeks.

Table 6b. Categories, types, items, strains and inoculation levels for Accuracy profile study for coliforms

Category	Types	Strain coliforms	Item	Level (cfu per g)
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	*Leclercia adecarboxylata CRA 5501 (previously known as Escherichia adecarboxylata)* CRA 5501 Isolated from skimmed milk powder	Milk powder	60-200
				10000-610000
				8500000-23000000
			Dessert powder	150-350
				15000-25000
				14000000-9000000
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	*Leliottia* amingena *previously known as Enterobacter amingenus* NCIMB 2118 isolated from seawater	Frozen white fish	15000-35000
				150000-300000
				7000000-16000000
			Chilled tuna steak	6000-8500
				5000000-1000000
				5000000-15000000
Produce and fruits (combined category fresh and processed)	Cut ready to eat	*Citrobacter amalonaticus* CRA 7458 isolated from beansprouts	Lettuce	300-600
				20000-60000
				1500000-5000000
			Spinach	350-900
				20000-50000

Category	Types	Strain coliforms	Item	Level (cfu per g)
				1000000-4000000
Meat and poultry (Combined category)	Fresh meats	*Citrobacter youngae* DSM 17578 isolated from meat	Raw ground beef	300-500
				8000-15000
				100000-600000
			Chicken breast fillets	300-500
				15000-50000
				900000-350000
Multi-components foods or meal components	Composite foods with raw /processed ingredients	*Escherichia hermanii* CRA 7477 isolated from sesame seeds	Sandwich	75-300
				30000-70000
				5000000-7000000
			Pasta salad	150-400
				50000-75000
				6000000-9000000
Pet food and animal feed	Wet food (cooked)	*Citrobacter youngae* NCTC 13709 isolated from meat scraps	Dog pate	1500-3000
				15000-30000
				800000-1500000
			Cat food with gravy	1500-3000
				30000-50000
				1000000-2000000
Environmental samples	Process water	*Kluyvera intermedia (previously known as Enterobacter intermedius)* NCTC12125 Isolated from surface water	Wash water	400-900
				6000-20000
				1500000-300000
			Cooling water	500-1000
				10000-60000
				1500000-8000000

For all matrices used in the study except pet food animal feed and dry milk samples, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis. For the wet pet food ,the samples were spiked with heat treated *E. coli*. Samples of dry milk were inoculated with freeze dried culture and stored at ambient for 2 weeks.

3.3.2 Calculations and interpretation of Accuracy profile study (E. coli)

All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in Figures 19 to 25 for *E. coli*.

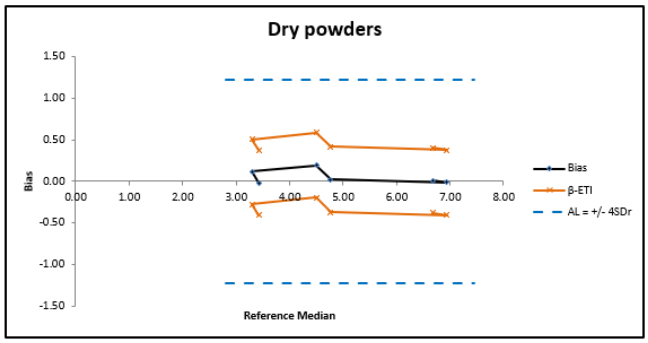
All raw data per category are given in Excel file 2021LR103: tab Accuracy Profile

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 19: Accuracy profile of dairy products (combined category; raw milk and heat processed) for Easy Plate EC method for E.coli

Matrices used = (1-3) and (3-6)

(Food) Category	Milk and dairy
(Food) Type	Dry powders



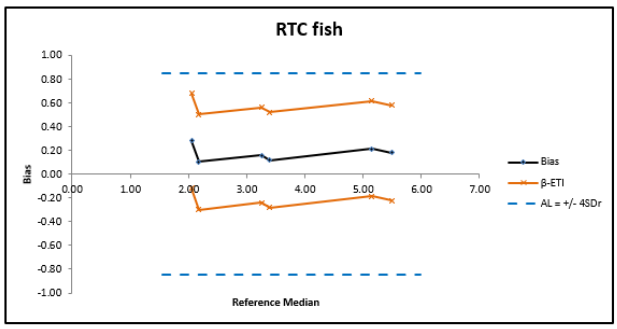
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
2 a-e	3.43	-0.016	-0.409	0.376	YES	YES
5 a-e	3.30	0.114	-0.279	0.507	NO	YES
1 a-e	4.51	0.194	-0.199	0.586	NO	YES
4 a-e	4.77	0.022	-0.371	0.414	YES	YES
3 a-e	6.94	-0.015	-0.408	0.378	YES	YES
6 a-e	6.68	0.009	-0.384	0.402	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.306	0.272	NO	+/- 1.224

Figure 20: Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate EC method for E.coli

Matrices used = White fish (7-9) and Tuna steaks (10-12)

(Food) Category	Fishery
(Food) Type	RTC fish



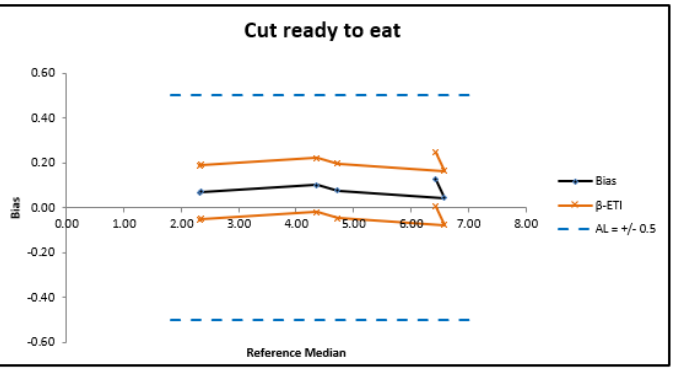
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7a-e	2.06	0.282	-0.120	0.683	NO	YES
10a-e	2.18	0.103	-0.299	0.504	NO	YES
11a-e	3.26	0.160	-0.242	0.561	NO	YES
8a-e	3.40	0.121	-0.281	0.522	NO	YES
12a-e	5.15	0.216	-0.186	0.617	NO	YES
9a-e	5.49	0.181	-0.221	0.582	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.212	0.278	NO	+/- 0.848

Figure 21: Accuracy profile for Produce and fruits for Easy Plate EC method for E.coli

Matrices used = Lettuce (13-15) and Spinach (16-18)

(Food) Category	Produce and fruits
(Food) Type	Cut ready to eat



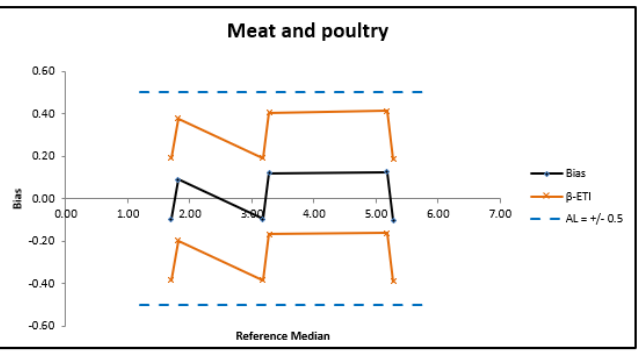
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
13a-e	2.32	0.067	-0.054	0.188	YES	YES
16a-e	2.35	0.071	-0.050	0.192	YES	YES
14a-e	4.36	0.101	-0.021	0.222	YES	YES
17a-e	4.72	0.075	-0.046	0.196	YES	YES
15a-e	6.58	0.043	-0.078	0.165	YES	YES
18a-e	6.43	0.125	0.004	0.246	YES	

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.081	0.084	YES	+/- 0.500

Figure 22: Accuracy profile for Meat and poultry for Easy Plate EC method for E.coli

Matrices used = Raw Chicken (19-21) and Beef (22-24)

(Food) Category	Meat and poultry
(Food) Type	Meat and poultry



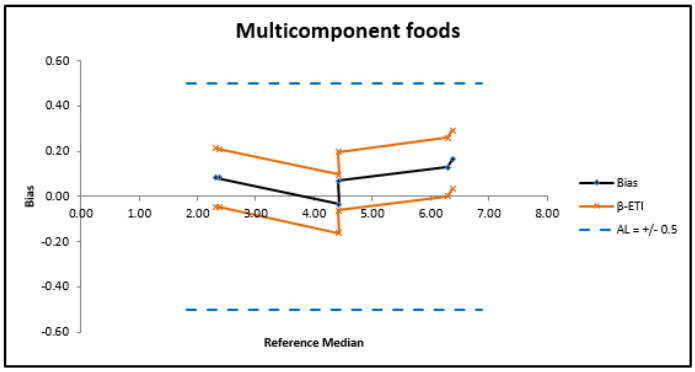
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
22a-e	1.70	-0.097	-0.384	0.190	YES	YES
19a-e	1.81	0.090	-0.197	0.377	YES	YES
23a-e	3.18	-0.097	-0.384	0.190	YES	YES
20a-e	3.28	0.119	-0.168	0.406	YES	YES
21a-e	5.18	0.125	-0.162	0.412	YES	YES
24a-e	5.28	-0.103	-0.390	0.185	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.198	0.199	NO	+/- 0.500

Figure 23: Accuracy profile for Multicomponent foods for Easy Plate EC method for E.coli

Matrices used = Sandwich (25-27) and Pasta salad (28-30)

(Food) Category	Multicomponent
(Food) Type	Multicomponent foods



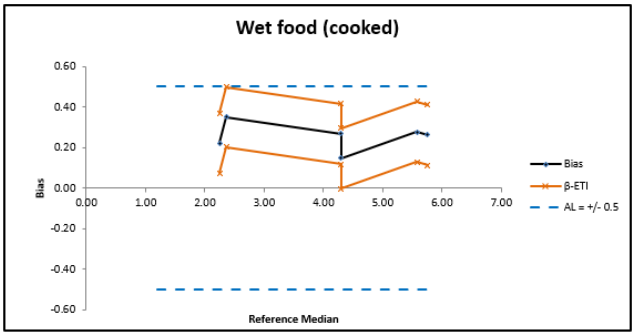
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
28a-e	2.38	0.082	-0.048	0.212	YES	YES
25a-e	2.32	0.084	-0.046	0.214	YES	YES
26a-e	4.43	-0.033	-0.163	0.097	YES	YES
29a-e	4.42	0.068	-0.062	0.198	YES	YES
30a-e	6.30	0.130	0.000	0.260	YES	YES
27a-e	6.38	0.164	0.034	0.294	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.105	0.090	YES	+/- 0.500

Figure 24: Accuracy profile for Pet food and animal feed for Easy Plate EC method for E.coli

Matrices used = Dog pate (31-33) cat food with gravy (34-36)

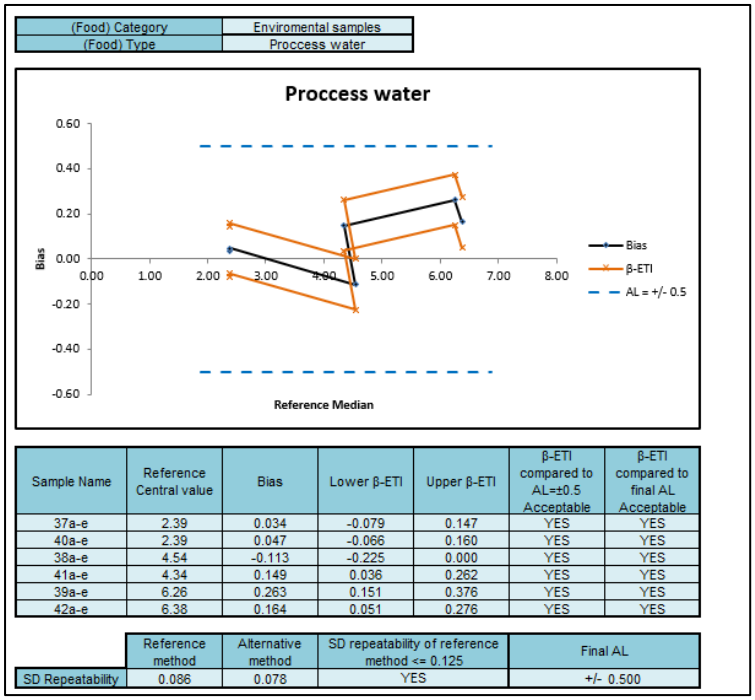
(Food) Category	Pet food and animal feed
(Food) Type	Wet food (cooked)



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
34a-e	2.26	0.222	0.073	0.371	YES	YES
31a-e	2.36	0.350	0.201	0.499	YES	YES
32a-e	4.30	0.267	0.118	0.416	YES	YES
35a-e	4.30	0.146	-0.003	0.295	YES	YES
36a-e	5.58	0.278	0.129	0.426	YES	YES
33a-e	5.76	0.261	0.112	0.410	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.202	0.103	NO	+/- 0.500

Figure 25: Accuracy profile for Environmental samples for Easy Plate EC method for E.coli
Matrices used = wash water (37-39) cooling water (40-42)



(Food) Category	Enviromental samples
(Food) Type	Proccess water

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
37a-e	2.39	0.034	-0.079	0.147	YES	YES
40a-e	2.39	0.047	-0.066	0.160	YES	YES
38a-e	4.54	-0.113	-0.225	0.000	YES	YES
41a-e	4.34	0.149	0.036	0.262	YES	YES
39a-e	6.26	0.263	0.151	0.376	YES	YES
42a-e	6.38	0.164	0.051	0.276	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.086	0.078	YES	+/- 0.500

Five of the seven categories met the AL of 0.5log (dairy, fresh produce, multicomponent foods, meat, petfood and environmental samples). Two categories (dairy products and fish and seafood) required the new AL to be calculated. All data met the new AL values of 0.848 and 1.224.

A high variability was noted on the reference method in the dairy category. This could be explained by the variability in die-off rate of the organisms in the dried product during storage between the samples which could contribute to a greater variation in levels of contamination between the replicates.

A positive bias was observed with pet food samples, which was also noted in the relative truenesss study. One possible explanation could be that for the alternative method the sample is that the alternative method is incubated at 37°C for the full incubation time, rather than the reference method where the majority of the incubation time is at 44°C which could slow the growth of the *E. coli*

3.3.2 Conclusion Accuracy profile study

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

3.3.3 Calculations and interpretation of Accuracy profile study (coliforms)

All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in Figures 26 to 32 for coliforms.

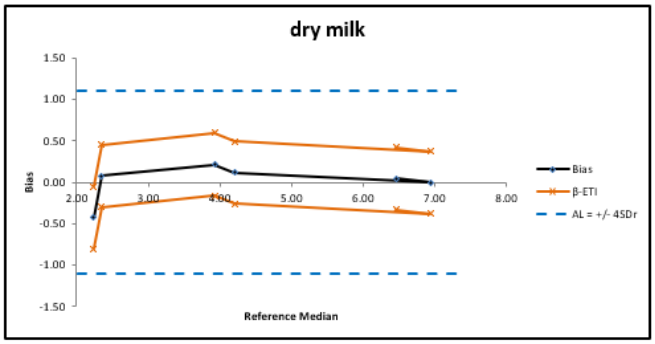
All raw data per category are given in Excel file 2021LR103: tab Accuracy Profile

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

Matrices used = (101-103) and (104-106)

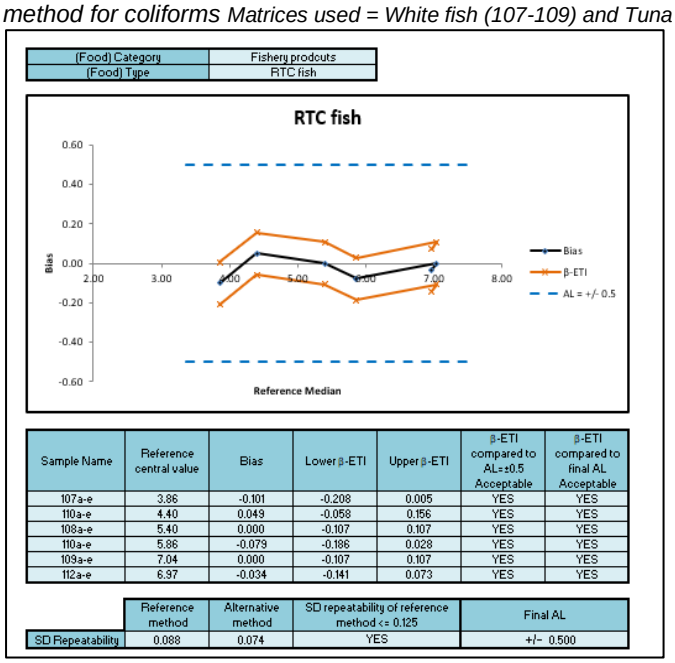
(Food) Category	milk and dairy products
(Food) Type	dry milk



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
101 a-e	2.24	-0.425	-0.802	-0.048	NO	YES
104 a-e	2.35	0.080	-0.297	0.457	YES	YES
#REF!	3.93	0.217	-0.160	0.593	NO	YES
102 a-e	4.20	0.118	-0.259	0.495	YES	YES
103 a-e	6.94	-0.005	-0.382	0.372	YES	YES
106a-e	6.46	0.043	-0.334	0.420	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.277	0.261	NO	+/- 1.108

Figure 27: Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate EC method for coliforms Matrices used = White fish (107-109) and Tuna steaks (110-112)



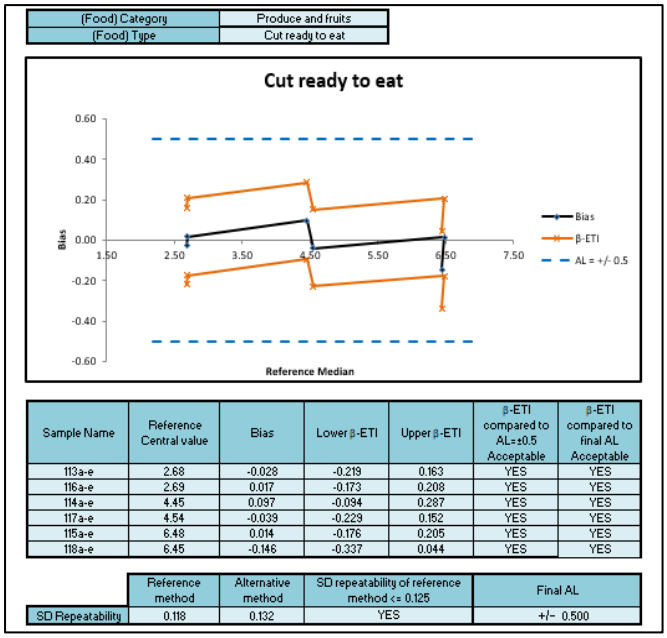
(Food) Category	Fishery prodcuts
(Food) Type	RTC fish

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
107a-e	3.86	-0.101	-0.208	0.005	YES	YES
110a-e	4.40	0.049	-0.058	0.156	YES	YES
108a-e	5.40	0.000	-0.107	0.107	YES	YES
110a-e	5.86	-0.079	-0.186	0.028	YES	YES
109a-e	7.04	0.000	-0.107	0.107	YES	YES
112a-e	6.97	-0.034	-0.141	0.073	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.088	0.074	YES	+/- 0.500

Figure 28 Accuracy profile for Produce and fruits for Easy Plate EC method for coliforms

Matrices used = Lettuce (113-115) and spinach (116-118)

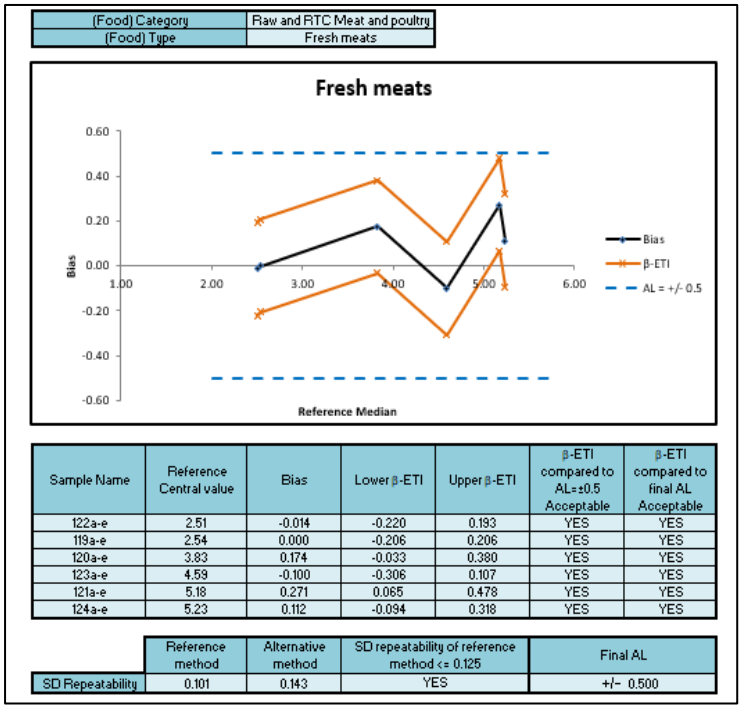


(Food) Category	Produce and fruits
(Food) Type	Cut ready to eat

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
113a-e	2.68	-0.028	-0.219	0.163	YES	YES
116a-e	2.69	0.017	-0.173	0.208	YES	YES
114a-e	4.45	0.097	-0.094	0.287	YES	YES
117a-e	4.54	-0.039	-0.229	0.152	YES	YES
115a-e	6.48	0.014	-0.176	0.205	YES	YES
118a-e	6.45	-0.146	-0.337	0.044	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.118	0.132	YES	+/- 0.500

Figure 29: Accuracy profile for Meat and poultry for Easy Plate EC method for coliforms
Matrices used = Raw Chicken (119-121) and Beef (122-124)

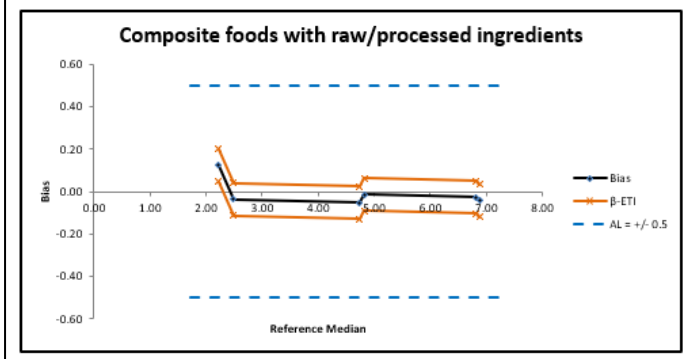


(Food) Category	Raw and RTC Meat and poultry
(Food) Type	Fresh meats

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
122a-e	2.51	-0.014	-0.220	0.193	YES	YES
119a-e	2.54	0.000	-0.206	0.206	YES	YES
120a-e	3.83	0.174	-0.033	0.380	YES	YES
123a-e	4.59	-0.100	-0.306	0.107	YES	YES
121a-e	5.18	0.271	0.065	0.478	YES	YES
124a-e	5.23	0.112	-0.094	0.318	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.101	0.143	YES	+/- 0.500

Figure 30: Accuracy profile for Multicomponent foods for Easy Plate EC method for coliforms
Matrices used = Sandwich (125-127) and Pasta salad (128-130)



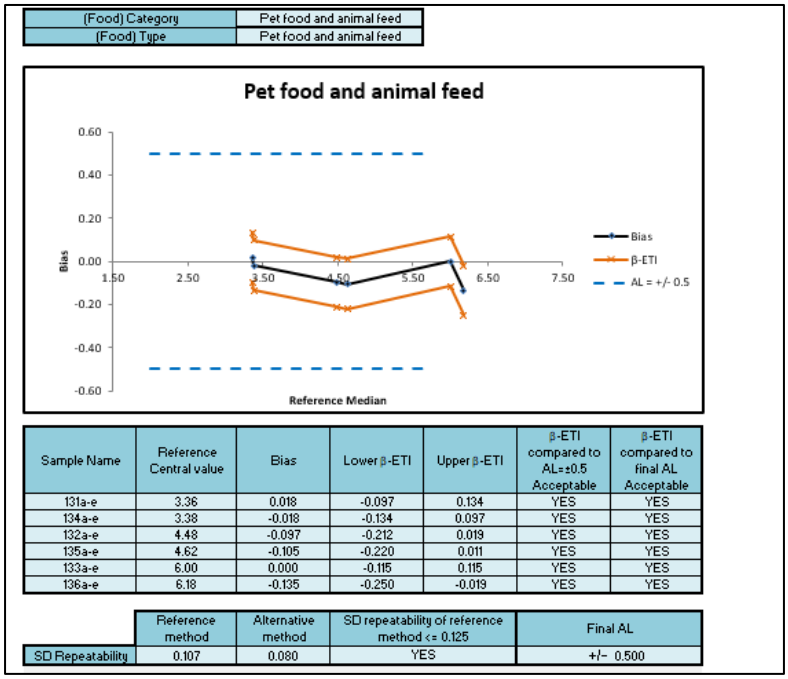
(Food) Category	Multicomponent
(Food) Type	Composite foods with

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
125a-e	2.22	0.125	0.048	0.201	YES	YES
128a-e	2.49	-0.037	-0.113	0.040	YES	YES
126a-e	4.73	-0.051	-0.128	0.025	YES	YES
129a-e	4.82	-0.013	-0.090	0.063	YES	YES
127a-e	6.81	-0.028	-0.104	0.049	YES	YES
130a-e	6.88	-0.043	-0.119	0.034	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.107	0.053	YES	+/- 0.500

Figure 31: Accuracy profile for Pet food and animal feed for Easy Plate EC method for coliforms

Matrices used = Dog pate (131-133) cat food with gravy (134-136)



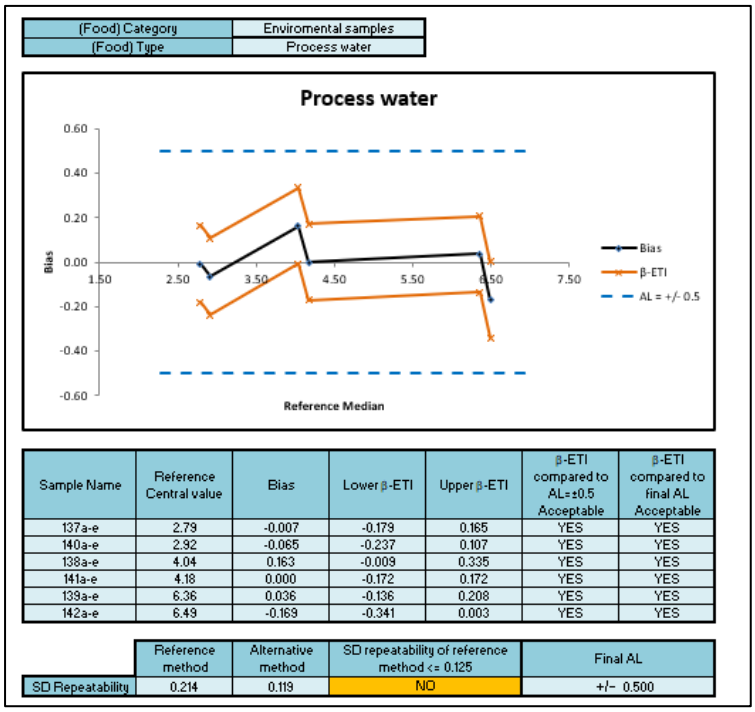
(Food) Category	Pet food and animal feed
(Food) Type	Pet food and animal feed

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
131a-e	3.36	0.018	-0.097	0.134	YES	YES
134a-e	3.38	-0.018	-0.134	0.097	YES	YES
132a-e	4.48	-0.097	-0.212	0.019	YES	YES
135a-e	4.62	-0.105	-0.220	0.011	YES	YES
133a-e	6.00	0.000	-0.115	0.115	YES	YES
136a-e	6.18	-0.135	-0.250	-0.019	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.107	0.080	YES	+/- 0.500

Figure 32: Accuracy profile for Environmental samples for Easy Plate EC method for coliforms

Matrices used = wash water (137-139) cooling water (140-142)



(Food) Category	Enviromental samples
(Food) Type	Process water

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
137a-e	2.79	-0.007	-0.179	0.165	YES	YES
140a-e	2.92	-0.065	-0.237	0.107	YES	YES
138a-e	4.04	0.163	-0.009	0.335	YES	YES
141a-e	4.18	0.000	-0.172	0.172	YES	YES
139a-e	6.36	0.036	-0.136	0.208	YES	YES
142a-e	6.49	-0.169	-0.341	0.003	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.214	0.119	NO	+/- 0.500

Six of the seven categories met the AL of 0.5log (dairy, fresh produce, fish and seafood, multicomponent foods, meat petfood and environmental samples). One categories (dairy products) required the new AL to be calculated. All data met the new AL values of 1.108.

The repeatability for the dairy samples was very high and this was caused by a high level of variability in the milk powder samples. One possible explanation was that the die off in the sample was variable leading to different levels of surviving organisms in the replicates.

3.3.4 Conclusion Accuracy profile study

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

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 *E. coli*:

50 pure cultures of *E. coli* 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 *E. coli*:

A minimum of 30 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.

Inclusivity coliforms:

50 pure cultures of coliforms were tested in this section of the study. Each strain was be grown overnight in a non-selective broth and diluted so that the inoculum level is 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 coliforms:

A minimum of 30 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 2021LR103 Quantitative data file: tabs Inclusivity and Exclusivity.

Inclusivity E. coli

For the inclusivity study, all 50 *E. coli* strains tested gave typical colonies on the alternate method. 49 out of the 50 *E. coli* strains tested gave typical colonies on the reference method.

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

Exclusivity E. coli

For the exclusivity strains, 28 out of the 30 non *E. coli* isolates tested gave the expected result with the reference and alternative methods. Two isolates (*Shigella boydii*, NCTC 11312) and (*Shigella sonnei*, NCTC 10352) gave colonies on both the reference and alternative methods. Further identification work with biochemical identification verified that the isolates were *Shigella* spp and *Shigella sonnei* respectively.

Conclusion

All 50 *E. coli* strains were correctly identified following the alternative method.

28 out of the 30 non *E. coli* isolates tested in the exclusivity panel gave the expected result with the reference and alternative methods.

The alternative method gave comparable performance to the reference method and is therefore selective and specific for *E. coli*.

Inclusivity Coliforms

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), *Klebsiella rhinoscleromatis* (CRA 4272). Three out of these four isolates giving

discrepant results were negative when run through the BGLBB confirmation test had 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.

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

Exclusivity Coliforms

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

For the exclusivity strains, 23 out of the 30 isolates tested gave the expected result with the reference and alternative methods.

One isolate *Shigella sonneii* (NCTC 10352) gave a positive results on both the reference and alternative methods. This strain was positive when subcultured into BGLBB therefore was a coliform and could be excluded from the exclusivity panel.

Six isolates *Shigella boydii* (NCTC 11312) and *Shigella sonneii* (ATCC 25931), *Shigella flexneri* (NCTC 9950), *Vibrio mimicus (*NCIMB 12702*), Vibrio parahaemolyticus* (NCTC 11435) and *Yersinia enterocolitica (*NCTC 11344*)* gave typical colonies on the reference method only, however on confirmation they did not produce gas when grown in BGLBB verifying that they were not coliforms.

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 30 non target strains in the exclusivity panel gave the anticipated result when following 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 EC for enumeration of *E. coli* and coliforms shows satisfactory results for Accuracy profile.
- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms shows satisfactory results for relative trueness
- The alternative method is selective and specific to *E. coli* and the coliform group

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 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.

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 *E.coli* and 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 6a – E. coli stability in the matrix

Day	Reference method cfu/g						Alternative method cfu/g					
	Low level		Medium level		*High level*		Low level		Medium level		*High level*	
	a	b	a	b	a	b	a	b	a	b	a	b
0	936	445	1.10 E+05	1.20 E+05	1.10 E+06	6.80 E+05	1.10 E+03	1.20 E+03	1.00 E+05	1.10 E+05	1.30 E+06	8.90 E+05
6	691	700	5.70 E+04	5.00 E+04	7.30 E+05	7.00 E+05	809	845	9.70 E+04	8.50 E+04	1.20 E+06	7.90 E+05
7	555	764	8.00 E+04	5.80 E+04	8.00 E+05	3.50 E+05	818	627	9.60 E+04	9.00 E+04	1.00 E+06	9.00 E+05

Table 6b - Coliform stability in the matrix

Day	Reference method cfu/g						Alternative method cfu/g					
	Low level		Medium level		*High level*		Low level		Medium level		*High level*	
	a	b	a	b	a	b	a	b	a	b	a	b
0	1.10E +03	1.20 E+03	1.20 E+05	1.10 E+05	1.20 E+06	6.70 E+05	2.10 E+03	1.96 E+03	1.96 E+05	2.20 E+05	2.11 E+06	1.64 E+06
6	809	982	5.60 E+04	6.50 E+04	9.90 E+05	9.60 E+05	1.35 E+03	1.41 E+03	1.29 E+05	1.26 E+05	1.65 E+06	1.30 E+06
7	473	645	6.70 E+04	6.50 E+04	7.40 E+05	5.10 E+05	1.44 E+03	945	1.42 E+05	1.18 E+05	1.50 E+06	1.12 E+06

Logistic conditions

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and 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

Collaborator	Temperature measured by probe (°C)	Receipt date and time	State of the package and samples at receipt	Analysis date
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.

The samples were expected to be delivered chilled and there were 3 participants (5, 7 and 12) that received their parcels above the 8°C tolerance set for the study. Analysis of the results obtained demonstrated that the slight difference in temperature did not impact on the counts obtained they were kept them the calculations

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 8a – Results obtained by the expert lab – E. coli

Level	Reference method per g	Alternative method g
Blank	<10	<10
Low	5.10E+02	6.30E+02
Low	1.30E+02	3.20E+02
Medium	1.00E+04	3.90E+03
Medium	6.20E+03	2.40E+04
High	5.40E+05	9.40E+05
High	3.70E+05	8.30E+05

Table 8b – Results obtained by the expert lab coliforms

Level	Reference method per g	Alternative method g
Blank	<10	<10
Low	6.90E+02	6.50E+02
Low	1.90E+02	3.30E+02
Medium	2.00E+04	1.10E+04
Medium	1.40E+04	2.60E+04
High	7.50E+05	1.00E+06
High	4.20E+05	8.50E+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 9a: Summary of the results of the interlaboratory study per analyte level – E. coli

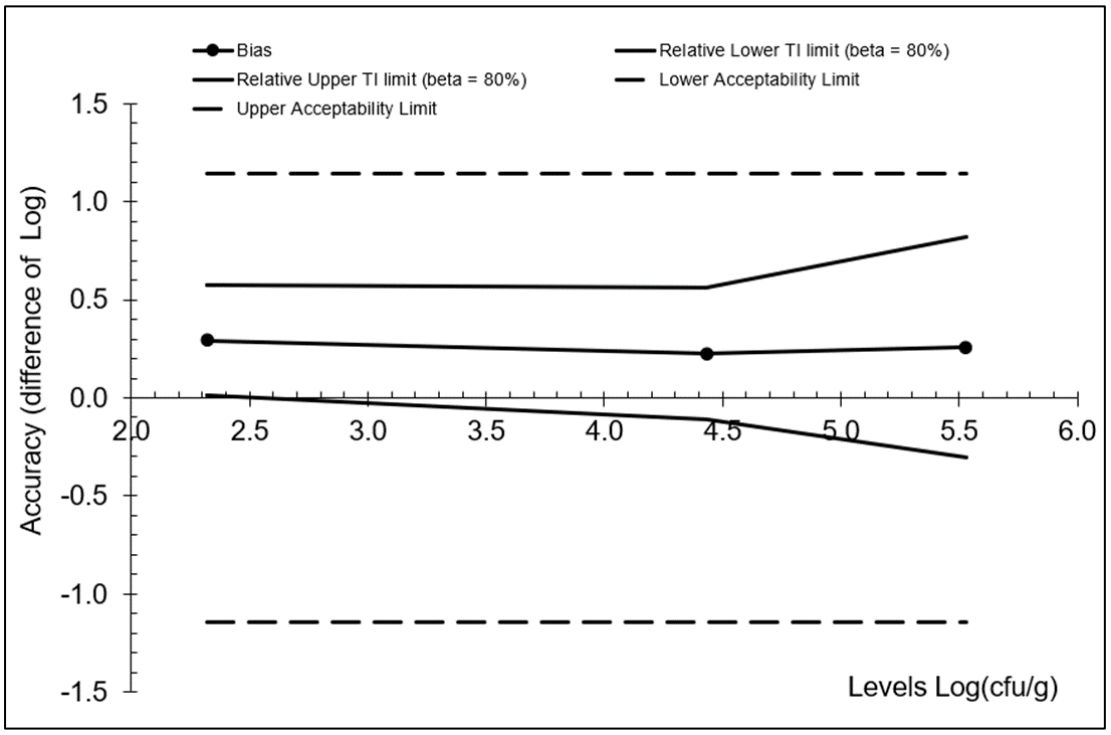
Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	2.5	2.3	2.6	2.4
2	low	2.6	2.3	2.6	2.6
4	low	2.3	2.4	2.8	2.8
5	low	2.5	2.1	2.7	2.7
6	low	2.7	2.4	2.8	2.6
7	low	2.7	2.5	2.8	2.9
9	low	2.0	1.9	2.6	2.3
10	low	2.0	1.3	2.6	2.4
11	low	2.4	2.5	2.8	2.8
12	low	2.5	2.3	2.6	2.4
1	medium	4.3	4.3	4.5	4.6
2	medium	4.3	5.1	4.7	4.7
4	medium	4.2	4.2	4.9	4.8
5	medium	4.3	4.5	4.8	4.9
6	medium	4.6	4.6	4.9	4.9
7	medium	4.6	4.5	4.6	4.8

9	medium	4.4	3.8	4.7	4.4
10	medium	3.9	4.0	4.5	4.5
11	medium	4.4	4.7	4.8	4.7
12	medium	4.3	4.3	4.5	4.6
1	high	5.3	5.5	5.7	5.8
2	high	5.3	5.4	5.6	5.7
4	high	5.6	5.7	6.1	6.1
5	high	5.8	6.3	6.3	6.2
6	high	5.9	6.3	6.1	6.2
7	high	5.7	5.6	5.9	5.8
8	high	5.3	5.1	4.7	5.1
9	high	5.1	5.2	5.7	5.7
10	high	5.2	5.3	5.7	5.5
11	high	5.3	5.6	5.9	5.9
12	high	5.3	5.5	5.7	5.8
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	

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

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	2.6	2.6
4	low	2.6	2.4	2.9	2.9
5	low	2.4	2.4	2.8	2.8
6	low	2.7	2.6	2.9	2.7
7	low	3.4	2.4	2.8	2.9
9	low	2.3	2.0	2.7	2.5
10	low	2.2	1.5	2.6	2.6
11	low	2.4	2.6	2.8	2.8
12	low	2.5	2.0	2.7	2.4
1	medium	4.5	4.4	4.7	4.7
2	medium	4.3	4.4	4.8	4.7
4	medium	4.5	4.4	4.0	4.9
5	medium	4.5	4.7	4.8	5.0
6	medium	4.7	4.7	5.0	5.0
7	medium	4.4	4.5	4.7	4.8
9	medium	4.3	4.1	4.7	4.4
10	medium	4.0	4.3	4.6	4.7
11	medium	4.6	4.8	4.9	4.8
12	medium	4.5	4.4	4.7	4.7
1	high	5.6	5.6	5.8	5.9
2	high	5.8	5.6	5.6	5.7
4	high	5.9	5.9	6.1	6.2
5	high	6.0	6.4	6.4	6.3
6	high	6.4	6.0	6.1	6.2
7	high	5.7	5.6	6.0	5.8
9	high	5.3	5.3	5.7	5.7
10	high	5.4	5.3	5.7	5.6
11	high	5.5	5.8	6.0	6.0
12	high	5.6	5.6	5.8	5.9
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 13a. Accuracy profile for Easy Plate EC (*E. coli*) from the ILS



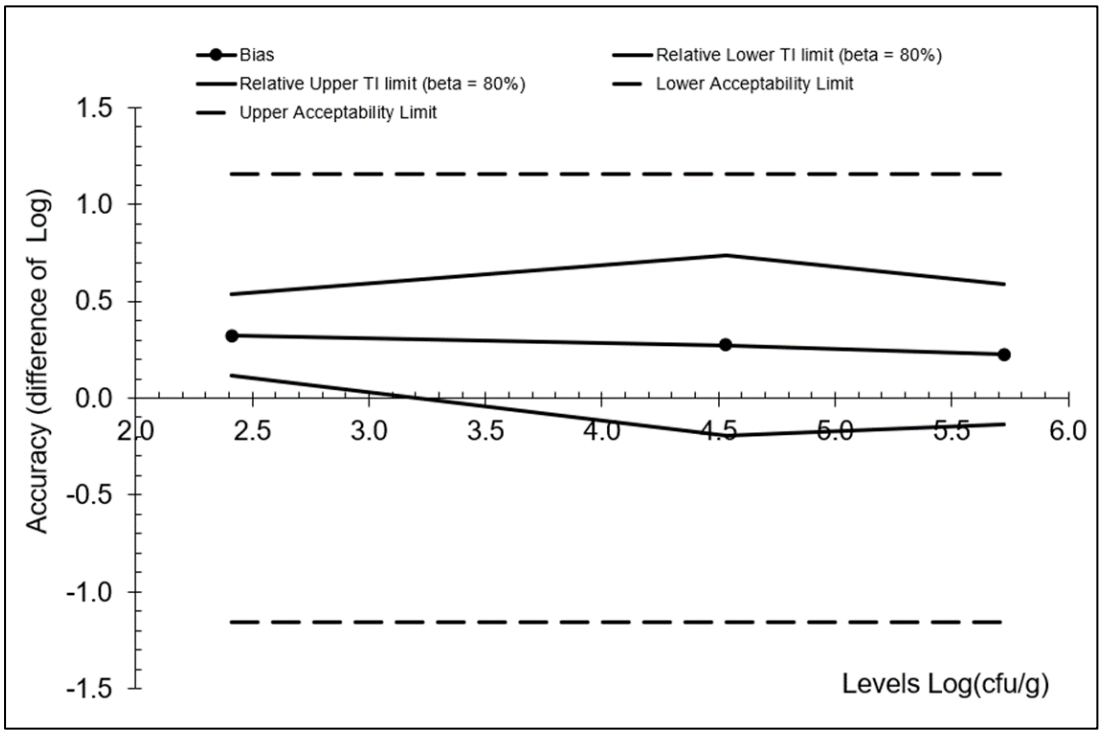
A review of the accuracy profile and statistical analysis revealed that there was a positive bias of 0.3 observed for all levels of contamination in the ILS samples. A root cause analysis was carried out to determine possible reasons for this.

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 melted and tempered 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 involve a pour plate where colonies growing in the agar could be exposed to more inhibitory compound with the agar.

A positive bias of approximately 0.2 was also noted in accuracy profile part of the study for fishery products.

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 medium level samples for the reference method which resulted in the recalculated AL of 1.14log

Figure 13b. Accuracy profile for Easy Plate EC (coliforms) from the ILS



A review of the accuracy profile and statistical analysis revealed that there was a positive bias of 0.3 observed for all levels 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.

No bias was also noted in the accuracy profile part of the study for fishery products. In the relative trueness a slight negative bias of -0.128 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 1.15log.

Table 10a. Statistical analysis of the ILS data according to the ISO spreadsheet Easy Plate EC (E. coli)

Accuracy profile			
Study Name	Free text		
Date	Free text		
Coordinator	Free text		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	1.14	1.14	1.14

TRUE

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.

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	2.321	4.431	5.529			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	2.614	4.657	5.784	2.321	4.431	5.529
Repeatability standard deviation (sr)	0.109	0.108	0.101	0.236	0.319	0.166
Between-labs standard deviation (sL)	0.168	0.208	0.376	0.238	0.168	0.300
Reproducibility standard deviation (sR)	0.200	0.235	0.390	0.335	0.361	0.343
Corrected number of dof	12.071	11.127	9.628	14.478	17.716	11.377
Coverage factor	1.412	1.422	1.441			
Interpolated Student t	1.356	1.363	1.376			
Tolerance interval standard deviation	0.2088	0.2452	0.4081			
Lower TI limit	2.331	4.323	5.223			
Upper TI limit	2.897	4.991	6.346			
Bias	**0.293**	**0.227**	**0.255**			
Relative Lower TI limit (beta = 80%)	**0.010**	**-0.108**	**-0.306**			
Relative Upper TI limit (beta = 80%)	**0.576**	**0.561**	**0.817**			
Lower Acceptability Limit	**-1.14**	**-1.14**	**-1.14**			
Upper Acceptability Limit	**1.14**	**1.14**	**1.14**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.347					

FALSE
TRUE

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

Table 10b. Statistical analysis of the ILS data according to the ISO spreadsheet Easy Plate EC (coliforms)

Accuracy profile			
Study Name	Free text		
Date	Free text		
Coordinator	Free text		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	1.15	1.15	1.15

TRUE

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.

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	2.410	4.531	5.725			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	2.735	4.803	5.951	2.410	4.531	5.725
Repeatability standard deviation (sr)	0.090	0.304	0.078	0.333	0.214	0.153
Between-labs standard deviation (sL)	0.119	0.149	0.239	0.184	0.270	0.284
Reproducibility standard deviation (sR)	0.149	0.338	0.252	0.381	0.344	0.322
Corrected number of dof	12.834	17.914	9.913	17.561	13.157	11.268
Coverage factor	1.405	1.370	1.437			
Interpolated Student t	1.351	1.331	1.373			
Tolerance interval standard deviation	0.1553	0.3481	0.2633			
Lower TI limit	2.525	4.340	5.589			
Upper TI limit	2.945	5.266	6.312			
Bias	**0.325**	**0.271**	**0.225**			
Relative Lower TI limit (beta = 80%)	**0.116**	**-0.192**	**-0.136**			
Relative Upper TI limit (beta = 80%)	**0.535**	**0.735**	**0.587**			
Lower Acceptability Limit	**-1.15**	**-1.15**	**-1.15**			
Upper Acceptability Limit	**1.15**	**1.15**	**1.15**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.350					

FALSE
TRUE

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

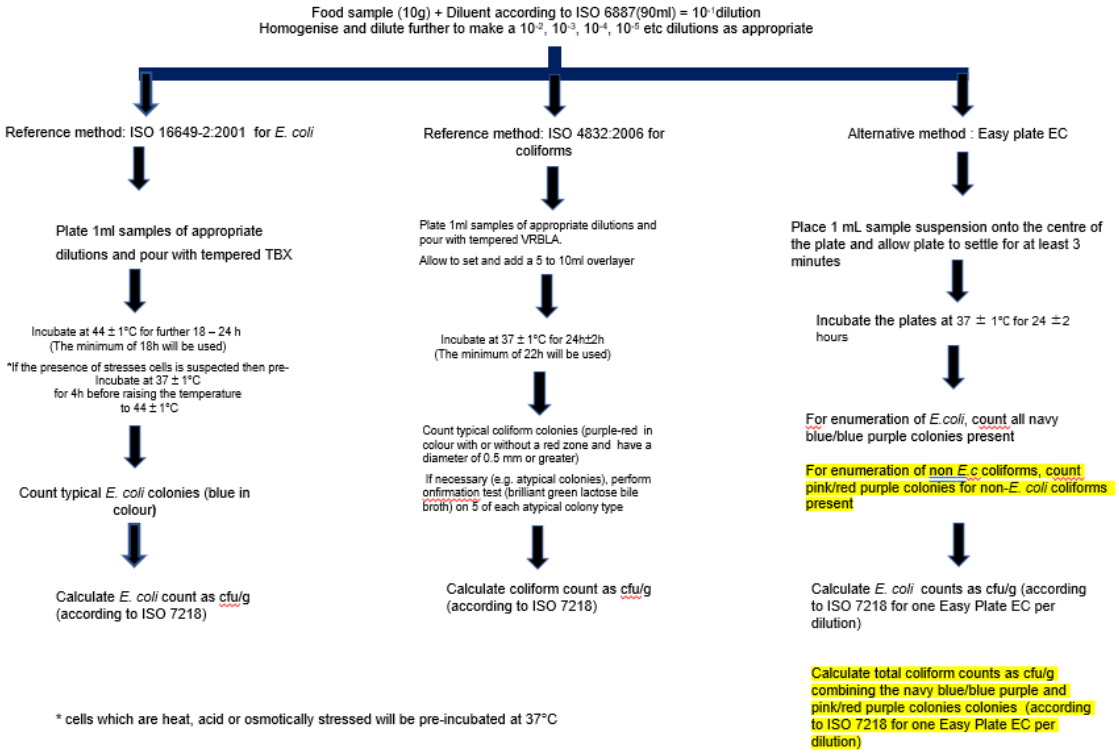
5 Overall conclusions of the MCS/ILS study

- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms shows satisfactory results for relative trueness.
- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms shows satisfactory results for accuracy profile.
- The alternative method Easy Plate EC for enumeration of *E. coli* and coliforms is selective and specific.
- The alternative method Easy Plate EC for enumeration of *E. coli*, and coliforms shows satisfactory performance in the ILS.
- The alternative method Easy Plate EC for enumeration of *E. coli* shows comparable performance to the reference method ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide
- The alternative method Easy Plate EC 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 04/07/23

Signature Suzanne Jordan

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



* cells which are heat, acid or osmotically stressed will be pre-incubated at 37°C

7 ANNEX B: Kit insert

Please refer to separate pdf document

8 ANNEX C: Raw data per category – screening of samples

Sample screening data for RT study

Sample number	**Item**	Reference method ISO 16649-2:2001		Alternative method Easy Plate EC E. coli	
		Count -1	Result cfu	Count -1	Result cfu per g
1	Raw milk	**0**	**<10**	**0**	**<10**
2	Raw salmon filet	**0**	**<10**	**0**	**<10**
3	Smoked salmon	**0**	**<10**	**0**	**<10**
4	Frozen seafood	**0**	**<10**	**0**	**<10**
5	Semi-dried fish	**0**	**<10**	**0**	**<10**
6	Cooked Shrimp	**0**	**<10**	**0**	**<10**
7	Crab meat	**0**	**<10**	**0**	**<10**
8	Bagged pre-cut lettuce	**0**	**<10**	**0**	**<10**
9	Shredded carrot	**0**	**<10**	**0**	**<10**
10	Chicken Sausages	**0**	**<10**	**0**	**<10**
11	Salami	**0**	**<10**	**0**	**<10**
12	Smoked salmon	**0**	**<10**	**0**	**<10**
14	Raw milk cheese	**0**	**<10**	**0**	**<10**
15	Tuna chunks	**0**	**<10**	**0**	**<10**
16	RTC Rice	**0**	**<10**	**0**	**<10**
17	Vegetable salad	**0**	**<10**	**0**	**<10**

Annex C: Calculations and interpretation of relative trueness – E. coli

Type	Code	log(Ref)	log(Alt)	Mean	Difference
Dusts					
Industrial kitchen sink corner	G11	2.464	2.505	2.485	0.041
Radiator	G12	3.531	3.653	3.592	0.122
Oven top	G13	5.544	5.431	5.488	-0.113
Base of equipment	G14	5.398	5.491	5.445	0.093
Bakery sink corner	G15	6.415	6.690	6.553	0.275
Process water					
Cooling water	G10	6.398	6.447	6.423	0.049
Wash water	G6	2.362	2.519	2.440	0.157
Bakery prep area	G7	3.041	3.462	3.252	0.421
Surface run off - industrial kitchen	G8	3.959	4.544	4.252	0.585
High pressure cooling water	G9	4.792	5.568	5.180	0.776
Surfaces (wipes, swabs)					
Metal - industrial kitchen sink	G1	2.592	2.653	2.623	0.061
Ceramic - sink splashback	G2	3.447	3.690	3.569	0.243
Rubber - equipment piping	G3	4.398	4.491	4.445	0.093
Plastic - equipment surface	G4	5.580	5.653	5.616	0.073
MDF - lab bench	G5	6.255	6.342	6.299	0.087
Crustaceans					
prawn cocktail	B11	2.431	2.255	2.343	-0.176
pickled cockles	B12	3.477	3.301	3.389	-0.176
Lemon & Garlic King Prawns	B13	4.230	4.531	4.381	0.301
Big & Juicy Crayfish Tails	B14	5.531	5.580	5.556	0.048
Chilli & Lime King Prawns	B15	6.079	6.431	6.255	0.352
Raw fish (unprocessed)					
Diced salmon fillets	B1	2.613	2.699	2.656	0.086
Tesco Cod loin	B2	3.763	3.699	3.731	-0.064
skinless basa fillets	B3	4.544	4.613	4.578	0.069
Wild Salmon Tesco	B4	5.556	5.643	5.600	0.087
Cornish hake	B5	6.556	6.672	6.614	0.116
RTE/RTC/RTRH fish and seafoods					
Frozen Tuna steaks	B10	6.301	6.398	6.349	0.097
smoked haddock loin	B6	2.531	2.342	2.437	-0.189
Honey Roast Salmon Flakes	B7	3.301	3.398	3.349	0.097
smoked mackerel fillets	B8	4.301	4.477	4.389	0.176
Herring Creamy Sauce	B9	5.279	5.462	5.371	0.184

Dry milk products					
Milk powder	A11	2.872	2.792	2.832	-0.080
dried skimmed milk	A12	4.204	4.204	4.204	0.000
Cake mix	A13	4.505	4.505	4.505	0.000
Pancake mix	A14	5.322	5.255	5.289	-0.067
Skimmed milk powder (less than 1% fat)	A15	6.380	6.505	6.443	0.125
Pasteurised milk and dairy products					
Skimmed milk	A10	5.663	5.806	5.734	0.143
whole milk	A6	6.544	6.748	6.646	0.204
Protein shake strawberry	A7	2.591	2.362	2.476	-0.229
Double cream	A8	3.756	3.415	3.585	-0.341
Vanilla ice cream	A9	4.690	4.230	4.460	-0.460
Raw milk and dairy products					
Raw milk (AMP)	A1	2.628	2.708	2.668	0.079
Raw milk (straightfromthecow)	A2	3.623	3.491	3.557	-0.132
Raw milk cheese 1	A3	4.591	4.681	4.636	0.090
Raw milk cheese 2	A4	5.531	5.580	5.556	0.048
No.1 Mountain Comté AOP	A5	6.681	6.708	6.694	0.026
Composite foods with substantial raw ingredients					
Fire pit triple grain salad	D1	3.146	3.176	3.161	0.030
prawn layered salad	D2	3.699	4.279	3.989	0.580
cheese spring onion sandwich	D3	4.991	5.342	5.167	0.351
Egg mayo sandwich	D4	6.204	6.362	6.283	0.158
egg and cress sandwich	D5	7.079	7.176	7.128	0.097
Mayonnaise based deli-salads					
Coleslaw with real mayo	D11	1.845	1.778	1.812	-0.067
Potato salad with mayo	D12	2.190	2.415	2.303	0.225
cheese coleslaw with mayo	D13	3.813	3.845	3.829	0.032
Egg mayonnaise	D14	4.653	4.820	4.736	0.166
Chicken, tomato and basil pasta with egg yolk and pasteurized egg mayo	D15	4.230	4.903	4.567	0.673
RTRH/RTE foods (chilled, frozen)					
Spinach and tomato quiche	D10	3.903	4.146	4.025	0.243
chicken tomato & basil pasta	D6	2.380	2.613	2.496	0.233
curry cod fillet & chips	D7	3.204	3.462	3.333	0.258
Roasted Vegetable Couscous	D8	4.398	4.519	4.458	0.121
Chicken Curry	D9	3.114	3.176	3.145	0.062
Animal feeds (poultry and fish)					
Wheatgerm sticks Food for all pond fish	F11	4.544	4.398	4.471	-0.146

King British Tropical fish mini pellets	F12	2.498	2.146	2.322	-0.352
Bug bites goldfish formula	F13	3.398	3.041	3.220	-0.357
Mixed corn	F14	5.681	5.699	5.690	0.018
Layers mash	F15	6.477	6.623	6.550	0.146
Dry Food					
dreamies cat treats	F1	1.978	2.362	2.170	0.384
Grain free Salmon with sweet potato minibites	F2	2.021	1.477	1.749	-0.544
perfect for cats duck treats	F3	4.732	4.826	4.779	0.094
mature dog biscuit bones	F4	5.699	5.833	5.766	0.134
chicken kitten food	F5	6.672	6.826	6.749	0.154
Wet food (raw and canned)					
dog food lamb supper	F10	5.398	5.699	5.548	0.301
gourmet cat food - mousse salmon& gravy	F6	5.863	5.833	5.848	-0.031
Adult dog food - smooth pate with chicken	F7	2.423	2.699	2.561	0.276
Tuna fillet natural cat food	F8	3.623	3.663	3.643	0.040
dog food Beef Supper with carrots & peas	F9	4.342	4.785	4.564	0.443
Cut ready-to-eat vegetables/leafy greens and sprouts					
Tesco Chilli & Lime King Prawns	C1	2.431	2.362	2.397	-0.070
shredded iceberg lettuce	C2	3.176	3.079	3.128	-0.097
sliced Carrot	C3	4.415	4.362	4.388	-0.053
Morrisons Green beans	C4	5.519	5.204	5.361	-0.314
Sprouted salad topper	C5	6.279	6.204	6.241	-0.075
Fresh fruit/Cut RTE fruit and vegetable products					
Super Blue (non heat treated, cold pressed) smoothie	C10	6.653	6.041	6.347	-0.612
Mango	C6	2.856	2.716	2.786	-0.140
Watermelon chunks	C7	3.908	3.699	3.804	-0.210
smooth freshly squeezed orange juice	C8	4.833	4.079	4.456	-0.753
super green (non heat treated, cold pressed) smoothie	C9	5.732	5.708	5.720	-0.025
Heat treated fruit and vegetables					
Wonder Green Juice	C11	1.845	2.000	1.923	0.155
Cranberry juice drink (heat treated) juice	C12	2.422	2.771	2.596	0.349
Juice burst orange (heat treated) juice	C13	3.934	3.940	3.937	0.005
V8 original vegetable juice (heat treated) smoothie	C14	4.982	5.079	5.031	0.097
Brilliant Beetroot Juice (heat treated) smoothie	C15	5.799	6.000	5.900	0.201
Raw poultry and meat cuts					
chicken meat	E1	6.255	6.544	6.400	0.289

diced beef	E2	2.398	2.813	2.605	0.415
pork loin chops	E3	3.431	3.806	3.619	0.375
Turkey mini fillet	E4	4.415	4.740	4.578	0.325
Swab taken from chicken	E5	5.477	5.813	5.645	0.336
Raw processed meat					
Raw diced beef 500g	E10	5.602	5.462	5.532	-0.140
Fire Pit 6 Beef Burgers	E6	6.491	6.462	6.477	-0.029
mini pork meatballs	E7	2.477	2.613	2.545	0.136
seasoned diced pork	E8	3.491	3.914	3.703	0.422
mince beef	E9	4.146	4.568	4.357	0.422
RTC processed poultry					
Fire pit piri piri chicken steaks	E11	3.431	3.415	3.423	-0.016
Southern chicken goujons	E12	4.477	4.255	4.366	-0.222
Spiced poultry	E13	2.756	2.602	2.679	-0.154
Turkey burgers	E14	4.230	4.279	4.255	0.048
Turkey escolopes ham and cheese	E15	4.724	4.041	4.383	-0.683

Annex C: Calculations and interpretation of relative trueness – coliforms

Item	Sample number	log(Ref)	log(Alt)	Mean	Difference
Dusts					
Industrial kitchen sink corner	N11	2.356	2.653	2.505	0.297
Radiator	N12	3.230	3.204	3.217	-0.026
Oven top	N13	4.146	3.845	3.996	-0.301
Base of equipment	N14	5.079	4.820	4.949	-0.260
Bakery sink corner	N15	6.362	5.851	6.106	-0.510
Process water					
High pressure cooling water	N10	7.041	6.839	6.940	-0.203
Cooling water	N6	3.079	2.771	2.925	-0.308
Wash water	N7	3.851	3.792	3.822	-0.059
Bakery prep area	N8	4.771	4.690	4.731	-0.081
Surface run off - industrial kitchen	N9	5.875	5.820	5.847	-0.056
Surfaces (wipes, swabs)					
Metal - industrial kitchen sink	N1	1.826	2.000	1.913	0.174
Ceramic - sink splashback	N2	2.582	3.146	2.864	0.564
Rubber - equipment piping	N3	2.602	2.892	2.747	0.290
Plastic - equipment surface	N4	4.531	5.146	4.839	0.615
MDF - lab bench	N5	7.176	7.146	7.161	-0.030
Crustaceans					
prawn cocktail	i11	2.342	2.342	2.342	0.000
brown shrimp	i12	3.342	3.230	3.286	-0.112
Lemon & Garlic King Prawns	i13	4.255	4.398	4.327	0.143
Big & Juicy Crayfish Tails	i14	5.279	5.255	5.267	-0.023
Chilli & Lime King Prawns	i15	6.000	6.301	6.151	0.301
Raw fish (unprocessed)					
Diced salmon fillets	i1	2.796	2.602	2.699	-0.194
Cod loin	i2	4.756	4.322	4.539	-0.434
skinless basa fillets	i3	6.204	5.519	5.861	-0.686
Raw Tiger Prawns (new)	i4	4.230	4.342	4.286	0.112
cornish hake	i5	5.740	5.544	5.642	-0.196
RTE/RTC/RTRH fish and seafoods					
Frozen Tuna steaks	i10	5.279	5.230	5.255	-0.048
smoked haddock loin	i6	2.695	2.505	2.600	-0.189
Honey Roast Salmon Flakes	i7	2.526	2.041	2.284	-0.485
Ready to eat smoked mackerel fillets	i8	3.544	3.431	3.488	-0.113
Lisner Herring Creamy Sauce	i9	4.301	4.301	4.301	0.000

					Dry milk products
original instant custard	H11	2.201	2.204	2.203	0.003
Pancake mix	H12	4.491	4.613	4.552	0.121
Dessert powder	H13	2.477	2.362	2.419	-0.115
Scone powder	H14	6.230	6.398	6.314	0.167
Instant Custard Powder 72g	H15	6.813	6.813	6.813	0.000
Raw milk and dairy products					
Raw milk (AMP)	H1	2.431	2.398	2.415	-0.033
Raw milk (straightfromthecow)	H2	3.176	3.398	3.287	0.222
Raw milk cheese 1	H3	4.342	4.633	4.488	0.291
Raw milk cheese 2	H4	5.041	5.041	5.041	0.000
Raw milk cheese 3 (No.1 Mountain Comté AOP)	H5	6.279	6.398	6.338	0.119
Dry milk					
cream cheese 'n' prawns	H6	2.389	2.556	2.473	0.167
Protein shake strawberry	H7	3.301	3.398	3.349	0.097
Double cream	H8	4.114	4.505	4.310	0.391
(Vanilla) ice cream	H9	4.869	5.146	5.008	0.277
Skimmed milk	H10	6.079	6.362	6.220	0.283
Composite foods with substantial raw ingredients					
Fire pit triple grain salad	K1	2.865	3.230	3.047	0.366
prawn layered salad	K2	3.851	4.176	4.014	0.325
first Fresh foods wolves, cheese spring onion sandwich	K3	4.653	5.230	4.942	0.577
Egg mayo sandwich	K4	5.724	6.114	5.919	0.390
Egg and cress sandwich	K5	6.813	7.230	7.022	0.418
Mayonnaise based deli-salads					
Coleslaw with real mayo	K11	2.190	2.146	2.168	-0.044
Potato salad with mayo, tesco brand	K12	3.146	3.230	3.188	0.084
cheese colesaw with mayo	K13	4.230	4.279	4.255	0.048
Egg mayonnaise	K14	5.398	5.362	5.380	-0.036
chicken, tomato and basil pasta with egg yolk and pasteurized egg mayo	K15	6.477	6.362	6.419	-0.115
RTRH/RTE foods (chilled, frozen)					
spinach and tomato quiche	K10	6.362	6.690	6.526	0.328
chicken tomato & basil pasta	K6	2.525	2.491	2.508	-0.034
cod fillet & chips	K7	3.826	3.954	3.890	0.128
Roasted Vegetable Couscous	K8	4.431	4.415	4.423	-0.016
Savers Chicken Curry	K9	5.431	5.633	5.532	0.202
Animal feeds (poultry and fish)					
sticks Food for all pond fish	M11	2.813	2.799	2.806	-0.014

King British Tropical fish mini pellets	M12	3.869	3.806	3.838	-0.063
Bug bites goldfish formula	M13	4.813	4.839	4.826	0.026
Mixed corn	M14	5.633	5.708	5.671	0.074
Layers mash	M15	6.732	6.851	6.792	0.119
Dry Food					
Tuna dreamies cat treats	M1	2.677	2.255	2.466	-0.421
Grain free Salmon with sweet potato minibites	M2	2.820	2.491	2.655	-0.328
kitty's scrummy treats snack pockets with turkey, beef and duck	M3	4.748	4.785	4.767	0.037
mature dog biscuit bones	M4	5.255	5.380	5.318	0.125
Pets at home chicken kitten food	M5	6.398	6.724	6.561	0.326
Wet food (raw and canned)					
Lily's kitchen dog food lamb supper	M10	6.322	6.255	6.289	-0.067
gourmet cat food - mousse with salmon&cascading gravy	M6	2.515	2.531	2.523	0.017
Adult dog food - smooth pate with chicken	M7	3.477	3.362	3.419	-0.115
Tuna fillet natural cat food	M8	4.462	4.362	4.412	-0.101
dog food Beef Supper with carrots & peas	M9	5.613	5.477	5.545	-0.136
Cut ready-to-eat vegetables/leafy greens and sprouts					
Sweet leaf salad	J1	5.255	5.041	5.148	-0.214
Tesco sliced Carrot	J2	2.863	2.415	2.639	-0.448
Sweet beets and little leaves	J3	4.699	4.690	4.695	-0.009
Good 4 U | Prouted salad topper	J4	7.732	7.732	7.732	0.000
Spinach, watercress & rocket salad	J5	4.778	4.398	4.588	-0.380
Fresh fruit/Cut RTE fruit and vegetable products					
Super Blue (non heat treated, cold pressed) smoothie	J10	4.230	4.748	4.489	0.518
Mango	J6	3.544	3.230	3.387	-0.314
Watermelon chunks	J7	5.716	5.792	5.754	0.076
smooth freshly squeezed orange juice	J8	3.079	3.672	3.376	0.593
super green (non heat treated, cold pressed) smoothie	J9	5.041	5.079	5.060	0.038
Heat treated fruit and vegetables					
Wonder Green Juice	J11	1.845	2.041	1.943	0.196
Cranberry juice drink (heat treated) juice	J12	4.041	3.886	3.964	-0.155
Juice burst orange (heat treated) juice	J13	4.114	4.477	4.296	0.363
V8 original vegetable juice (heat treated) smoothie	J14	5.176	5.740	5.458	0.564
Beetroot Juice (heat treated) smoothie	J15	6.447	6.748	6.598	0.301
Raw poultry and meat cuts					
chicken meat	L1	6.398	6.556	6.477	0.158
diced beef	L2	2.538	2.342	2.440	-0.195

pork loin chops	L3	3.322	3.204	3.263	-0.118
Turkey minifillet,	L4	4.462	4.415	4.439	-0.047
Swab taken from chicken	L5	5.362	5.380	5.371	0.018
Raw processed meat					
Raw diced beef 500g t	L10	7.279	6.869	7.074	-0.410
Fire Pit 6 Beef Burgers	L6	2.555	2.672	2.614	0.117
mini pork meatballs	L7	4.230	3.699	3.965	-0.531
seasoned diced pork	L8	6.146	5.531	5.839	-0.615
Bag of mince beef	L9	4.041	3.380	3.711	-0.661
RTC processed poultry					
Fire pit piri piri chicken steaks	L11	2.562	2.519	2.540	-0.044
Southern chicken goujons	L12	3.544	3.447	3.496	-0.097
Spiced poultry	L13	4.447	4.591	4.519	0.144
Turkey burgers	L14	5.531	5.663	5.597	0.131
Turkey escalope ham and cheese	L15	6.544	6.653	6.599	0.109

9 Annex D: Summary tables Accuracy profile study

Milk and dairy products (combined category raw and heat processed Milk and dairy products) *E. coli*

(Food) Category 2		Milk and dairy										
(Food) Type 2		Dry powders										
			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
2 a-e	Milk powder	low	2.50E+03	2.90E+03	9.10E+03	1.60E+03	2.70E+03	2.60E+03	2.80E+03	8.90E+03	1.70E+03	2.60E+03
5 a-e	Dessert powder	low	7.80E+03	5.30E+03	1.90E+03	2.00E+03	1.60E+03	7.50E+03	4.50E+03	1.70E+03	2.60E+03	1.40E+03
1a-e	Milk powder	interm ediate	4.50E+04	3.20E+04	2.40E+04	2.00E+04	6.50E+04	5.40E+04	3.70E+04	5.00E+04	4.50E+04	5.00E+04
4 a-e	Dessert powder	interm ediate	6.80E+04	3.60E+04	4.60E+04	6.10E+04	5.90E+04	7.30E+04	6.70E+04	5.90E+04	6.20E+04	5.20E+04
3 a-e	Milk powder	high	8.80E+06	1.50E+07	2.30E+07	1.40E+06	3.00E+06	8.50E+06	1.60E+07	2.30E+07	1.70E+06	2.80E+06
6 a-e	Dessert powder	high	4.80E+06	2.50E+06	6.20E+06	1.80E+06	8.80E+06	5.00E+06	4.40E+06	4.90E+06	2.30E+06	8.30E+06

Fishery products combined category: raw, RTE RTRH, RTC *E. coli*

(Food) Category 2		Fishery										
(Food) Type 2		RTC fish										
			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
7a-e	Tuna	low	2.E+02	1.E+02	9.E+01	1.E+02	8.E+01	2.E+02	3.E+02	2.E+02	3.E+02	2.E+02
10a-e	white fish	low	4.E+01	3.E+01	2.E+02	2.E+02	2.E+02	1.E+02	5.E+01	4.E+02	3.E+02	2.E+02
11a-e	white fish	interm ediate	2.5E+03	1.8E+03	2.6E+03	1.4E+03	1.5E+03	2.6E+03	2.5E+03	2.7E+03	2.8E+03	2.6E+03
8a-e	Tuna	interm ediate	2.5E+03	1.6E+03	2.5E+03	2.6E+03	2.2E+03	3.3E+03	2.6E+03	5.0E+03	4.0E+03	3.2E+03
12a-e	white fish	high	1.4E+05	1.5E+05	1.7E+05	7.0E+04	8.3E+04	2.3E+05	2.5E+05	3.5E+05	1.7E+04	1.4E+05
9a-e	Tuna	high	4.7E+05	4.3E+05	2.5E+05	1.3E+05	3.1E+05	7.7E+05	7.2E+05	4.7E+05	2.4E+05	3.6E+05

Produce and fruits (combined category fresh and processed) *E. coli*

(Food) Category 8		Produce and fruits										
(Food) Type 8		Cut ready to eat										
			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
13a-e	Lettuce	low	1.80E+02	2.80E+02	2.10E+02	2.10E+02	2.55E+02	2.85E+02	2.45E+02	2.45E+02	1.85E+02	3.30E+02
16a-e	Spinach	low	2.30E+02	1.85E+02	2.25E+02	2.65E+02	1.90E+02	3.40E+02	2.30E+02	3.55E+02	2.65E+02	2.40E+02
14a-e	Lettuce	interm ediate	2.00E+04	2.30E+04	2.40E+04	1.80E+04	2.40E+04	3.00E+04	2.90E+04	2.40E+04	2.70E+04	2.90E+04
17a-e	Spinach	interm ediate	4.60E+04	5.00E+04	5.30E+04	7.20E+04	6.50E+04	6.30E+04	4.90E+04	6.40E+04	7.60E+04	5.60E+04
15a-e	Lettuce	high	3.80E+06	3.50E+06	4.40E+06	5.50E+06	3.70E+06	3.50E+06	3.40E+06	4.20E+06	5.20E+06	4.40E+06
18a-e	Spinach	high	2.50E+06	2.20E+06	2.70E+06	3.20E+06	4.40E+06	2.00E+06	3.70E+06	3.90E+06	2.90E+06	3.60E+06

Multi-component foods or meal components *E. coli*

(Food) Category 5		Multicomponent										
(Food) Type 5		Multicomponent foods										
			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
28a-e	Pasta salad	low	1.85E+02	2.65E+02	2.25E+02	2.50E+02	2.40E+02	3.10E+02	2.90E+02	2.35E+02	3.15E+02	2.30E+02
25a-e	Sandwhich	low	2.10E+02	1.60E+02	2.90E+02	1.95E+02	2.25E+02	2.10E+02	2.75E+02	2.65E+02	2.45E+02	2.55E+02
26a-e	Sandwhich	interm ediate	2.90E+04	2.70E+04	2.30E+04	1.80E+04	3.20E+04	2.20E+04	2.50E+04	3.90E+04	3.10E+04	2.40E+04
29a-e	Pasta salad	interm ediate	4.40E+04	2.65E+04	1.30E+04	2.60E+04	2.70E+04	3.10E+04	3.80E+04	2.30E+04	4.10E+04	2.50E+04
30a-e	Pasta salad	high	1.60E+06	2.00E+06	2.20E+06	1.70E+06	2.00E+06	3.40E+06	2.00E+06	2.70E+06	2.10E+06	2.90E+06
27a-e	Sandwhich	high	2.20E+06	2.00E+06	2.50E+06	2.40E+06	3.10E+06	2.90E+06	2.50E+06	3.50E+06	4.50E+06	4.00E+06

Raw and ready to cook RTC meat and poultry *E. coli*

(Food) Category 4		Meat and poultry										
(Food) Type 4		Meat and poultry										
			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
22a-e	beef mince	low	5.E+01	6.E+01	4.E+01	1.E+01	1.E+02	6.E+01	5.E+01	4.E+01	1.E+01	3.E+01
19a-e	Chicken breast	low	8.E+01	7.E+01	5.E+01	9.E+01	5.E+01	6.E+01	8.E+01	9.E+01	9.E+01	6.E+01
23a-e	beef mince	interm ediate	1.E+03	1.E+03	2.E+03	2.E+03	4.E+03	9.E+02	6.E+02	2.E+03	1.E+03	3.E+03
20a-e	Chicken breast	interm ediate	1.E+03	1.E+03	2.E+03	2.E+03	2.E+03	3.E+03	3.E+03	2.E+03	3.E+03	2.E+03
21a-e	Chicken breast	high	1.E+05	1.E+05	2.E+05	2.E+05	2.E+05	2.E+05	2.E+05	3.E+05	9.E+04	2.E+05
24a-e	beef mince	high	1.E+05	2.E+05	3.E+05	3.E+05	2.E+05	1.E+05	2.E+05	2.E+05	2.E+05	1.E+05

Pet food and animal feed *E. coli*

(Food) Category 6		Pet food and animal feed										
(Food) Type 6		Wet food (cooked)										
			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
34a-e	Cat food with gravy	low	2.15E+02	1.80E+02	1.30E+02	1.95E+02	1.75E+02	3.35E+02	2.95E+02	3.00E+02	3.65E+02	2.00E+02
31a-e	Dog pate	low	3.75E+02	1.85E+02	2.20E+02	2.30E+02	2.40E+02	5.30E+02	5.15E+02	5.25E+02	4.40E+02	3.95E+02
32a-e	Dog pate	interm ediate	2.00E+04	1.60E+04	2.60E+04	2.50E+04	1.90E+04	3.30E+04	2.70E+04	5.90E+04	3.70E+04	3.90E+04
35a-e	Cat food with gravy	interm ediate	1.10E+04	2.10E+04	3.50E+04	2.00E+04	1.10E+04	2.70E+04	4.40E+04	6.40E+04	2.80E+04	2.70E+04
36a-e	Cat food with gravy	high	2.10E+05	3.80E+05	3.50E+05	6.70E+05	4.20E+05	5.70E+05	7.50E+05	7.20E+05	8.70E+05	7.10E+05
33a-e	Dog pate	high	3.70E+06	9.10E+05	5.70E+05	4.50E+05	5.40E+05	1.00E+06	1.10E+06	1.04E+06	8.70E+05	1.20E+06

Environmental samples (food or feed production) *E. coli*

(Food) Category 7		Enviromental samples										
(Food) Type 7		Proccess water										
			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
37a-e	Wash water	low	2.00E+02	2.82E+02	2.45E+02	2.64E+02	2.10E+02	3.18E+02	3.90E+02	2.27E+02	2.47E+02	2.65E+02
40a-e	Cooling Water	low	3.40E+02	3.36E+02	1.91E+02	2.27E+02	2.45E+02	2.55E+02	2.73E+02	2.00E+02	2.91E+02	3.64E+02
38a-e	Wash water	interm ediate	3.90E+04	4.00E+04	3.50E+04	2.90E+04	2.50E+04	3.80E+04	2.90E+04	2.70E+04	2.30E+04	2.60E+04
41a-e	Cooling Water	interm ediate	2.10E+04	2.40E+04	2.20E+04	2.90E+04	2.10E+04	3.80E+04	3.10E+04	3.00E+04	3.70E+04	3.10E+04
39a-e	Wash water	high	2.20E+06	1.40E+06	1.70E+06	2.50E+06	1.80E+06	2.70E+06	3.30E+06	3.70E+06	3.90E+06	2.90E+06
42a-e	Cooling Water	high	2.50E+06	1.60E+06	2.40E+06	2.20E+06	2.70E+06	2.70E+06	3.00E+06	4.10E+06	3.50E+06	3.60E+06

Milk and dairy products (combined category raw and heat processed Milk and dairy products) coliforms

(Food) Category 1		milk and dairy products										
(Food) Type 1		dry milk										
			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
101a-e	Milk powder	Low	1.86E+02	1.41E+02	1.73E+02	1.60E+02	2.36E+02	6.50E+01	6.00E+01	1.05E+02	3.00E+01	1.27E+02
104a-e	Dessert Powder	Low	2.14E+02	2.45E+02	2.23E+02	2.27E+02	1.60E+02	2.36E+02	2.68E+02	3.18E+02	2.81E+02	2.05E+02
102 a-e	Milk powder	Med	1.00E+04	8.00E+03	5.50E+03	1.70E+04	8.50E+03	1.40E+04	9.50E+03	5.30E+03	1.60E+04	1.40E+04
105a-e	Dessert Powder	Med	1.60E+04	1.50E+04	1.60E+04	1.90E+04	2.10E+04	2.30E+04	2.10E+04	2.50E+04	2.10E+04	2.00E+04
103 a-e	Milk powder	High	8.50E+05	1.90E+07	9.50E+06	8.70E+06	1.60E+06	1.40E+06	1.60E+07	1.00E+07	8.60E+06	2.00E+06
106a-e	Dessert Powder	High	2.90E+06	1.50E+06	2.20E+06	8.50E+06	4.70E+06	1.40E+06	3.20E+06	2.80E+06	9.00E+06	4.40E+06

Fishery products combined category: raw, RTE RTRH, RTC coliforms

(Food) Category 1		Fishery prodcuts										
(Food) Type 1		RTC fish										
			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
107a-e	Tuna	low	7.70E+03	7.20E+03	6.80E+03	6.20E+03	8.50E+03	6.60E+03	4.50E+03	6.10E+03	5.20E+03	5.70E+03
110a-e	White fish	low	2.70E+04	3.50E+04	1.80E+04	2.50E+04	2.50E+04	3.00E+04	2.70E+04	3.10E+04	2.60E+04	2.80E+04
108a-e	Tuna	interm ediate	2.70E+05	2.50E+05	2.30E+05	2.20E+05	2.70E+05	1.90E+05	2.50E+05	2.20E+05	2.50E+05	2.50E+05
110a-e	White fish	interm ediate	7.40E+05	6.90E+05	7.00E+05	1.00E+06	7.20E+05	6.30E+05	7.50E+05	5.10E+05	6.00E+05	5.40E+05
109a-e	White fish	high	1.20E+07	1.00E+07	1.10E+07	1.60E+07	7.10E+06	9.30E+06	1.10E+07	1.30E+07	1.30E+07	8.70E+06
112a-e	Tuna	high	7.90E+06	9.30E+06	9.40E+06	1.50E+07	9.30E+06	5.00E+06	9.50E+06	8.60E+06	1.00E+07	8.40E+06

Produce and fruits (combined category fresh and processed) coliforms

(Food) Category 8	Produce and fruits
(Food) Type 8	Cut ready to eat

			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
113a-e	Lettuce	Low	3.85E+02	4.65E+02	4.80E+02	5.00E+02	5.15E+02	4.30E+02	4.95E+02	3.25E+02	4.50E+02	4.75E+02
116a-e	Spinach	Low	5.70E+02	6.65E+02	4.90E+02	4.15E+02	4.30E+02	5.55E+02	8.15E+02	5.10E+02	3.45E+02	3.95E+02
114a-e	Lettuce	Med	2.30E+04	2.50E+04	3.10E+04	3.80E+04	2.80E+04	3.70E+04	3.50E+04	3.20E+04	3.50E+04	5.70E+04
117a-e	Spinach	Med	3.80E+04	3.50E+04	2.60E+04	2.30E+04	3.50E+04	2.70E+04	3.20E+04	4.50E+04	2.90E+04	4.90E+04
115a-e	Lettuce	High	3.10E+06	2.20E+06	1.70E+06	3.50E+06	3.00E+06	2.00E+06	5.00E+06	2.10E+06	3.40E+06	3.10E+06
118a-e	Spinach	High	1.80E+06	1.20E+06	2.80E+06	3.50E+06	3.50E+06	1.50E+06	1.50E+06	2.00E+06	3.50E+06	2.90E+06

Multi-component foods or meal components coliforms

(Food) Category 5	Multicomponent
(Food) Type 5	Composite foods with raw/processed

			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
125a-e	Sandwich	Low	165	190	160	75	240	220	245	210	235	190
128a-e	Pasta salad	Low	215	315	325	195	310	285	195	285	255	335
126a-e	Sandwich	Med	6.50E+04	3.70E+04	3.70E+04	6.50E+04	5.40E+04	4.80E+04	4.80E+04	4.60E+04	6.20E+04	5.60E+04
129a-e	Pasta salad	Med	7.20E+04	6.50E+04	6.90E+04	5.20E+04	6.60E+04	6.90E+04	6.40E+04	5.40E+04	6.40E+04	6.90E+04
127a-e	Sandwich	High	5.60E+06	6.10E+06	6.70E+06	6.50E+06	6.90E+06	5.30E+06	5.40E+06	6.50E+06	6.10E+06	6.10E+06
130a-e	Pasta salad	High	6.50E+06	7.50E+06	8.40E+06	6.50E+06	8.00E+06	6.60E+06	6.00E+06	7.00E+06	6.80E+06	7.30E+06

Raw and ready to cook RTC meat and poultry coliforms

(Food) Category 4	Raw and RTC Meat and poultry
(Food) Type 4	Fresh 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
122a-e	Chicken	Low	3.20E+02	4.75E+02	3.15E+02	4.20E+02	3.20E+02	2.75E+02	3.60E+02	3.10E+02	3.30E+02	3.00E+02
119a-e	Beef	Low	3.50E+02	4.80E+02	3.50E+02	3.10E+02	4.55E+02	3.50E+02	4.60E+02	3.35E+02	3.65E+02	3.40E+02
120a-e	Beef	Med	6.70E+03	6.20E+03	7.20E+03	6.30E+03	8.30E+03	1.00E+04	1.30E+04	6.80E+03	5.90E+03	1.20E+04
123a-e	Chicken	Med	3.90E+04	4.60E+04	3.30E+04	4.50E+04	2.80E+04	3.40E+04	3.10E+04	3.20E+04	1.90E+04	2.90E+04
121a-e	Beef	High	1.20E+05	1.70E+05	1.50E+05	2.00E+05	1.40E+05	1.0E+05	2.80E+05	5.60E+05	3.50E+05	2.20E+05
124a-e	Chicken	High	1.70E+05	1.60E+05	2.90E+05	9.50E+04	1.70E+05	2.70E+05	2.20E+05	3.20E+05	2.00E+05	2.10E+05

Pet food and animal feed coliforms

(Food) Category 6	Pet food and animal
(Food) Type 6	Pet food and animal feed

			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
131a-e	Dog Pate	Low	2.50E+03	1.90E+03	2.10E+03	2.60E+03	2.30E+03	2.70E+03	2.00E+03	1.90E+03	2.40E+03	2.80E+03
134a-e	Cat Food	Low	1.90E+03	2.50E+03	2.40E+03	3.10E+03	1.90E+03	2.50E+03	2.30E+03	2.80E+03	2.00E+03	2.00E+03
132a-e	Dog Pate	Med	1.30E+04	3.00E+04	3.30E+04	3.00E+04	2.00E+04	1.70E+04	2.60E+04	2.30E+04	2.40E+04	2.50E+04
135a-e	Cat Food	Med	3.30E+04	4.20E+04	4.50E+04	4.80E+04	4.10E+04	3.30E+04	3.50E+04	3.70E+04	3.10E+04	3.00E+04
133a-e	Dog Pate	High	8.60E+05	9.40E+05	1.60E+06	1.00E+06	1.40E+06	8.50E+05	1.00E+06	1.20E+06	7.20E+05	1.50E+06
136a-e	Cat Food	High	1.50E+06	1.30E+06	1.40E+06	1.50E+06	2.40E+06	1.10E+06	1.00E+06	1.20E+06	1.10E+06	1.60E+06

Environmental samples (food or feed production) coliforms

(Food) Category 7		Enviromental samples										
(Food) Type 7		Process water										
			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
137a-e	Cooling water	Low	615	820	670	610	565	460	605	550	730	605
140a-e	Wash water	Low	825	955	695	545	825	770	710	685	840	635
138a-e	Cooling water	Med	7.50E+03	1.20E+04	7.00E+04	6.20E+03	1.10E+04	1.60E+04	1.50E+04	1.60E+04	1.60E+04	1.70E+04
141a-e	Wash water	Med	1.50E+04	1.50E+04	1.10E+04	1.80E+04	1.50E+04	1.50E+04	5.90E+04	2.70E+04	1.50E+04	1.50E+04
139a-e	Cooling water	High	2.50E+06	2.40E+06	1.70E+06	2.30E+06	2.10E+06	2.50E+06	2.50E+06	2.40E+06	1.90E+06	2.60E+06
142a-e	Wash water	High	2.00E+06	7.90E+06	3.10E+06	3.50E+06	1.50E+06	1.80E+06	2.10E+06	2.20E+06	2.90E+06	2.00E+06

10 ANNEX E: Raw data from the ILS Easy plate EC *E. coli*

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
1	1	Low	2.5	2.6	27/02/23
	2	Medium	4.3	4.5	27/02/23
	3	High	5.3	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.3	2.4	27/02/23
	6	Medium	4.3	4.6	27/02/23
	7	High	5.5	5.8	27/02/23
2	1	Low	2.6	2.6	27/02/23
	2	Medium	4.3	4.7	27/02/23
	3	High	5.3	5.6	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.3	2.6	27/02/23
	6	Medium	5.1	4.7	27/02/23
	7	High	5.4	5.7	27/02/23
4	1	Low	2.3	2.8	27/02/23
	2	Medium	4.2	4.9	27/02/23
	3	High	5.6	6.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.8	27/02/23
	6	Medium	4.2	4.8	27/02/23
	7	High	5.7	6.1	27/02/23
5	1	Low	2.5	2.7	27/02/23
	2	Medium	4.3	4.8	27/02/23
	3	High	5.8	6.3	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.1	2.7	27/02/23
	6	Medium	4.5	4.9	27/02/23
	7	High	6.3	6.2	27/02/23
6	1	Low	2.7	2.8	27/02/23
	2	Medium	4.6	4.9	27/02/23
	3	High	5.9	6.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.6	27/02/23
	6	Medium	4.6	4.9	27/02/23
	7	High	6.3	6.2	27/02/23
7	1	Low	2.7	2.8	27/02/23
	2	Medium	4.6	4.6	27/02/23
	3	High	5.7	5.9	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.5	2.9	27/02/23

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
	6	Medium	4.5	4.8	27/02/23
	7	High	5.6	5.8	27/02/23
9	1	Low	2.6	2.3	27/02/23
	2	Medium	5.3	4.3	27/02/23
	3	High	5.3	4.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.1	27/02/23
	6	Medium	4.4	4.1	27/02/23
	7	High	5.1	5.1	27/02/23
10	1	Low	2.0	2.6	27/02/23
	2	Medium	4.4	4.7	27/02/23
	3	High	5.1	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	1.9	2.3	27/02/23
	6	Medium	3.8	4.4	27/02/23
	7	High	5.2	5.7	27/02/23
11	1	Low	2.0	2.6	27/02/23
	2	Medium	3.9	4.5	27/02/23
	3	High	5.2	5.7	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	1.3	2.4	27/02/23
	6	Medium	4.0	4.5	27/02/23
	7	High	5.3	5.5	27/02/23
12	1	Low	2.4	2.8	27/02/23
	2	Medium	4.4	4.8	27/02/23
	3	High	5.3	5.9	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.5	2.8	27/02/23
	6	Medium	4.7	4.7	27/02/23
	7	High	5.6	5.9	27/02/23
Expert lab	1	Low	2.7	2.8	27/02/23
	2	Medium	4.0	3.6	27/02/23
	3	High	5.7	6.0	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.1	2.5	27/02/23
	6	Medium	3.8	4.4	27/02/23
	7	High	5.6	5.9	27/02/23

Raw data from the ILS Easy plate EC coliforms

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	5.8	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.0	2.4	27/02/23
	6	Medium	4.4	4.7	27/02/23
	7	High	5.6	5.9	27/02/23
2	1	Low	2.6	2.6	27/02/23
	2	Medium	4.3	4.8	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	2.9	27/02/23
	2	Medium	4.5	4.0	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	6.2	27/02/23
5	1	Low	2.4	2.8	27/02/23
	2	Medium	4.5	4.8	27/02/23
	3	High	6.0	6.4	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.8	27/02/23
	6	Medium	4.7	5.0	27/02/23
	7	High	6.4	6.3	27/02/23
6	1	Low	2.7	2.9	27/02/23
	2	Medium	4.7	5.0	27/02/23
	3	High	6.4	6.1	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.7	27/02/23
	6	Medium	4.7	5.0	27/02/23
	7	High	6.0	6.2	27/02/23
7	1	Low	3.4	2.8	27/02/23
	2	Medium	4.4	4.7	27/02/23
	3	High	5.7	5.6	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.4	2.9	27/02/23

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
	6	Medium	4.5	4.8	27/02/23
	7	High	6.0	5.8	27/02/23
9	1	Low	2.7	2.9	27/02/23
	2	Medium	5.6	5.9	27/02/23
	3	High	5.6	6.0	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.8	27/02/23
	6	Medium	4.8	4.9	27/02/23
	7	High	5.6	5.8	27/02/23
10	1	Low	2.3	2.7	27/02/23
	2	Medium	4.3	4.7	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	4.4	27/02/23
	7	High	5.3	5.7	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	2.6	27/02/23
	6	Medium	4.3	4.7	27/02/23
	7	High	5.3	5.6	27/02/23
12	1	Low	2.4	2.8	27/02/23
	2	Medium	4.6	4.9	27/02/23
	3	High	5.5	6.0	27/02/23
	4	Blank	<1	<1	27/02/23
	5	Low	2.6	2.8	27/02/23
	6	Medium	4.8	4.9	27/02/23
	7	High	5.8	6.0	27/02/23
Expert lab	1	Low	2.8	2.8	27/02/23
	2	Medium	4.3	4.0	27/02/23
	3	High	5.9	6.0	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