ISO 16140-2:2016 validation of Kikkoman Biochemifa Company Easy Plate AC, for the enumeration of aerobic plate count in a broad range of foods, plus pet food and animal feed and environmental samples

MicroVal study number: 2021LR102

Method/Kit name: Easy Plate AC

Report version: Summary report

MicroVal Expert Laboratory: Campden BRI

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Foreword

The protocol 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 AC

Validation standard: Microbiology of the food chain— Method validation

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

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

Reference method: ISO 4833-1:2013 Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30°C by the pour plate technique

Scope of validation: Broad range of foods plus pet food and anmal feed and environmental samples covering

- Dairy (raw and processed)
- Fishery products
- Fresh and procesed produce
- Multicomponent products
- Meat and poultry products
- Pet food
- 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

And, in areobic plate count studies, eg:

- 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 total aerobic count in 7 different categories was carried out by Campden BRI as the MicroVal Expert Laboratory.

Alternative method

Easy Plate AC is a prepared microbiological culture plate made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate AC method is intended to indicate the level of aerobic bacteria in food and beverage products. After incubation at 30°C ±1°C for 48h ±3h, the aerobic colonies are visible as red colonies on the Easy Plate AC growth medium.

Reference method

ISO 4833-1:2013; Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30 degrees C by the pour plate technique

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

Scope of the validation study : A broad range of foods, pet food and animal feed environmental samples

Categories included:

- Dairy (raw and processed)
- Fishery products
- Fresh and procesed produce
- Multicomponent products
- Meat and poultry products
- Pet food
- Environmental samples

Criteria evaluated during the MCS/ILS study:

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

The final conclusion on the Method Comparison Study is summarised below:

The alternative method Easy Plate AC shows comparable performance to the reference method ISO 4833-1:2013 for the enumeration of total aerobic count in a broad range of foods, pet food and animal feed and environmental samples.

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 method is 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 shall be 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 insert for the alternative method (Easy Plate AC) is given in Annex B.

The alternative method principle is based on chromogenic media. Easy Plate AC is a prepared microbiological culture plate made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate AC method is intended to indicate the level of aerobic bacteria in food and beverage products. After incubation for 48h ±3h, the aerobic colonies are visible as red colonies on the Easy Plate AC growth medium. For the validation study, the minimum of 45h incubation time was used for the Easy plate AC.

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 (examples)	Number of samples analysed	Number of samples with interpretable results	ISO 6887 and Diluent used
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 sodium citrate solution
	Pasteurised milk and milk based products	Milk, ice-cream, milk based drinks	5	5	6887-5 PSD
	Dry milk products	Milk powder, dessert powder	5	5	6887-5 PSD
	Total		15	15	
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon fillet, tuna	5	5	6887-3 PSD
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods	5	5	6887-3 PSD
	Crustaceans	Shrimp, crab	5	5	6887-3 PSD
	Total		15	15	
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot	5	5	6887-4 PSD
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	5	6887-4 PSD
	Heat treated fruit and vegetables	Pasteurised smoothies/juice, blanched frozen vegetables	5	5	6887-4 PSD
	Total		15	15	
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, sandwiches	5	5	6887-1 PSD
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products,	5	5	6887-1 PSD
	Mayonnaise based deli-salads	Vegetable salad	5	5	6887-1 PSD
	Total		15	15	
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	5	5	6887-2 PSD
	Raw processed meat	Frozen burger patties, pork meat balls,	5	5	6887-2 PSD

Category	Types	Items (examples)	Number of samples analysed	Number of samples with interpretable results	ISO 6887 and Diluent used
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	5	6887-2 PSD
	Total		15	15	
Pet food and animal feed	Dry Food	Pellets, kibbles, treats	5	5	6887-4 PSD
	Wet food (raw and canned)	Pates, sausages	5	5	6887-2 PSD
	Animal feeds (poultry and fish)	seasoned chicken, turkey meat balls,	5	5	6887-4 PSD
	Total		15	15	
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	Equipment, floors, walls	5	5	6887-1 ISO 18593:2018 PSD
	Process water	Wash water, cooling water	5	5	6887-1 PSD
	Dusts	Bakery and food manufacturing environment	5	5	6887-1 ISO 18593:2018 PSD
	Total		15	15	
Total samples			**105**	**105**	

Key PSD = peptone salt diluent

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

All samples were screened for naturally contamination to ensure suitable levels of contamination for the study.

For 6 categories (Milk and Dairy, Fishery, Produce, Raw and RTC meats, multicomponent and environmental) all samples were natually contaminated. The pet food and animal feed category was artificially contaminated due to the low level of naturally present organisms in the samples tested.

Artificial procedures were used to inoculate 20 samples, 5 heat treated fruit and vegetables samples in the produce and fruits category, 5 Dry Food, 5 Wet food (raw and canned) and 5 Animal feeds (poultry and fish) in the pet food and animal feed category. The heat treated fruit and vegetables and Wet food (raw and canned) samples were spiked with heat treated strains (10min @55°C). The injury level achieved was between 0.55 and 0.92 logs (see raw data file for details).

Five Dry Food and Animal feeds (poultry and fish) samples were seeded with lyophilised strains and the samples were stored for 2 weeks at ambient prior to analysis.

Four isolates (*Enterobacter intermedius, Enterococcus casseliflavus, Enterococcus mundtii and Enterococcus faecium*) were used for artificial inoculations These cultures preferably originated from comparable sample types as the ones to be inoculated. Each particular strain was used to contaminate up to 5 different items.

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

3.2.3 Protocols applied during the validation study.

A single protocol was applied for the study.
Reference method plates were incubated at 30±1ºC for 72±3h.

Alternative method was incubated at 30±1ºC for 48±3h.

In all cases the minimum incubation times were used.
No confirmations were needed for the alternative and reference method.

3.2.4 Test results

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

3.2.5 Calculation and interpretation of relative trueness study

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x).
Figures 1 to 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)

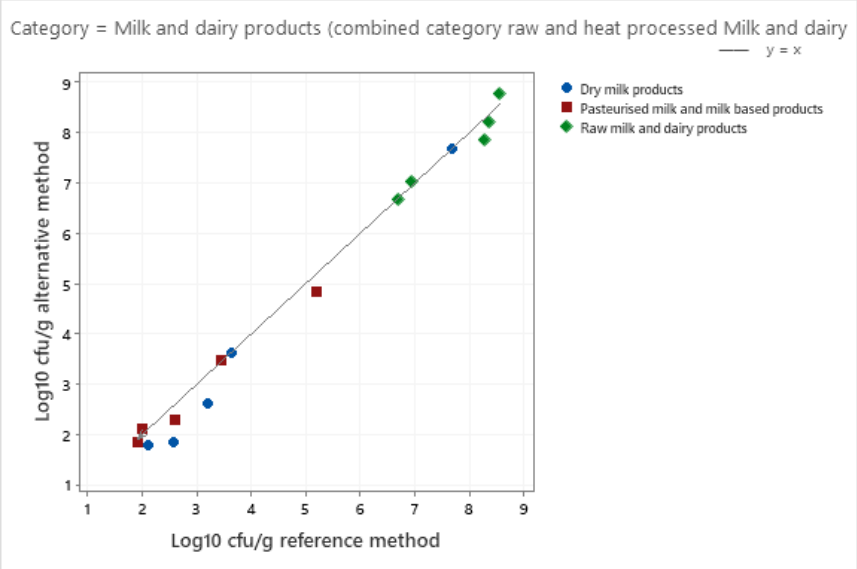


Figure 2 - Scatter plot of the reference method versus alternative method results for Fishery products, Combined category: raw, RTE, RTRH, RTC

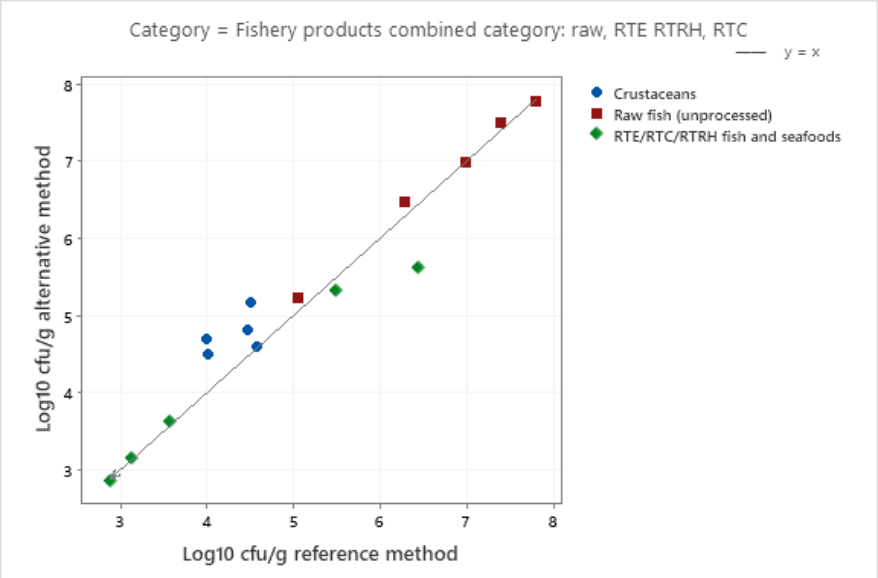


Figure 3 - Scatter plot of the reference method versus alternative method results for Produce and fruits (combined category fresh and processed)

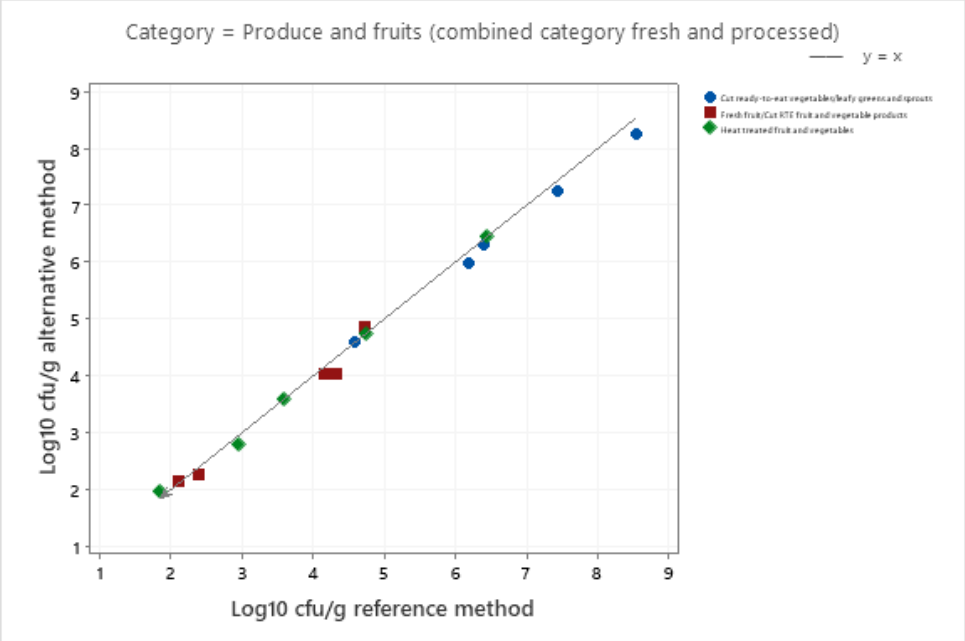


Figure 4 - Scatter plot of the reference method versus alternative method results for Raw and RTC Meat and poultry (Combined category)

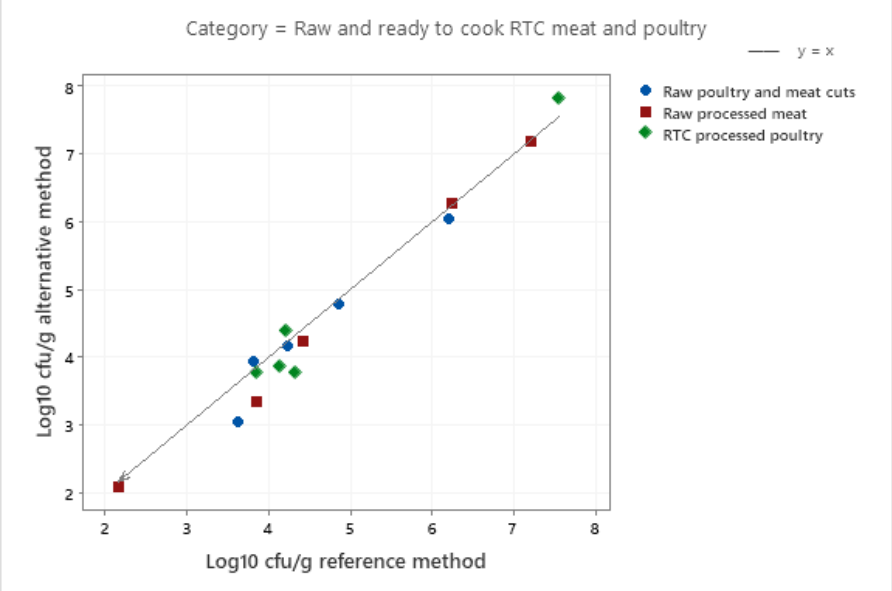


Figure 5 - Scatter plot of the reference method versus alternative method results for Multicomponent

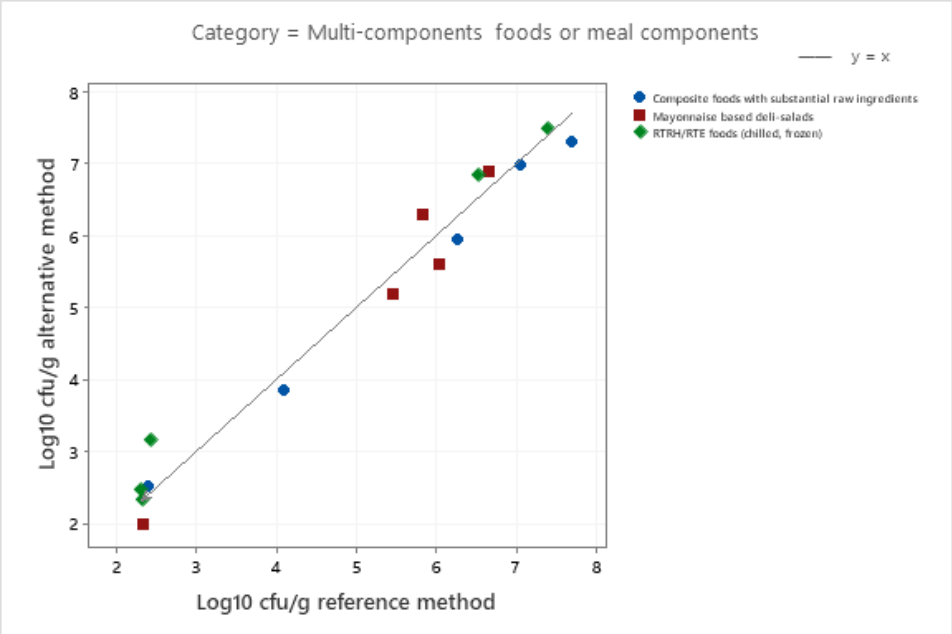


Figure 6 - Scatter plot of the reference method versus alternative method results for Pet food and animal feed

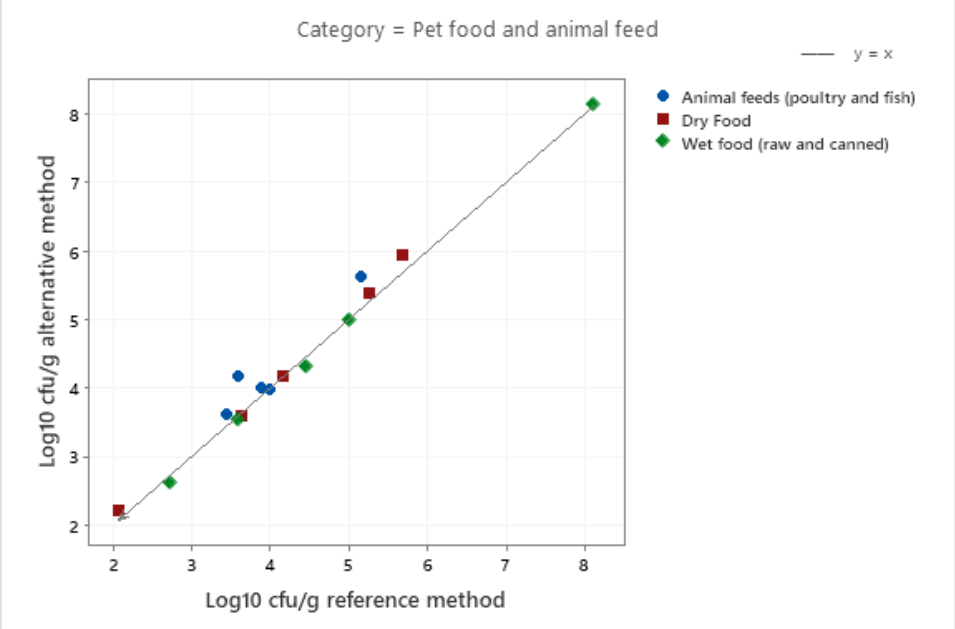


Figure 7 - Scatter plot of the reference method versus alternative method results for Environmental samples

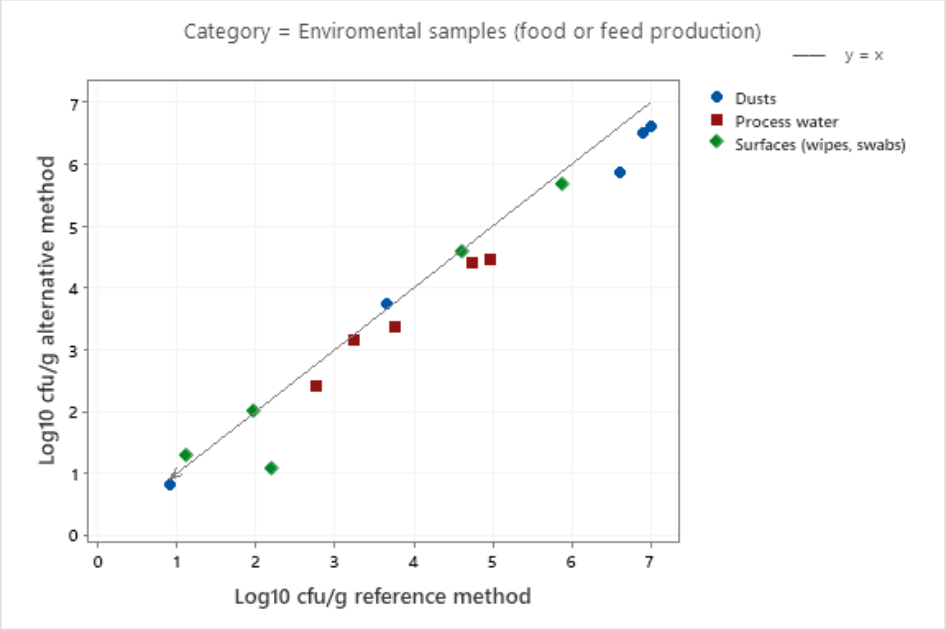
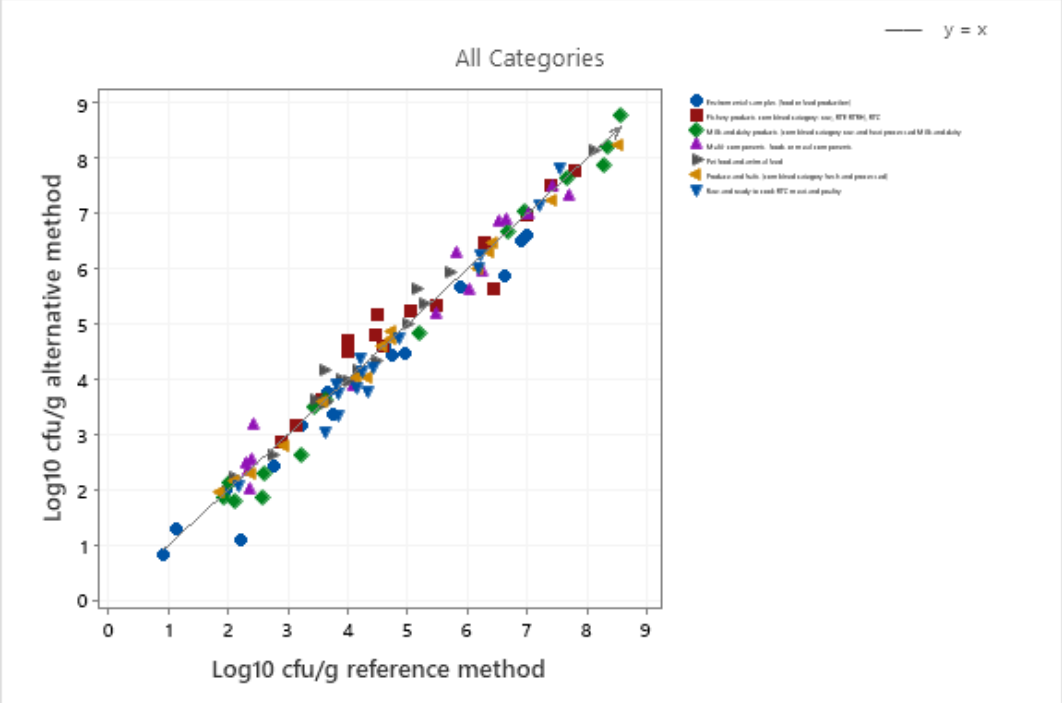


Figure 8 - Scatter plot of the reference method versus alternative method results for all categories



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results.

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

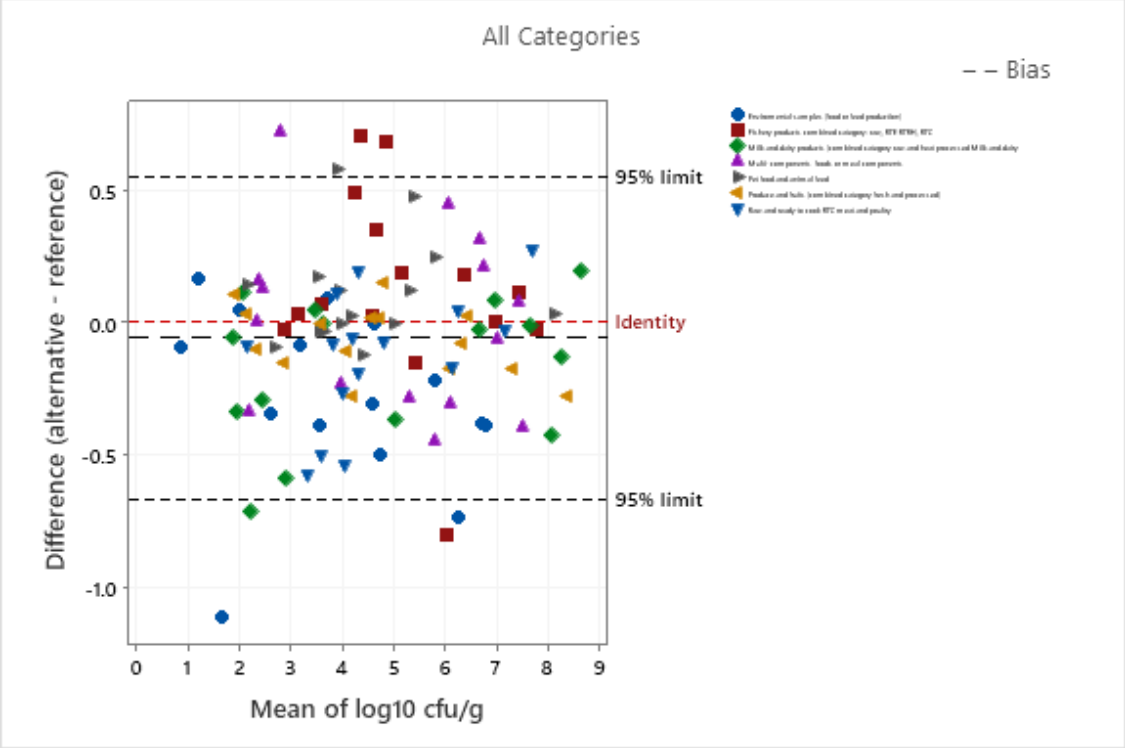
A summary of the calculated values per category is provided in Table 2

Table 2 - Summary of the calculated values per category

Category	**N**	$\bar{D}$	**SD**	**95% Lower limit**	**95% Upper limit**
Milk and dairy products	15	-0.282	0.336	-1.027	0.462
Fishery products	15	0.122	0.364	-0.684	0.928
Produce and fruit	15	-0.167	0.272	-0.769	0.435
Raw and RTC Meat and poultry	15	0.000	0.341	-0.757	0.756
Multicomponent	15	0.110	0.200	-0.334	0.554
Pet food and animal feed	15	-0.066	0.131	-0.356	0.224
Environmental	15	-0.126	0.254	-0.689	0.436
All categories	**105**	-0.058	0.306	-0.669	0.552

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

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



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

Table 3 – data which are outside of the accepted limits

Category	**Type**	**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	RTRH/RTE foods (chilled, frozen)	54	2.422	3.146	2.784	0.725	0.552
Pet food and animal feed	Animal feeds (poultry and fish)	87	3.591	4.176	3.884	0.585	0.552
Fishery products combined category: raw, RTE RTRH, RTC	Crustaceans	27	3.996	4.699	4.347	0.703	0.552
		30	4.491	5.176	4.834	0.685	0.552
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	94	2.188	1.079	1.633	-1.108	-0.669
	Dusts	102	6.613	5.875	6.244	-0.738	-0.669
Milk and dairy products (combined category raw and heat processed Milk and dairy	Dry milk products	13	2.556	1.845	2.201	-0.711	-0.669
Fishery products combined category: raw, RTE RTRH, RTC	RTE/RTC/RTRH fish and seafoods	23	6.431	5.633	6.032	-0.798	-0.669

Comments

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. Four points were slightly higher than the upper limit of 0.552 and three points were slightly lower than the lower limit of -0.669. One point (sample 94) had a difference of -1.108 between the reference and alternative method.

The samples covered a diverse range of foods items with different groups of naturally present organisms present and therefore these results show good agreement between the two methods for enumeration of total aerobic count with a slight negative bias for the alternate method with an overall bias from all the categories of -0.058.

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.

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 4)

Each sample was bulk inoculated and five replicate test portions examined from the bulk sample/ individually inoculated as a separate test portion, with the exception of salad where single test portions were inoculated.

The tested categories, types and items are provided in Table 4

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

Category	Types	Strain	Item	Level	Test portions
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	*Bacillus cereus* CRA 1724 from Dried milk	Milk powder	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Dessert powder	Low 10^2	5
				Medium 10^4	5

Category	Types	Strain	Item	Level	Test portions
				High 10^6	5
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	*Pseudomonas fragi* CRA7222 from spoiled fish	Frozen white fish	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Chilled tuna steak	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
Produce and fruits (combined category fresh and processed)	Cut ready to eat	*E. coli* CRA3379 from spinach	Lettuce	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Grated carrot	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
Raw and RTC Meat and poultry (Combined category)	Fresh meats	*Citrobacter freundii* CRA403 from chicken	Raw stir fry beef strips	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Chicken breast fillets	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
Multicomponent	Composite foods with raw /processed ingredients	*Hafnia alvei* CRA7417 from pate	Sandwich	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Pasta salad	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
Pet food and animal feed	Wet food (cooked)	*Staph aureus* CRA 1246 from pork sausage	Dog pate	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Cat food with gravy	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
Environmental samples	Process water	*Pseudomonas fluorescens CRA 7774* from wash house	Wash water	Low 10^2	5
				Medium 10^4	5
				High 10^6	5
			Cooling water	Low 10^2	5
				Medium 10^4	5
				High 10^6	5

For all matrices, except dessert powder, milk powder,the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis. For dessert and milk powder, a lyophilised culture were used and mixed into the samples prior to testing. The wet pet food and heat processed foods samples were inoculated with heat stressed cultures, with a minimum of 0.5 log injury.

3.3.2 Calculations and interpretation of accuracy profile study

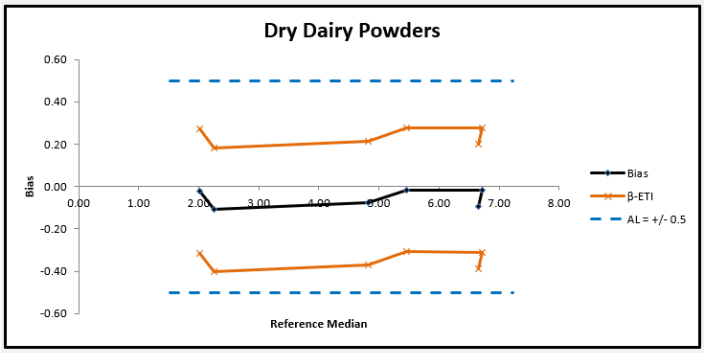
All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in Figures 10 to 16.

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 10: Accuracy profile of dairy products (dry dairy powders) for Easy Plate AC method

Matrices used = Dry milk (1-3) and dessert powder (3-6)

(Food) Category	Dairy products
(Food) Type	Dry Dairy Powders



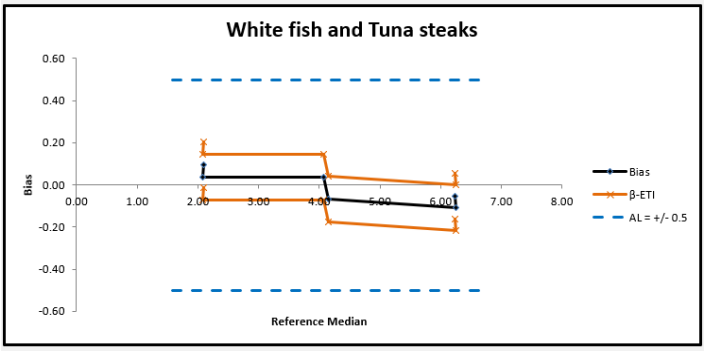
Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
1	2.02	-0.021	-0.314	0.272	YES	YES
4	2.26	-0.109	-0.402	0.184	YES	YES
2	4.83	-0.078	-0.371	0.215	YES	YES
5	5.46	-0.015	-0.308	0.278	YES	YES
3	6.72	-0.017	-0.310	0.276	YES	YES
6	6.66	-0.095	-0.388	0.198	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.374	0.203	NO	+/- 0.500

Figure 11: Accuracy profile of Fishery products for Easy Plate AC method

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

(Food) Category	Fishery products (Combined
(Food) Type	White fish and Tuna steaks



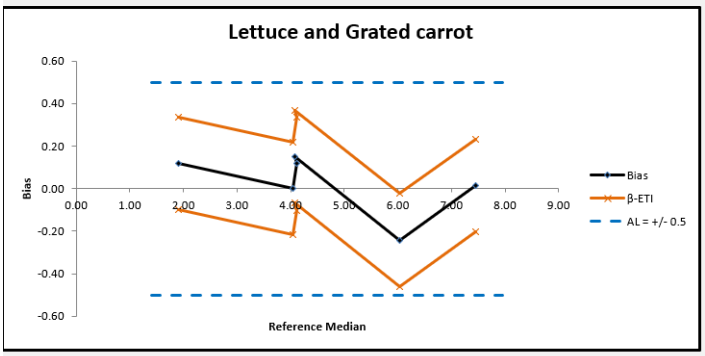
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7	2.10	0.093	-0.015	0.202	YES	YES
10	2.08	0.035	-0.074	0.143	YES	YES
8	4.08	0.035	-0.074	0.143	YES	YES
11	4.15	-0.067	-0.175	0.041	YES	YES
9	6.26	-0.109	-0.217	-0.001	YES	YES
12	6.23	-0.054	-0.163	0.054	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.079	0.075	YES	+/- 0.500

Figure 12: Accuracy profile for Produce and fruits for Easy Plate AC method

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

(Food) Category	Produce and fruits (combined
(Food) Type	Lettuce and Grated carrot



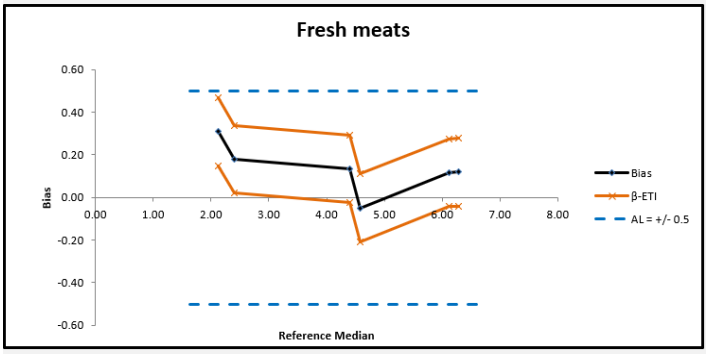
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
13	1.90	0.118	-0.100	0.336	YES	YES
16	4.04	0.000	-0.218	0.218	YES	YES
14	4.11	0.117	-0.101	0.334	YES	YES
17	4.08	0.151	-0.067	0.369	YES	YES
15	6.04	-0.242	-0.460	-0.024	YES	YES
18	7.45	0.015	-0.203	0.233	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.118	0.151	YES	+/- 0.500

Figure 13: Accuracy profile for Meat and poultry for Easy Plate AC method

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

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



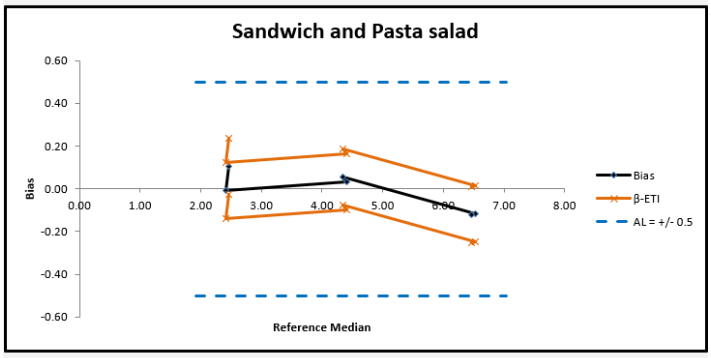
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19	2.13	0.309	0.150	0.468	YES	YES
22	2.41	0.179	0.020	0.338	YES	YES
20	4.40	0.134	-0.025	0.292	YES	YES
23	4.58	-0.048	-0.207	0.110	YES	YES
21	6.11	0.117	-0.042	0.275	YES	YES
24	6.28	0.119	-0.040	0.278	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.103	0.110	YES	+/- 0.500

Figure 14: Accuracy profile for Multicomponent for Easy Plate AC method

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

(Food) Category	Multicomponent
(Food) Type	Sandwich and Pasta salad



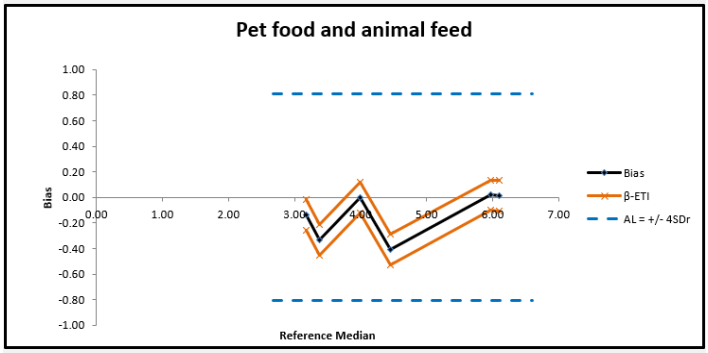
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25	2.46	0.106	-0.026	0.237	YES	YES
28	2.41	-0.008	-0.140	0.123	YES	YES
26	4.41	0.032	-0.099	0.164	YES	YES
29	4.34	0.056	-0.076	0.187	YES	YES
27	6.53	-0.117	-0.248	0.015	YES	YES
30	6.46	-0.120	-0.251	0.011	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.125	0.091	YES	+/- 0.500

Figure 15: Accuracy profile for Pet food and animal feed for Easy Plate AC method

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

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



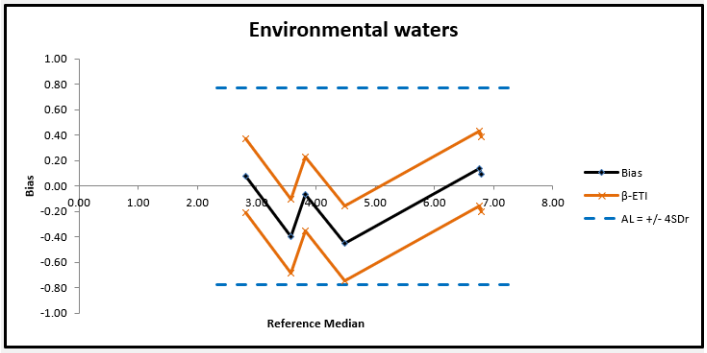
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
31	3.18	-0.135	-0.255	-0.015	YES	YES
34	3.37	-0.332	-0.451	-0.212	YES	YES
32	4.00	0.000	-0.120	0.120	YES	YES
35	4.45	-0.406	-0.526	-0.286	NO	YES
33	5.98	0.018	-0.102	0.138	YES	YES
36	6.10	0.014	-0.106	0.133	YES	

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.202	0.083	NO	+/- 0.808

Figure 16: Accuracy profile for Environmental samples for Easy Plate AC method

Matrices used = Wash (37-39) and cooling waters (40-42)

(Food) Category	Environmental
(Food) Type	Environmental waters



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
37	2.82	0.080	-0.213	0.373	YES	YES
40	3.58	-0.395	-0.688	-0.102	NO	YES
38	3.82	-0.064	-0.357	0.229	YES	YES
41	4.49	-0.450	-0.743	-0.157	NO	YES
39	6.76	0.136	-0.157	0.429	YES	YES
42	6.80	0.093	-0.200	0.386	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.193	0.203	NO	+/- 0.772

Five of the seven categories met the AL of 0.5log (dairy, fresh produce, fish and seafood, multicomponent foods, meat). Two categories (petfood and environmental samples) required the new AL to be calculated. All data met the new AL values of 0.808 and 0.772.

The pet foods showed a slight negative bias for the alternate method although all samples met the recalculated AL value. Other categories did not show any systematic bias between methods.

For some of the categories a larger repeatability was observed (including for the dry powders pet food and environmental samples). One possible explanation for this was that the cultures were stressed prior to inoculation, particular for the dry powders pet food and environmental samples.

3.3.3 Conclusion accuracy profile study

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

3.4 Inclusivity and exclusivity study

As this is a non-selective counting method, an Inclusivity/Exclusivity study is not required.

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 does not have to be calculated for the alternative method in this study.

3.6 Conclusion (MCS)

- The alternative method Easy Plate AC for enumeration of total aerobic count shows satisfactory results for accuracy profile.
- The alternative method Easy Plate AC for enumeration of total aerobic count shows satisfactory results for relative trueness.
- Easy Plate AC shows comparable performance to the reference method for enumeration of total aerobic count in a broad range of foods, pet foods and animal feeds and environmental samples.

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 12 laboratories.

Matrix and strain used

Smoked salmon was inoculated with *Staphylococcus aureus* CRA 1208 (isolated from smoked fish)

Sample preparation

Samples were prepared and inoculated on 9 November 2022 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 9 November 2021 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 14 November 2021. Stability studies had been conducted to show that the required level of target organisms would be present after 6 and 7 days frozen storage. The expert lab analysed a set of samples on Monday 14 November 2022.

Analysis of Samples

Collaborative study laboratories and the expert laboratory carried out the analyses on Monday 14 November 2022. The analyses by the reference method and the alternative method were performed on the same day.

4.2 Experimental parameters controls

Detection of Staphylococcus aureus in the matrix before inoculation

In order to ensure absence of *Staphylococcus aureus* 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 *S. aureus* after 6 and 7 days storage at -18°C. Samples were thawed under controlled conditions prior to analysis. The data shows good stability under the storage regime tested (Table 6).

Table 6 - Staphylococcus aureus stability in the matrix for aerobic plate count

	Reference method cfu/g						Alternative method cfu/g					
	Low level		Medium level		*High level*		Low level		Medium level		*High level*	
Day	a	b	a	b	a	b	a	b	a	b	a	b
0	3.30E+03	3.10E+03	2.90E+04	3.10E+04	1.70E+06	2.20E+06	1.40E+03	1.40E+03	1.90E+04	1.00E+04	8.10E+05	8.90E+05
6	1.80E+03	2.30E+03	3.00E+04	3.00E+04	2.00E+06	2.50E+06	1.20E+03	1.40E+03	1.40E+04	1.60E+04	7.40E+05	1.20E+06
7	3.50E+03	2.70E+03	2.90E+04	2.50E+04	2.40E+06	1.40E+06	1.60E+03	1.60E+03	2.40E+04	1.30E+04	9.9E+05	1.30E+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	10.4	10/11/2022,15:20	Good condition,	14/11/22
2	11.5	10/11/22, 14:00	Acceptable	14/11/22
3	1.4	10/11/22, 14:30	Good condition, frozen	14/11/22
4	6.5	10/11/22, ca 10.15	No issues	14/11/22
5	No data supplied			14/11/22
5	9.8	10/11/22 11:30	Condition of package was fine; samples and temp probe were defrosted	14/11/22
6	6.8	10/11/22, 12:00	sample still frozen	14/11/22
7	13	10/11/22, 14:10	satisfactory	14/11/22
8	7.2	10/11/22, 13:30	data logger not	14/11/22
9	12.1	11/11/22, 15:00	samples not found	14/11/22
10	No data supplied			14/11/22
11	8.9	10/11/22,12:30	temp control defrosted	14/11/22

Collaborator	Temperature measured by probe (°C)	Receipt date and time	State of the package and samples at receipt	Analysis date
12	9.9	10/11/22, no time given	water vial not	14/11/22

No issues were encountered during the transport or at receipt for 12 out of 12 collaborators. All the samples were delivered on time and in appropriate conditions to 11 laboratories. The parcel delivered to lab 9 was held at ambient for longer prior to frozen storage therefore the results were excluded from the analysis

Temperatures during shipment and at receipt were all correct at all 11 other labs participating in the study.

A further 3 labs were removed from the data analysis for the following reasons:

- Lab 4- incorrect incubation time used.
- Lab 8 noted issues with discrimination of food particles at lower dilutions.
- Lab 10 – samples cross contaminated.

4.3 Calculation and summary of data

MicroVal Expert laboratory results

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

Table 8 – Results obtained by the expert lab.

Level	Reference method cfu per g	Alternative method cfu per g
Blank	<10	<10
Low	470	540
Low	8.30E+03	6.30E+03
Medium	4.20E+05	7.00E+05
Medium	4.10E+05	6.20E+05
High	1.40E+06	6.40E+06
High	4.50E+06	6.80E+06

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 17 and the statistical analysis of the data shown in Table 9.

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

		Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
Collaborator	Level	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	2.43	2.53	2.30	2.58
2	low	4.04	2.20	3.86	2.36
3	low	3.90	2.64	3.53	2.63
4	low	3.00	3.54	2.94	3.04
5	low	2.53	2.59	2.48	2.51
6	low	3.11	3.18	3.08	3.18
7	low	3.85	2.87	3.66	2.83
8	low	3.11	4.41	2.74	3.17
9	low	3.57	2.52	3.57	2.26
10	low	4.15	4.00	3.93	3.89
11	low	2.45	2.64	2.34	2.72
12	low	2.43	2.53	2.30	2.58
1	medium	5.78	5.88	5.88	5.80
2	medium	5.67	5.94	5.86	5.99
3	medium	6.04	5.80	5.81	5.81
4	medium	5.73	5.81	5.92	5.83
5	medium	6.04	5.89	6.11	5.94
6	medium	5.89	5.88	5.86	5.92
7	medium	5.76	5.83	5.98	5.79
8	medium	5.85	5.86	5.90	5.88
9	medium	5.81	5.87	5.86	5.79
10	medium	6.00	6.04	6.08	6.00
11	medium	5.69	5.66	5.59	5.72
12	medium	5.78	5.88	5.88	5.80
1	high	6.87	6.93	6.98	6.87
2	high	6.73	6.73	6.87	6.81
3	high	7.11	7.08	7.08	7.15
4	high	6.90	6.71	6.99	6.76

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
5	high	7.20	7.00	7.18	7.08
6	high	6.93	6.98	6.92	6.92
7	high	6.92	6.68	6.89	6.77
8	high	6.84	6.92	7.00	6.00
9	high	9.11	6.67	9.08	6.85
10	high	7.00	6.99	7.04	6.99
11	high	6.65	6.74	6.77	6.86
12	high	6.87	6.93	6.98	6.87

Figure 17. Accuracy profile of Easy Plate AC from the ILS

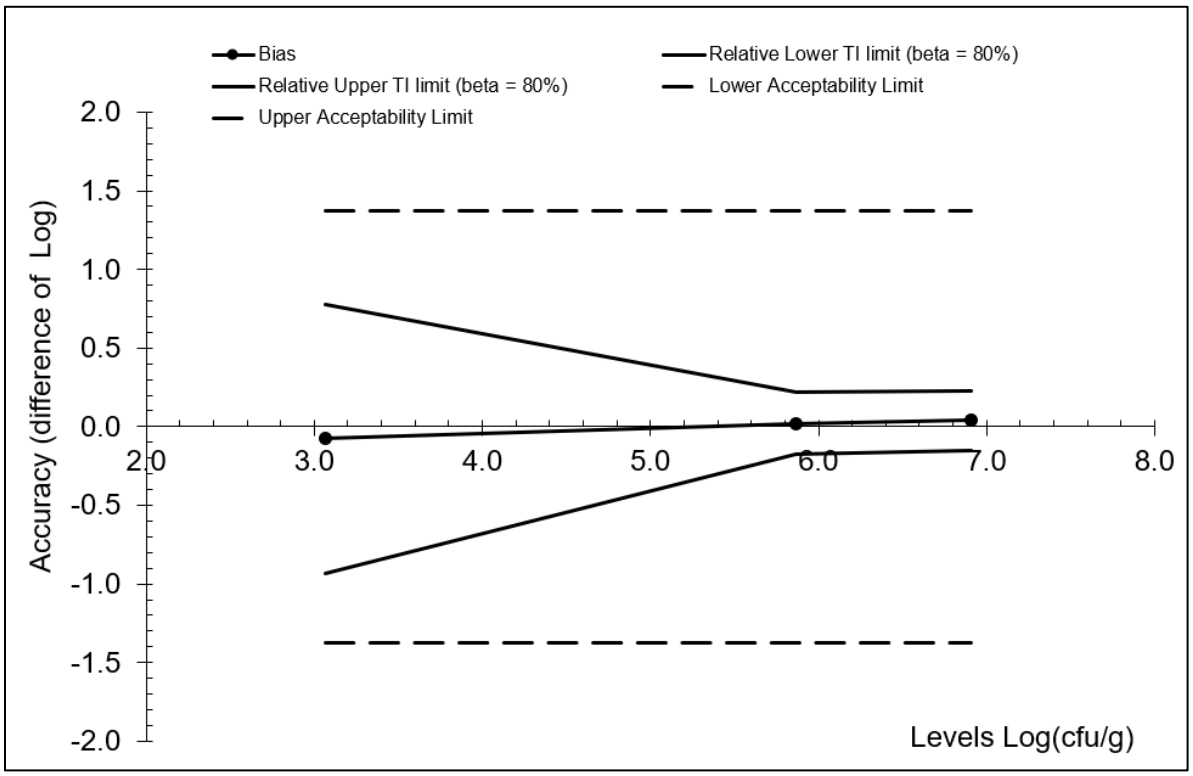


Table 18. Statistical analysis of the ILS data according to the ISO spreadsheet

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**3.070**	**5.863**	**6.910**			
Number of participants (K)	8	8	8	8	8	8
Average for alternative method	2.992	5.884	6.949	3.070	5.863	6.910
Repeatability standard deviation (sr)	0.499	0.085	0.059	0.611	0.103	0.085
Between-labs standard deviation (sL)	0.347	0.109	0.117	0.318	0.081	0.145
Reproducibility standard deviation (sR)	0.608	0.138	0.131	0.689	0.131	0.168
Corrected number of dof	12.985	10.131	8.580	13.904	12.497	9.032
Coverage factor	1.405	1.439	1.465			
Interpolated Student t	1.350	1.371	1.389			
Tolerance interval standard deviation	0.6323	0.1449	0.1381			
Lower TI limit	2.139	5.685	6.757			
Upper TI limit	3.846	6.083	7.140			
Bias	**-0.077**	**0.021**	**0.039**			
Relative Lower TI limit (beta = 80%)	**-0.931**	**-0.178**	**-0.153**			
Relative Upper TI limit (beta = 80%)	**0.777**	**0.220**	**0.231**			
Lower Acceptability Limit	**-1.37**	**-1.37**	**-1.37**			
Upper Acceptability Limit	**1.37**	**1.37**	**1.37**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.416					

TRUE
TRUE

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

A review of the accuracy profile and statistical analysis revealed that there was a higher than expected variability in the aerobic plate values observed for the low level of contamination in the ILS samples. A root cause analysis was carried out to determine possible reasons for this. The log difference in the counts obtained in the

reference method for the expert lab ranged from -0.22 to 0.12 and was -0.06 and 0.12 for the low level samples respectively. 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.

It was noted that the reproducibility of the low level samples was significantly higher than at the other levels being 0.611 for the reference method and 0.499 for the Easy Plate AC, compared to <0.1 recorded for both the medium and high count respectively.

Heat treated samples were used during the study due to the high level of background seen in the batch of smoked salmon used for the study. The higher variability in bacterial load could be explained by the difference in kill of the natural flora in the sample that would impact on the level of organisms able to grow at 30°C during the analysis.

5 Overall conclusions of the MCS/ILS study

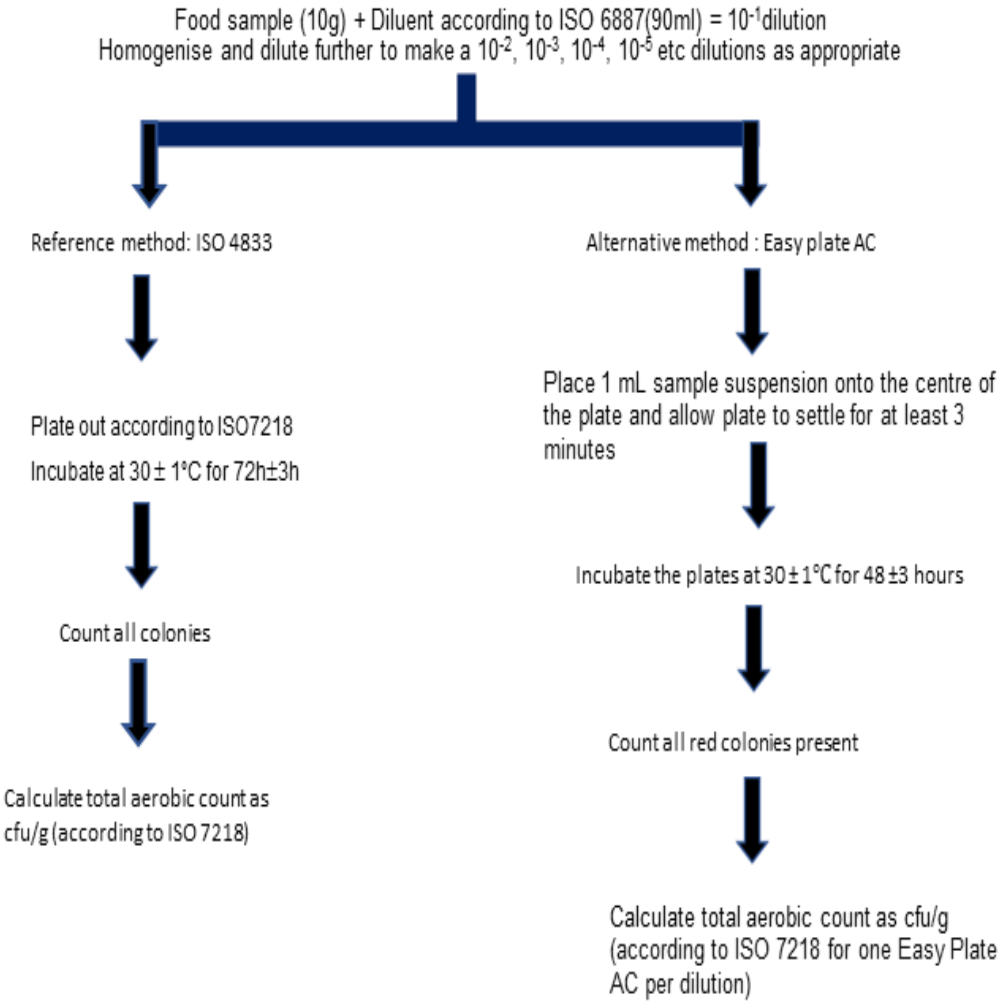
- The alternative method Easy Plate AC for enumeration of *s* shows satisfactory results for relative trueness.
- The alternative method Easy Plate AC for enumeration of aerobic bacteria shows satisfactory results for accuracy profile.
- The alternative method Easy Plate AC for enumeration of aerobic bacteria is selective and specific.
- The alternative method Easy Plate AC for enumeration of aerobic bacteria shows satisfactory performance in the ILS.

Date 06/06/23

Signature Suzanne Jordan

Dr Suzanne Jordan

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



7 ANNEX B: Kit insert.

Please refer to separate pdf document

8 ANNEX C: Calculations and interpretation of relative trueness

Row	Type	Sample code	log(Ref)	log(Alt)	Mean	Difference
Dust						
1	Industrial kitchen sink corner	101	0.903	0.813	0.858	-0.090
2	Radiator	102	6.613	5.875	6.244	-0.738
3	Oven top	103	3.653	3.748	3.701	0.095
4	Base of equipment	104	6.991	6.602	6.797	-0.389
5	Bakery sink corner	105	6.886	6.505	6.696	-0.381
Process water						
6	Cooling water	96	3.230	3.146	3.188	-0.084
7	water	97	4.959	4.462	4.711	-0.497
8	Bakery prep area	98	4.724	4.415	4.570	-0.309
9	Surface run off - industrial kitchen	99	3.748	3.362	3.555	-0.386
10	High pressure cooling water	100	2.756	2.415	2.585	-0.341
Surfaces (wipes, swabs)						
11	Metal - industrial kitchen sink	91	4.602	4.602	4.602	0.000
12	Ceramic - sink splashback	92	1.114	1.279	1.196	0.165
13	Rubber - equipment piping	93	5.886	5.672	5.779	-0.214
14	Plastic - equipment surface	94	2.188	1.079	1.633	-1.108
15	work bench	95	1.954	2.000	1.977	0.046
Crustaceans						
16	prawn cocktail	26	4.000	4.491	4.246	0.491
17	pickled cockles	27	3.996	4.699	4.347	0.703
18	Lemon & Garlic King Prawns	28	4.462	4.813	4.638	0.351
19	Big & Juicy Crayfish Tails	29	4.568	4.591	4.580	0.023
20	Fisherman Brown Shrimp	30	4.491	5.176	4.834	0.685
Raw fish (unprocessed)						
21	Diced salmon fillets	16	7.380	7.491	7.436	0.111
22	Cod Fillet	17	6.279	6.462	6.371	0.184
23	skinless basa fillets	18	7.792	7.763	7.778	-0.029
24	plaice fillets	19	6.978	6.982	6.980	0.005
25	Cornish hake	20	5.041	5.230	5.136	0.189
RTE/RTC/RTRH fish and seafoods						
26	smoked haddock loin	21	5.477	5.322	5.400	-0.155
27	Honey Roast Salmon Flakes	22	3.556	3.623	3.590	0.067
28	Ready to eat smoked mackerel fillets	23	6.431	5.633	6.032	-0.798
29	mexican smoked mackeral	24	3.114	3.146	3.130	0.032
30	Frozen Tuna steaks	25	2.878	2.851	2.865	-0.027

Row	Type	Sample code	log(Ref)	log(Alt)	Mean	Difference
Dry milk products						
31	Milk powder	11	7.672	7.663	7.667	-0.009
32	dried skimmed milk	12	2.114	1.778	1.946	-0.336
33	scone mix	13	2.556	1.845	2.201	-0.711
34	Scone powder mix	14	3.633	3.633	3.633	0.000
35	American Pancake & Waffle mix	15	3.204	2.613	2.908	-0.591
Pasteurised milk and milk based products						
36	Sainsbury's Grated mozzarella	6	5.204	4.839	5.021	-0.365
37	Euro shopper Protein shake strawberry	7	1.903	1.845	1.874	-0.058
38	Vanilla ice cream	8	2.591	2.301	2.446	-0.290
39	Double cream	9	2.000	2.114	2.057	0.114
40	Skimmed milk	10	3.431	3.477	3.454	0.046
Raw milk and dairy products						
41	Raw milk supplier 1	1	6.954	7.041	6.998	0.087
42	Raw milk supplier 2	2	6.699	6.672	6.686	-0.027
43	Raw milk cheese 1	3	8.301	7.875	8.088	-0.426
44	Mountain Comté AOP	4	8.362	8.230	8.296	-0.131
45	Saint-Marcellin IGP	5	8.580	8.778	8.679	0.198
Composite foods with substantial raw ingredients						
46	triple grain salad	46	4.079	3.845	3.962	-0.234
47	egg mayo sandwich	49	2.389	2.519	2.454	0.129
48	Tesco egg and cress	50	7.699	7.301	7.500	-0.398
49	Supergreen salad	106	7.041	6.978	7.010	-0.064
50	Crunchy peanut & sesame slaw	107	6.255	5.949	6.102	-0.306
Mayonnaise based deli-salads						
51	Colesaw with real mayo	56	2.338	2.000	2.169	-0.338
52	Egg mayonnaise	59	5.826	6.279	6.052	0.453
53	chicken, tomato and basil pasta with egg yolk and pasturized egg mayo	60	6.663	6.875	6.769	0.212
54	Coronation coleslaw	110	6.041	5.591	5.816	-0.450
55	Burger slaw	111	5.462	5.176	5.319	-0.286
RTRH/RTE foods (chilled, frozen)						
56	chicken tomato & basil pasta	51	2.320	2.322	2.321	0.002
57	cod fillet & chips	52	6.531	6.845	6.688	0.314
58	Roasted Vegetable Couscous	53	2.301	2.462	2.382	0.161
59	Savers Chicken Curry	54	2.422	3.146	2.784	0.725
60	spinach and tomato quiche	55	7.415	7.491	7.453	0.076
Animal foods (poultry and fish)						
61	Food for all pond fish	86	3.436	3.613	3.524	0.177

Row	Type	Sample code	log(Ref)	log(Alt)	Mean	Difference
62	British Tropical fish mini pellets	87	3.591	4.176	3.884	0.585
63	goldfish formula	88	5.146	5.623	5.385	0.477
64	Mixed corn	89	3.978	3.973	3.975	-0.005
65	Layers mash	90	3.875	4.000	3.938	0.125
Dry Food						
66	Beef cat treats	76	2.061	2.204	2.132	0.143
67	Grain free Salmon with sweet potato minibites	77	5.681	5.929	5.805	0.248
68	perfect for cats duck treats	78	5.255	5.380	5.318	0.125
69	mature dog biscuit bones	79	3.633	3.602	3.618	-0.031
70	chicken kitten food	80	4.146	4.176	4.161	0.030
Wet food (raw and canned)						
71	gourmet cat food - mousse with salmon&cascading gravy	81	8.114	8.146	8.130	0.032
72	Adult dog food - smooth pate with chicken	82	3.580	3.544	3.562	-0.036
73	Tuna fillet natural cat food	83	2.716	2.623	2.670	-0.093
74	dog food Beef Supper with carrots & peas	84	4.991	4.987	4.989	-0.004
75	dog food lamb supper	85	4.447	4.322	4.385	-0.125
Cut ready-to-eat vegetables/leafy greens and sprouts						
76	Shredded Iceberg Lettuce	31	6.176	6.000	6.088	-0.176
77	Grated Carrot	32	8.531	8.255	8.393	-0.276
78	Green beans	33	7.431	7.255	7.343	-0.176
79	Peeled Brussels Sprouts	34	4.580	4.602	4.591	0.022
80	Rocket	35	6.380	6.301	6.341	-0.079
Fresh fruit/Cut RTE fruit and vegetable products						
81	whole Blueberries	36	4.322	4.041	4.182	-0.281
82	Watermelon chunks	37	4.724	4.875	4.800	0.151
83	smooth freshly squeezed orange juice	38	4.146	4.041	4.094	-0.105
84	super green (non heat treated, cold pressed) smoothie	39	2.380	2.279	2.329	-0.101
85	Super Blue (non heat treated, cold pressed) smoothie	40	2.114	2.146	2.130	0.032
Heat treated fruit and vegetables						
86	Wonder Green Juice	41	6.447	6.477	6.462	0.030
87	Cranberry juice drink	42	1.845	1.954	1.900	0.109
88	Juice burst orange (heat treated juice)	43	2.937	2.785	2.861	-0.151
89	V8 original vegetable juice	44	3.580	3.580	3.580	0.000
90	Brilliant Beetroot Juice	45	4.732	4.748	4.740	0.016
Raw poultry and meat cuts						

Row	Type	Sample code	log(Ref)	log(Alt)	Mean	Difference
91	chicken meat	61	4.851	4.778	4.815	-0.073
92	diced beef	62	6.204	6.041	6.123	-0.163
93	pork loin chops	63	4.230	4.176	4.203	-0.054
94	Turkey minifillet	64	3.613	3.041	3.327	-0.571
95	Swab taken from chicken	65	3.813	3.929	3.871	0.117
Raw processed meat						
96	Fire Pit 6 Beef Burgers	66	4.415	4.230	4.323	-0.185
97	mini pork meatballs	67	2.161	2.079	2.120	-0.082
98	Lamb and Mint kebabs	68	3.839	3.342	3.591	-0.496
99	Bag of mince beef	69	7.204	7.176	7.190	-0.028
100	Raw diced beef 500g	70	6.230	6.279	6.255	0.048
RTC processed poultry						
101	Fire pit piri piri chicken steaks	71	4.322	3.785	4.054	-0.537
102	Southern chicken goujons	72	3.839	3.763	3.801	-0.075
103	Spiced poultry	73	7.556	7.833	7.694	0.276
104	Turkey burgers	74	4.137	3.875	4.006	-0.262
105	Turkey escalopes with ham and cheese	75	4.204	4.398	4.301	0.194

9 ANNEX D: Summary tables accuracy profile study

(Food) Category 1	Dairy products
(Food) Type 1	Dry Dairy Powders

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
1	Milk powder	low	8.5E+01	1.9E+02	7.5E+01	1.1E+02	1.1E+03	1.0E+02	1.2E+02	1.0E+02	1.2E+02	9.0E+01
4	Dessert Powder	low	8.5E+01	1.6E+02	3.0E+02	1.8E+02	3.1E+03	1.4E+02	1.4E+02	1.1E+02	1.6E+02	1.7E+02
2	Milk powder	interme diate	7.0E+04	2.5E+04	3.7E+04	1.2E+05	6.7E+04	4.5E+04	2.9E+04	8.3E+04	1.4E+05	5.6E+04
5	Dessert Powder	interme diate	3.0E+05	4.2E+05	5.4E+04	2.9E+05	1.4E+05	2.8E+05	4.3E+05	6.8E+04	3.4E+05	1.7E+05
3	Milk powder	high	5.9E+06	4.3E+06	1.2E+07	3.9E+06	5.3E+06	6.4E+06	4.5E+06	1.3E+07	3.7E+06	5.1E+06
6	Dessert Powder	high	4.6E+06	2.9E+06	5.1E+06	5.3E+06	3.6E+06	3.7E+06	2.5E+06	6.4E+06	5.6E+06	3.6E+06

(Food) Category 2	Fishery products
(Food) Type 2	White fish and Tuna steaks

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7	Fish	low	1.1E+02	1.3E+02	1.2E+02	1.6E+02	1.9E+02	1.8E+02	1.7E+02	1.6E+02	1.2E+02	1.2E+02
10	Tuna	low	1.2E+02	1.5E+02	1.1E+02	1.2E+02	1.4E+02	9.5E+01	1.1E+02	1.7E+02	1.4E+02	1.3E+02
8	Fish	interme diate	1.1E+04	1.3E+04	1.2E+04	1.6E+04	1.1E+04	1.5E+04	1.5E+04	1.2E+04	1.3E+04	1.3E+04
11	Tuna	interme diate	1.4E+04	1.2E+04	1.4E+04	1.8E+04	1.3E+04	1.5E+04	1.1E+04	1.2E+04	1.3E+04	1.2E+04
9	Fish	high	1.6E+06	1.8E+06	1.3E+06	1.8E+06	2.1E+06	1.4E+06	1.4E+06	1.3E+06	1.2E+06	1.8E+06
12	Tuna	high	1.7E+06	1.1E+06	1.7E+06	1.9E+06	1.8E+06	1.5E+06	9.3E+05	1.6E+06	1.3E+06	1.5E+06

(Food) Category 3	Produce and fruits (combined category
(Food) Type 3	Lettuce and Grated carrot

			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
13	Lettuce	low	1.1E+02	8.0E+01	1.2E+02	6.5E+01	8.0E+01	1.1E+02	1.9E+02	1.0E+02	8.0E+01	1.3E+02
16	Carrot	low	8.6E+03	1.4E+04	7.8E+03	1.1E+04	1.4E+04	1.1E+04	1.6E+04	8.2E+03	1.0E+04	1.4E+04
14	Lettuce	interme diate	8.2E+03	9.8E+03	1.5E+04	1.3E+04	2.2E+04	9.4E+03	1.2E+04	2.0E+04	1.7E+04	3.3E+04
17	Carrot	interme diate	1.1E+04	1.0E+04	1.5E+04	1.3E+04	1.2E+04	1.3E+04	1.8E+04	1.6E+04	1.7E+04	1.7E+04
15	Lettuce	high	1.5E+06	1.1E+06	6.6E+05	9.0E+05	1.4E+06	1.0E+06	6.3E+05	2.6E+05	5.5E+05	8.8E+05
18	Carrot	high	3.2E+07	2.5E+07	2.5E+07	3.8E+07	2.8E+07	2.5E+07	2.9E+07	2.9E+07	2.7E+07	3.7E+07

(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
19	Beef	low	1.3E+02	1.4E+02	1.4E+02	1.1E+02	1.6E+02	2.9E+02	2.8E+02	2.6E+02	2.8E+02	2.1E+02
22	Chicken	low	2.7E+02	3.3E+02	2.5E+02	1.5E+02	2.6E+02	5.3E+02	3.9E+02	5.2E+02	3.4E+02	3.5E+02
20	Beef	interme diate	2.1E+04	2.3E+04	2.5E+04	2.6E+04	4.3E+04	3.4E+04	1.2E+04	2.8E+04	3.7E+04	4.2E+04
23	Chicken	interme diate	3.2E+04	3.5E+04	7.8E+04	4.6E+04	3.8E+04	2.9E+04	2.9E+04	4.1E+04	3.7E+04	3.4E+04
21	Beef	high	1.2E+06	1.2E+06	1.6E+06	1.3E+06	1.7E+06	1.7E+06	1.2E+06	1.7E+06	1.3E+06	1.9E+06
24	Chicken	high	2.0E+06	1.7E+06	2.3E+06	1.9E+06	1.9E+06	2.8E+06	2.1E+06	2.5E+06	2.5E+06	2.5E+06

(Food) Category 5	Multicomponent
(Food) Type 5	Sandwich and Pasta salad

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
25	Sandwich	low	2.9E+02	2.6E+02	5.3E+02	3.2E+02	2.8E+02	3.7E+02	3.6E+02	5.3E+02	3.7E+02	3.8E+02
28	Pasta Salad	low	2.5E+02	3.5E+02	3.1E+02	2.6E+02	2.1E+02	2.6E+02	3.1E+02	3.4E+02	2.4E+02	2.4E+02
26	Sandwich	interme diate	3.8E+04	3.5E+04	2.6E+04	2.4E+04	2.4E+04	2.8E+04	3.1E+04	2.9E+04	2.1E+04	2.8E+04
29	Pasta Salad	interme diate	2.1E+04	2.2E+04	2.1E+04	3.5E+04	3.1E+04	3.2E+04	3.5E+04	2.1E+04	2.5E+04	2.3E+04
27	Sandwich	high	2.5E+06	3.5E+06	3.5E+06	2.3E+06	3.4E+06	2.5E+06	2.6E+06	2.5E+06	3.2E+06	2.8E+06
30	Pasta Salad	high	1.2E+06	2.9E+06	3.1E+06	1.5E+06	3.5E+06	1.1E+06	2.2E+06	2.8E+06	2.2E+06	2.5E+06

(Food) Category 8	Pet food and animal feed
(Food) Type 8	Pet food and animal feed

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
31	Cat food	low	8.8E+02	3.5E+03	1.5E+03	8.2E+02	1.8E+03	1.4E+03	5.6E+02	1.1E+03	1.3E+03	1.0E+03
34	Dog pate	low	2.4E+03	1.8E+03	2.9E+03	2.2E+03	4.7E+03	1.0E+03	1.2E+03	9.7E+02	1.2E+03	1.1E+03
32	Cat food	interme diate	1.1E+04	8.6E+03	8.7E+03	1.2E+04	1.0E+04	1.0E+04	1.1E+04	9.0E+03	1.1E+04	9.9E+03
35	Dog pate	interme diate	1.4E+04	4.0E+04	9.1E+03	2.8E+04	4.0E+04	9.5E+03	1.0E+04	1.3E+04	1.3E+04	1.1E+04
33	Cat food	high	1.6E+06	1.0E+06	9.5E+05	8.5E+05	7.1E+05	1.2E+06	1.5E+06	9.1E+05	9.2E+05	9.9E+05
36	Dog pate	high	1.4E+06	1.3E+06	3.7E+06	1.1E+06	1.1E+06	1.4E+06	1.4E+06	1.2E+06	1.2E+06	1.3E+06

(Food) Category 7	Environmental
(Food) Type 7	Environmental waters

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
37	Cooling water	low	8.1E+02	7.3E+02	5.6E+02	6.3E+02	6.7E+02	8.9E+02	7.6E+02	8.1E+02	8.0E+02	7.8E+02
40	Wash water	low	3.0E+03	2.5E+03	4.0E+03	4.0E+03	3.8E+03	1.5E+03	1.3E+03	1.6E+03	1.6E+03	1.5E+03
38	Cooling water	interme diate	6.2E+03	8.0E+03	6.0E+03	6.6E+03	8.3E+03	5.7E+03	5.8E+03	5.1E+03	6.7E+03	5.5E+03
41	Wash water	interme diate	2.6E+04	3.0E+04	4.2E+04	4.8E+04	3.1E+04	1.0E+04	1.1E+04	1.0E+04	1.5E+04	1.3E+04
39	Cooling water	high	5.7E+06	1.1E+06	1.0E+06	7.2E+06	6.8E+06	7.8E+06	9.5E+05	1.1E+06	7.9E+06	7.8E+06
42	Wash water	high	6.6E+06	4.8E+06	5.4E+06	7.1E+06	6.3E+06	7.6E+06	8.4E+06	7.8E+06	1.0E+07	7.0E+06

10 ANNEX E: Raw data from the ILS

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
1	1	Low	2.43	2.3	14/11/22
	2	Medium	5.78	5.88	14/11/22
	3	High	6.87	6.98	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.53	2.58	14/11/22
	6	Medium	5.88	5.8	14/11/22
	7	High	6.93	6.87	14/11/22
2	1	Low	4.04	3.86	14/11/22
	2	Medium	5.67	5.86	14/11/22
	3	High	6.73	6.87	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.2	2.36	14/11/22
	6	Medium	5.94	5.99	14/11/22
	7	High	6.73	6.81	14/11/22
3	1	Low	3.9	3.53	14/11/22
	2	Medium	6.04	5.81	14/11/22
	3	High	7.11	7.08	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.64	2.63	14/11/22
	6	Medium	5.8	5.81	14/11/22
	7	High	7.08	7.15	14/11/22
4	1	Low	3.00	2.94	14/11/22
	2	Medium	5.73	5.92	14/11/22
	3	High	6.9	6.99	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	3.54	3.04	14/11/22
	6	Medium	5.81	5.83	14/11/22
	7	High	6.71	6.76	14/11/22
5	1	Low	6.04	6.11	14/11/22
	2	Medium	7.20	7.18	14/11/22
	3	High	3.11	3.08	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	5.89	5.94	14/11/22
	6	Medium	7	7.08	14/11/22
	7	High	3.18	3.18	14/11/22
6	1	Low	6.93	6.92	14/11/22
	2	Medium	3.85	3.66	14/11/22
	3	High	5.76	5.98	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	6.98	6.92	14/11/22
	6	Medium	2.87	2.83	14/11/22
	7	High	5.83	5.79	14/11/22

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
7	1	Low	3.85	3.66	14/11/22
	2	Medium	5.76	5.98	14/11/22
	3	High	6.92	6.89	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.87	2.83	14/11/22
	6	Medium	5.83	5.79	14/11/22
	7	High	6.68	6.77	14/11/22
8	1	Low	3.11	2.74	14/11/22
	2	Medium	5.85	5.9	14/11/22
	3	High	6.84	7	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	4.41	3.17	14/11/22
	6	Medium	5.86	5.88	14/11/22
	7	High	6.92	6	14/11/22
9	1	Low	3.57	3.57	14/11/22
	2	Medium	5.81	5.86	14/11/22
	3	High	9.11	9.08	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.52	2.26	14/11/22
	6	Medium	5.87	5.79	14/11/22
	7	High	6.67	6.85	14/11/22
10	1	Low	4.15	3.93	14/11/22
	2	Medium	6	6.08	14/11/22
	3	High	7	7.04	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	4	3.89	14/11/22
	6	Medium	6.04	6	14/11/22
	7	High	6.99	6.99	14/11/22
11	1	Low	2.45	2.34	14/11/22
	2	Medium	5.69	5.59	14/11/22
	3	High	6.65	6.77	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.64	2.72	14/11/22
	6	Medium	5.66	5.72	14/11/22
	7	High	6.74	6.86	14/11/22
12	1	Low	2.43	2.3	14/11/22
	2	Medium	5.78	5.88	14/11/22
	3	High	6.87	6.98	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	2.53	2.58	14/11/22
	6	Medium	5.88	5.8	14/11/22
	7	High	6.93	6.87	14/11/22

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
Expert lab	1	Low	2.67	2.73	14/11/22
	2	Medium	5.62	5.85	14/11/22
	3	High	6.15	6.81	14/11/22
	4	Blank	<1	<1	14/11/22
	5	Low	3.92	3.80	14/11/22
	6	Medium	5.61	5.79	14/11/22
	7	High	6.65	6.83	14/11/22