

**Method Comparison Study Report for the ISO 16140-2:2016 validation of Neogen One Plate Total Viable Count (OP TVC), for the enumeration of aerobic plate count in a broad range of foods**

MicroVal study number: 2022LR112

Method/Kit name: Neogen One Plate Total Viable Count (OP TVC)

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## Foreword

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

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Method/Kit name: Neogen One Plate Total Viable Count (OP TVC)

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

Certification organization: LRQA

### List of abbreviations

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

And, in aerobic plate count studies, eg:

-	BPW	Buffered Peptone Water
-	MRD	Maximum Recovery Diluent
-	NA	Nutrient Agar
-	PCA	Plate count Agar

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## 1 Introduction

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

The alternative method was:

One Plate for Total Viable Count (OP-TVC) offers a rapid method for the enumeration of total aerobic mesophiles using traditional culture methodology. A supplement is added to the agar base post-sterilisation which colours colonies red, which improves the ability to identify and enumerate colonies. After incubation at 30°C ±1°C for 36-48 hours, all colonies are counted. The agar can be used as a surface plating technique with 0.1ml volumes or a pour plating technique with 1ml volumes plated.

Reference method was: 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 the validation study was: A broad range of foods.

Categories included:

- Heat processed dairy
- Raw fishery products
- Fresh and heat procesed produce
- Multicomponent products
- Raw meat and poultry products

Criteria to be evaluated during the study:

- Method Comparison Study (MCS)
  - Relative Trueness study
  - Accuracy profile study
  - Inclusivity and exclusivity study
- Interlaboratory Study (ILS)

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

- The alternative method One Plate TVC for enumeration of aerobic plate count shows satisfactory results for relative trueness for both plating formats and incubation times
- The alternative One Plate TVC for enumeration of aerobic plate count shows satisfactory results for accuracy profile for both plating formats and incubation times

- The alternative One Plate TVC for enumeration of aerobic plate count is selective and specific for both plating formats and incubation times

## 2 Method protocols

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

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

### 2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 6887-series parts 1, 2, 3, 4 and 5. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution were also 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 at least two relevant plates were available for use in calculations.

### 2.2 Alternative method

See the flow diagram in Annex A

The alternative method principle is based on chromogenic media.

One Plate for Total Viable Count (OP-TVC) offers a rapid method for the enumeration of total aerobic mesophiles using traditional culture methodology. A supplement is added to the agar base post-sterilisation which colours colonies red, which improves the ability to identify and enumerate colonies. The agar can be used with a surface plating technique with 0.1ml volumes and a pour plating technique with 1ml volumes plated. After incubation at 30°C ±1°C for 36-48 hours, all colonies are counted. The enumeration of aerobic plate count using OP TVC was calculated from one dilution on a single plate. During the study multiple dilutions were plated for each food item. When two counts within the acceptable counting range were available, then the dilution closest to 150 cfu/plate was selected for the calculation.

In this validation study, the minimum incubation time of 36 hours and the maximum incubation time of 48 hours was used for the alternative method (OP TVC). Four plating and incubation time combinations were evaluated during this study; 0.1ml spread plate for 36h, 0.1ml spread plate for 48h, 1ml pour plate for 36h and 1ml pour plate for 48h.

### 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 Method 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 contaminated samples. Different categories, types and items were tested for this.

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

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

#### 3.2.1 Number of samples

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

**Table 1 – Categories, types and number of samples analyzed**

Category	Types	Items	No of samples	ISO 6887 and Diluent used
Pasteurised dairy products	Pasteurised milk and milk based products	Pasteurised whole milk and skimmed milk	5	6887-5 MRD
	Pasteurised dairy products	ice-cream, milk based drinks	5	6887-5 MRD
	Dry milk products	Milk powder, dessert powder	5	6887-5 MRD

Category	Types	Items	No of samples	ISO 6887 and Diluent used
Raw fishery products	Raw fish (unprocessed)	Raw salmon filet, tuna	5	6887-3 MRD
	RTC fish and seafoods	Fish cakes, fish fingers	5	6887-3 MRD
	Raw crustaceans	Shrimp, crab	5	6887-3 MRD
Produce and fruits (combined category: fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot, radish sprouts, alfalfa	5	6887-4 MRD
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	6887-4 MRD
	Heat treated fruit and vegetables	Pasteurised smoothies/juice, blanched frozen vegetables	5	6887-4 MRD
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, sandwiches	5	6887-1 MRD
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products,	5	6887-1 MRD
	Mayonnaise based deli-salads	Vegetable salad	5	6887-1 MRD
Raw and Ready to Cook RTC Meat and poultry (combined: raw meat and poultry)	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	5	6887-2 MRD
	Raw processed meat	Frozen burger patties, pork meat balls, seasoned raw meat, lamb mince	5	6887-2 MRD
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	6887-2 MRD

75 samples were analyzed, leading to 75 exploitable results.

### 3.2.2 Test sample preparation

100% of the food items used in this study were Naturally contaminated.

### 3.2.3 Protocols applied during the validation study

#### Incubation time

Reference method plates were incubated at 30±1°C for 72±3h.

Alternative method was incubated at  $30\pm1^{\circ}\text{C}$  for two times 36h and 48h.

No confirmations were needed for the alternative and reference method.

### 3.2.4 Test results

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

### 3.2.5 Calculation and interpretation of relative trueness study

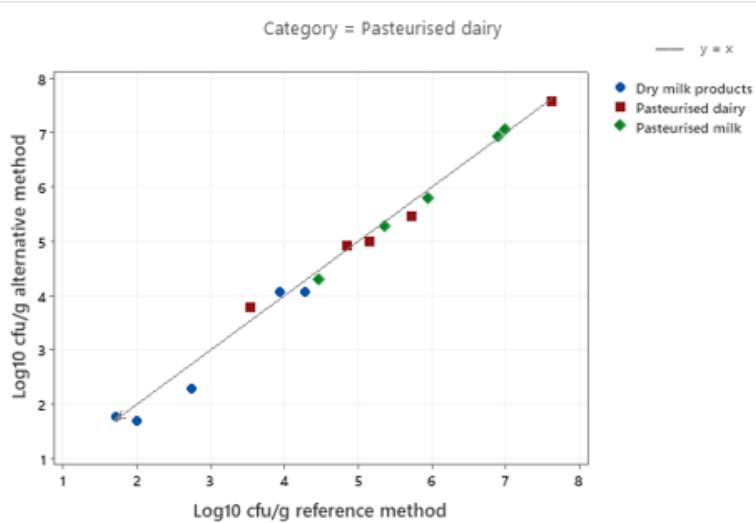
The calculations are provided in Annex B.

#### **0.1ml spread plate with 36h incubation**

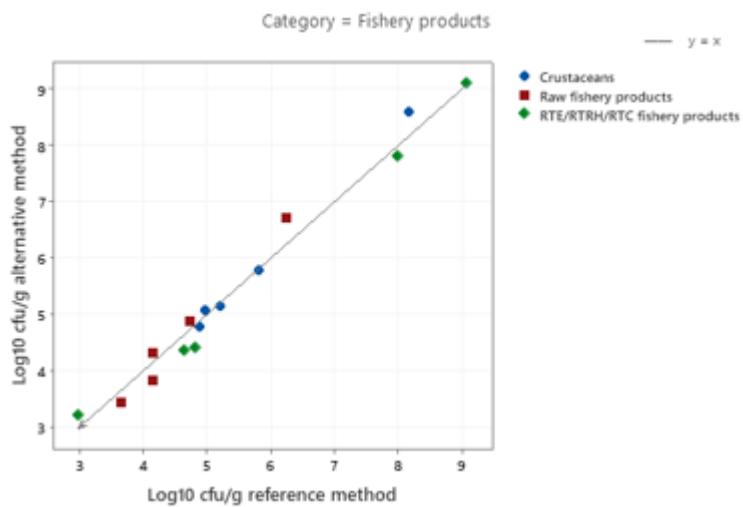
The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ). Data for OPTVC 0.1ml pour plates incubated at  $30^{\circ}\text{C}$  for 36h are given in figure 1a-e.

The Figure 1f shows the scatter plot for all the categories.

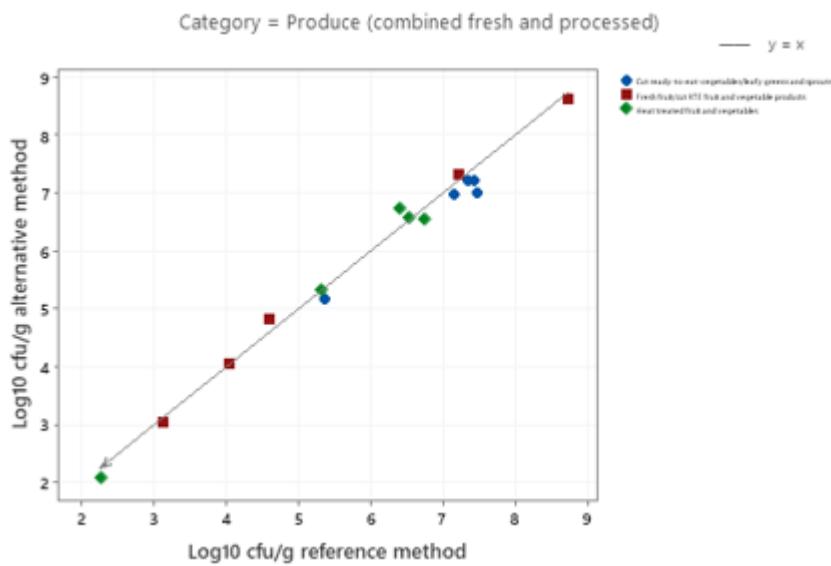
**Figure 1a - Scatter plot of the reference method versus OPTVC results for the pasteurised Dairy products 0.1ml spread plate at 36h incubation**



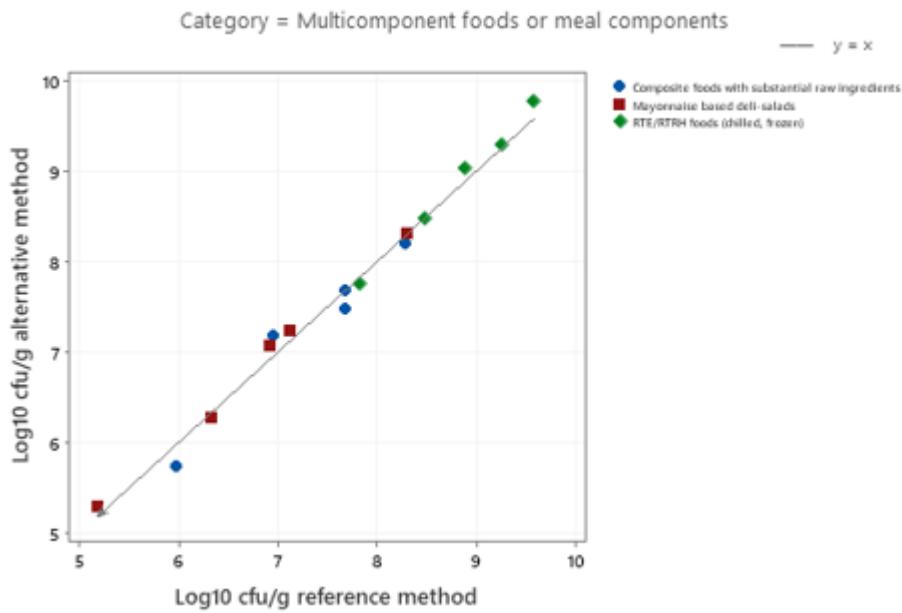
**Figure 1b - Scatter plot of the reference method versus OPTVC results for the Raw fishery products 0.1ml spread plate at 36h incubation**



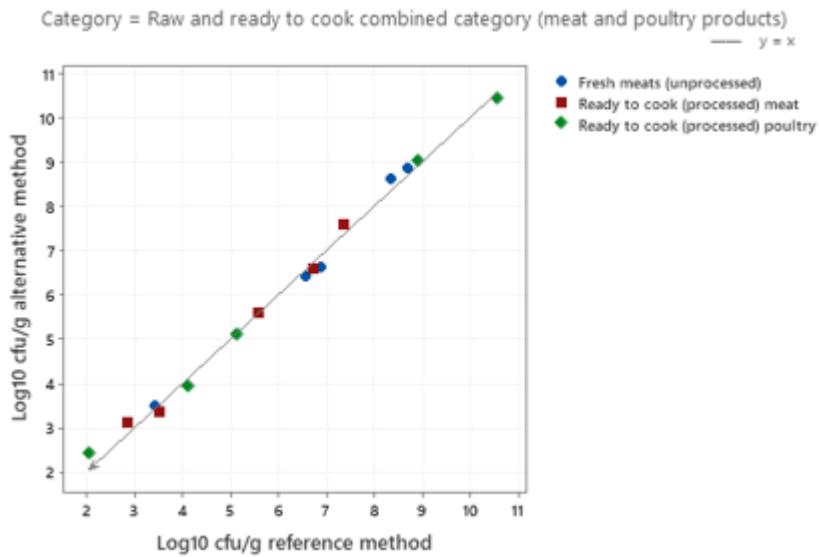
**Figure 1c - Scatter plot of the reference method versus OPTVC results for the Produce and fruits (combined category fresh and processed) 0.1ml spread plate with 36h incubation**



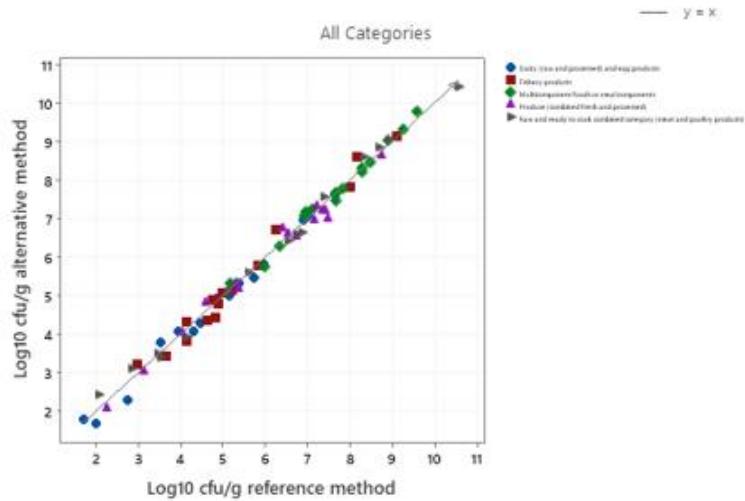
**Figure 1d - Scatter plot of the reference method versus OPTVC results for the Multi-component foods or meal components 0.1ml spread plate with 36h incubation**



**Figure 1e - Scatter plot of the reference method versus OPTVC results for the Raw and Ready to cook RTC Meat and poultry 0.1ml spread plate with 36h incubation**



**Figure 1f - Scatter plot of the reference method versus OPTVC results for all the categories using 0.1ml spread plate with 36h incubation**



According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plots show. The scatter plots show good agreement between the reference and alternative methods with minimal bias of -0.010 observed for the 5 food categories tested.

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

**Table 2 - Summary of the calculated values per category for 0.1ml spread plate with 36h incubation**

Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Pasteurised dairy products	15	-0.069	0.190	-0.490	0.352
Fishery products	15	0.008	0.265	-0.579	0.596
Multicomponent foods or meal components	15	0.027	0.140	-0.283	0.337
Produce (combined fresh and processed)	15	-0.063	0.200	-0.506	0.380
Raw and ready to cook combined category (meat and poultry products)	15	0.044	0.192	-0.381	0.469
<b>All Categories</b>	<b>75</b>	<b>-0.010</b>	<b>0.201</b>	<b>-0.415</b>	<b>0.394</b>

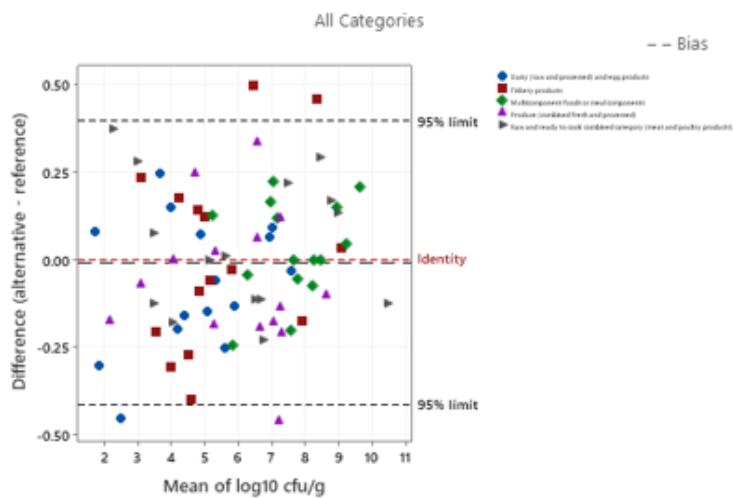
$\bar{D}$  : Average difference

SD: standard deviation of differences

n: number of samples

The Bland-Altman difference plot for all the samples is given Figure 1g.

**Figure 1g – Bland-Altman difference plot for all the samples for 0.1ml spread plate at 36h incubation**



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 0.1ml spread plate at 36h incubation**

Sample	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Lower / Upper limits	Comments
basa fillets	T16	6.23	6.72	6.48	0.49	<b>0.39</b>	Natural contamination
Cooked& Peeled King Prawns	T27	8.15	8.60	8.37	0.46	<b>0.39</b>	
non fat skim milk	T75	2.73	2.28	2.50	-0.45	<b>-0.42</b>	
mixed leaf salad	T34	7.46	7.00	7.23	-0.46	<b>-0.42</b>	

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 4 data points from a total of 75 data points which were outside of the accepted limits. The samples with values outside of the accepted limits belonged to three out of the five food categories indicating that these results are random outliers within the analysis.

Additional analysis showed that all four samples with differences outside the calculated limits are within 0.5 log. There is no indication of systematic bias in this study, with an equal number of values outside the limits showing either positive or negative log differences. As a result of the good agreement between the reference and alternative methods, the calculated acceptability limits are relatively narrow at -0.42 and 0.39 log with a minimal bias of -0.010 observed.

### 3.2.6 Conclusion (RT study OPTVC 0.1ml spread plate with 36h incubation)

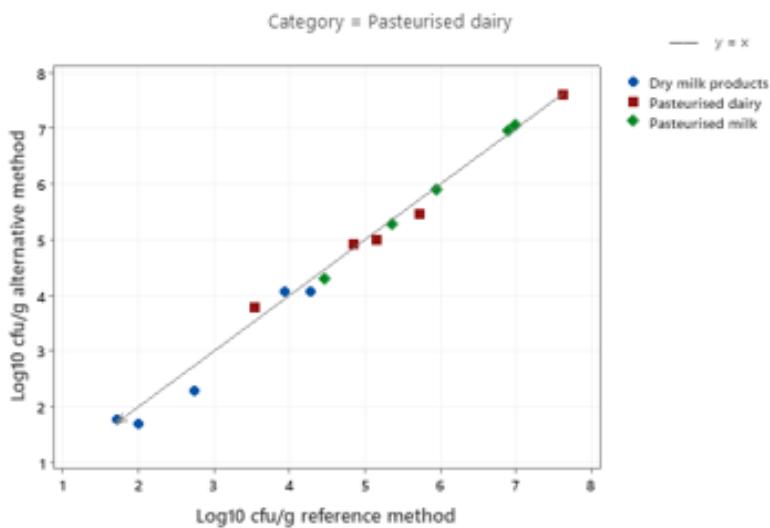
The relative trueness of the Alternative method is satisfied as it shows comparative performance to the reference method. Whilst the expectation of not more than 1 in 20 data points outside of the acceptability limits was not met there is no trend indication of systematic bias regarding sample type. The bias on each category is minimal.

### 0.1ml spread plates with 48h incubation

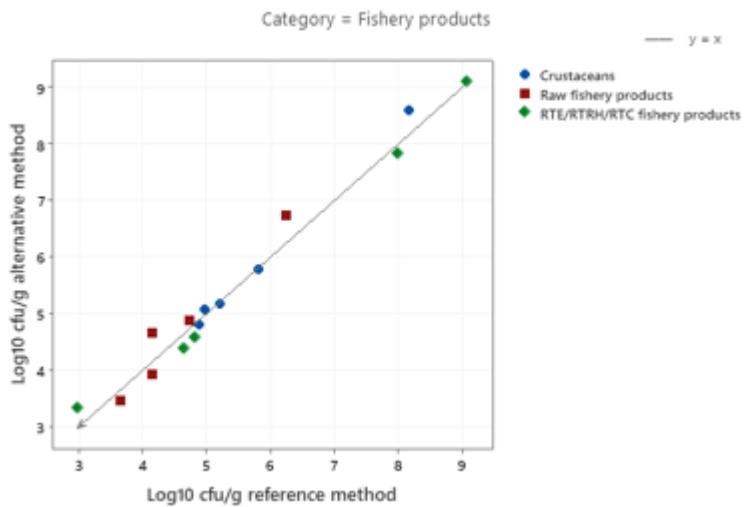
The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ). Data for OPTVC 0.1ml spread plates incubated at 30°C for 48h are given in figure 2a-e.

Figure 2f shows the scatter plot for all the categories.

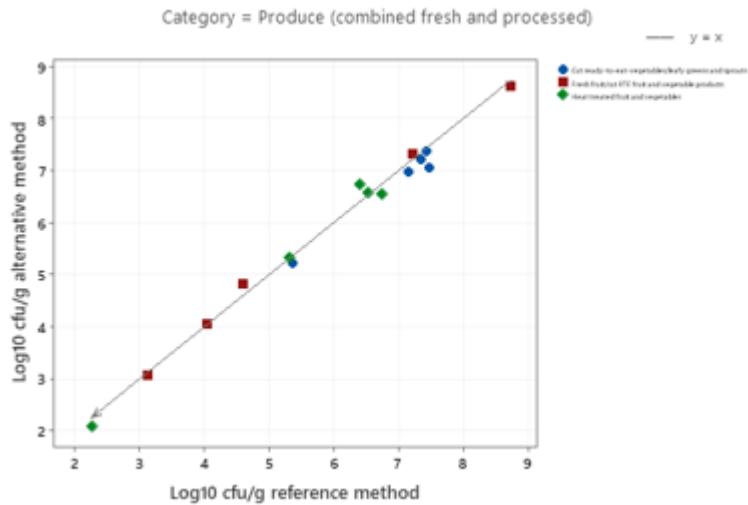
**Figure 2a - Scatter plot of the reference method versus OPTVC results for the pasteurised Dairy products 0.1ml spread plate with 48h incubation**



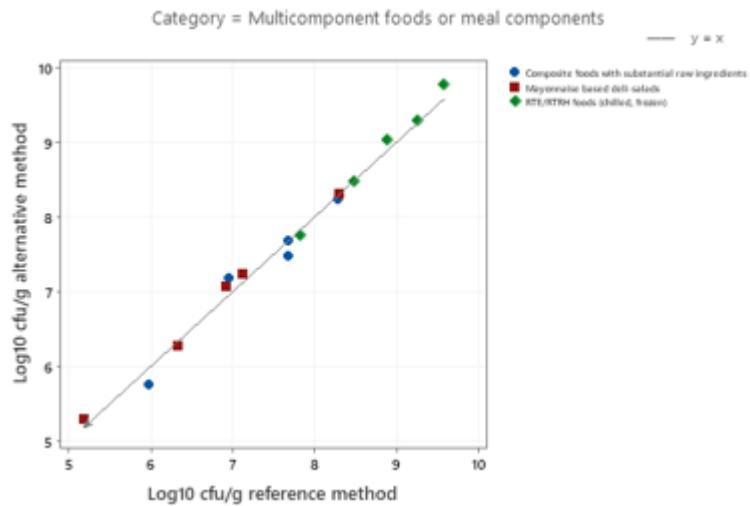
**Figure 2b - Scatter plot of the reference method versus OPTVC results for the raw fishery products 0.1ml spread plate with 48h incubation**



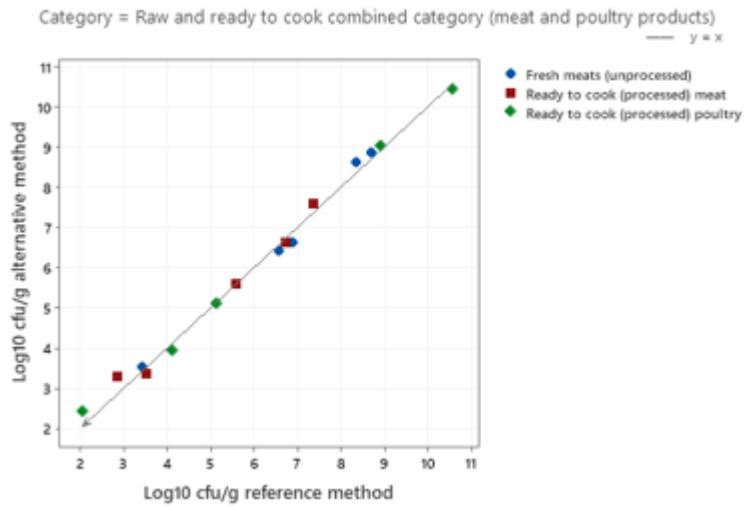
**Figure 2c - Scatter plot of the reference method versus OPTVC results for the Produce and fruits (combined category fresh and processed) 0.1ml spread plate with 48h incubation**



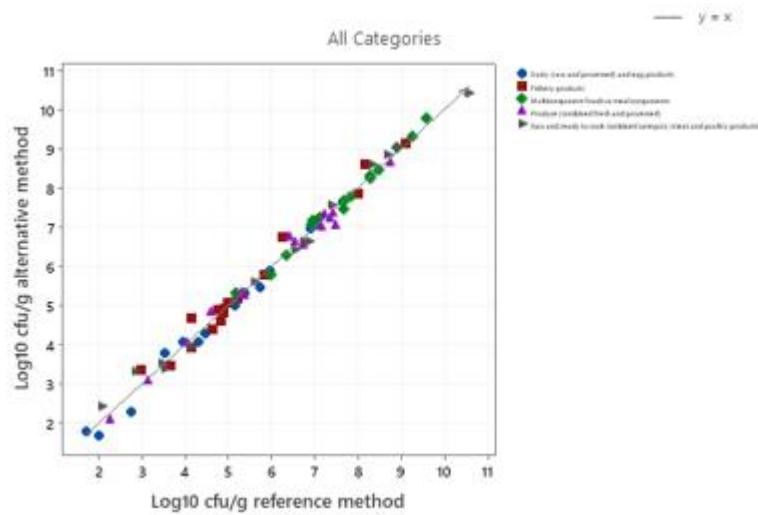
**Figure 2d - Scatter plot of the reference method versus OPTVC results for the Multi-component foods or meal components 0.1ml spread plate with 48h incubation**



**Figure 2e - Scatter plot of the reference method versus OPTVC results for the Raw and Ready to cook RTC Meat and poultry 0.1ml spread plate with 48h incubation**



**Figure 2f - Scatter plot of the reference method versus OPTVC results for all the categories for 0.1ml spread plate with 48h incubation**



According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plot show good agreement between the reference and alternative methods with minimal bias of 0.011 observed for the 5 food categories.

A summary of the calculated values per category is provided in Table 4.

**Table 4 - Summary of the calculated values per category for 0.1ml spread plate with 48h incubation**

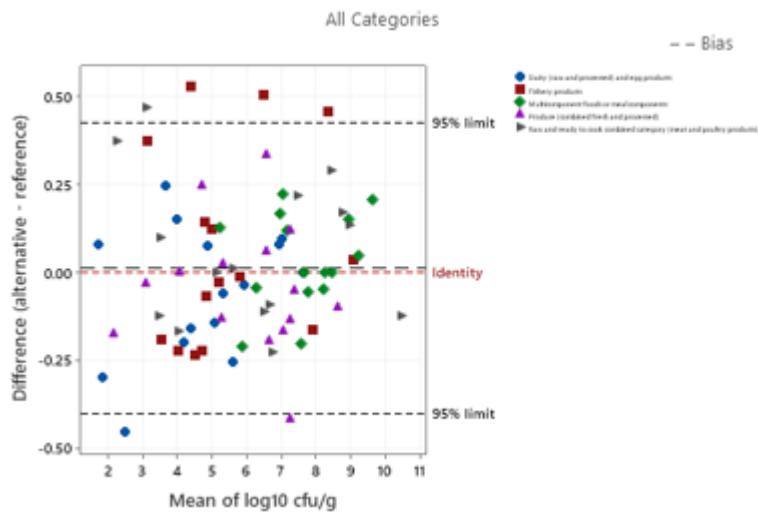
Category.	n	Dbar	sD	95% Lower limit	95% Upper limit
Pasteurised dairy products	15	-0.060	0.191	-0.482	0.363
Fishery products	15	0.067	0.276	-0.544	0.679
Multicomponent foods or meal components	15	0.031	0.134	-0.267	0.329
Produce (combined fresh and processed)	15	-0.043	0.187	-0.457	0.371
Raw and ready to cook combined category (meat and poultry products)	15	0.061	0.212	-0.408	0.529
<b>All Categories</b>	<b>75</b>	<b>0.011</b>	<b>0.206</b>	<b>-0.403</b>	<b>0.425</b>

$\bar{D}$  : Average difference

SD: standard deviation of differences n: number of samples

The Bland-Altman difference plot for all the samples is given Figure 2g.

**Figure 2g – Bland-Altman difference plot for all the samples for 0.1ml spread plate with 48h incubation**



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 0.1ml spread plate at 48h incubation**

Type	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Lower / Upper limits	Comments
beef burgers	T9	2.83	3.30	3.07	0.47	<b>0.43</b>	Naturally contaminated
basa fillets	T16	4.15	4.67	4.41	0.53	<b>0.43</b>	
sea bass fillets	T17	6.23	6.73	6.48	0.50	<b>0.43</b>	
Peeled King Prawns	T27	8.15	8.60	8.37	0.45	<b>0.43</b>	
non fat skim milk	T75	2.73	2.28	2.50	-0.45	<b>-0.40</b>	
mixed leaf salad	T34	7.46	7.04	7.25	-0.42	<b>-0.40</b>	

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 6 data points from a total of 75 data points which were outside of the accepted limits. The samples with values outside of the accepted limits belonged to four out of the five food categories indicating that these results are random outliers within the analysis.

Additional analysis showed that five out of the six samples with differences outside the calculated limits are within 0.5 log. There is no indication of systematic bias in this study, with a minimal bias of 0.011 observed for the 5 food categories. As a result of the good agreement between the reference and alternative methods, the calculated acceptability limits are relatively narrow at -0.40 and 0.43 log.

### 3.2.7 Conclusion (RT study OPTVC 0.1ml spread plate with 48h incubation)

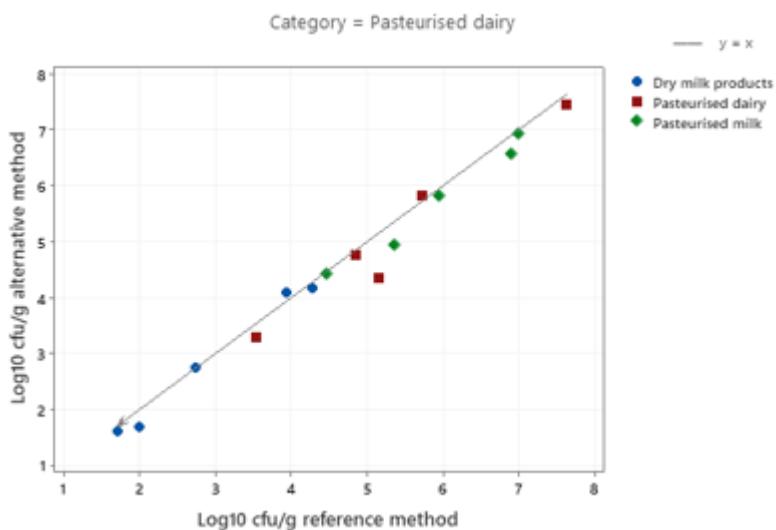
The relative trueness of the Alternative method is satisfied as it shows comparative performance to the reference method. Whilst the expectation of not more than 1 in 20 data points outside of the acceptability limits was not met, there is no trend indication of systematic bias regarding sample type. The bias on each category is minimal.

### 1ml pour plate with 36h incubation

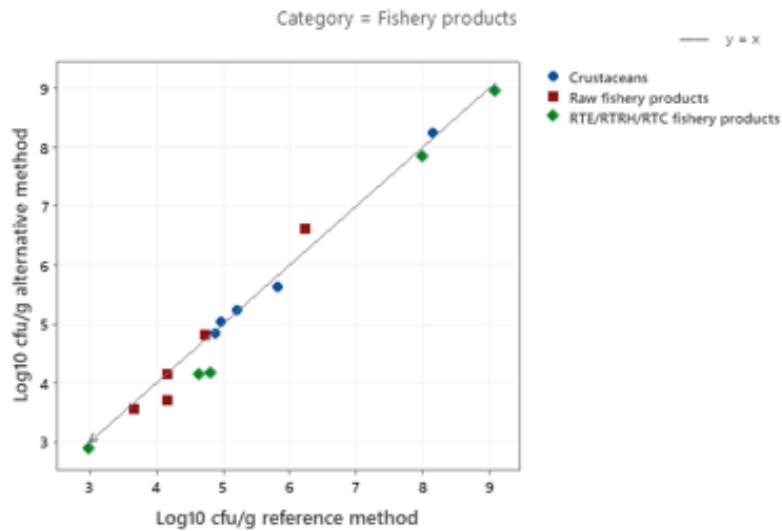
The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ). Data for OPTVC 1ml pour plates incubated at 30°C for 36h are given in figure 3a-e

Figure 3f shows the scatter plot for all the categories.

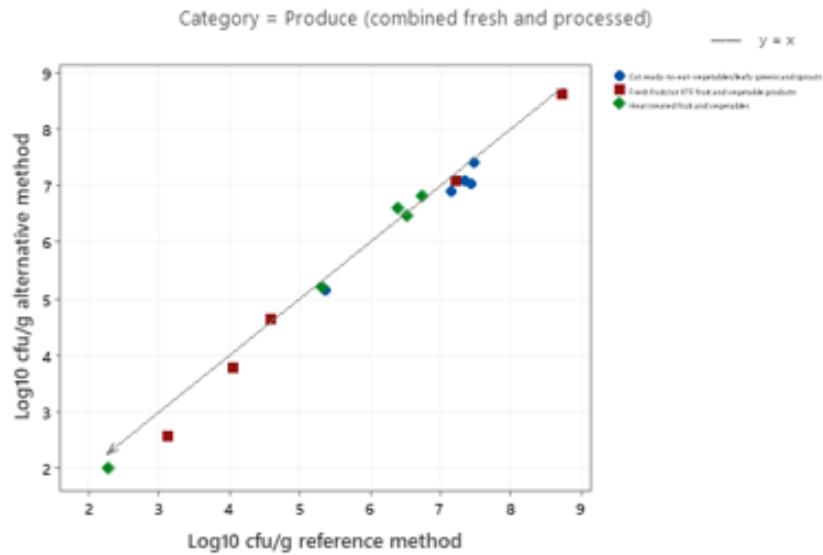
**Figure 3a - Scatter plot of the reference method versus OPTVC pour plate method results for the pasteurised Dairy products 1ml pour plate with 36h incubation**



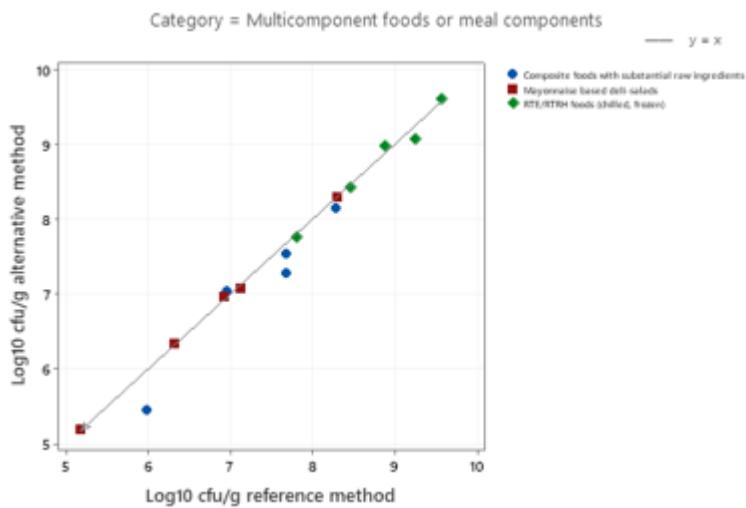
**Figure 3b - Scatter plot of the reference method versus OPTVC pour plate method results for the raw fishery products category 1ml pour plate at 36h incubation**



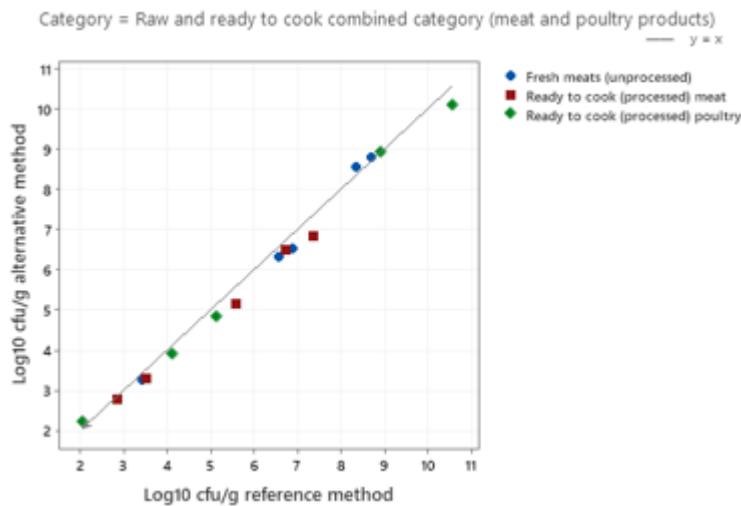
**Figure 3c - Scatter plot of the reference method versus OPTVC pour plate method results for the Produce and fruits (combined category fresh and processed) 1ml pour plate at 36h incubation**



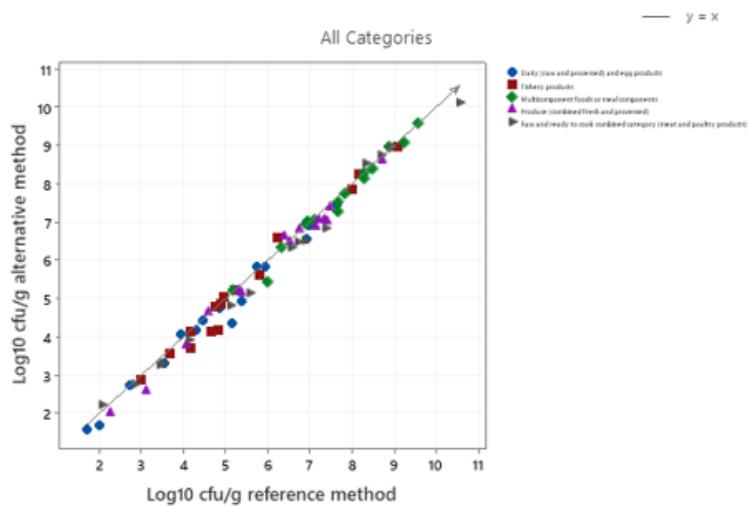
**Figure 3d - Scatter plot of the reference method versus OPTVC pour plate method results for the Multi-component foods or meal components 1ml pour plate at 36h incubation**



**Figure 3e - Scatter plot of the reference method versus OPTVC pour plate method results for the Raw and Ready to cook RTC Meat and poultry 1ml pour plate at 36h incubation**



**Figure 3f - Scatter plot of the reference method versus OPTVC pour plate method results for all the categories using 1ml pour plate at 36h incubation**



According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plot show good agreement between the reference and alternative methods with negative bias of -0.135 observed for the 5 food categories. One possible explanation for the negative bias is the shorter incubation time compared to the reference method. This conclusion is supported by the 48h results, where an improvement is seen in the negative bias reported for the 5 food categories at 48h compared to 36h incubation time .

A summary of the calculated values per category is provided in table 6.

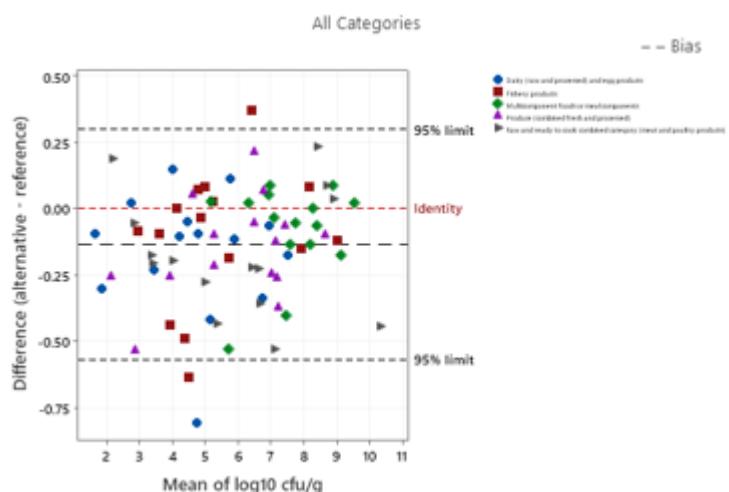
**Table 6- Summary of the calculated values per category for 1ml pour plate at 36h incubation**

Category	n	Dbar	sD	95% Lower limit	95% Upper limit
Pasteurised dairy products	15	-0.167	0.235	-0.688	0.354
Raw fishery products	15	-0.106	0.255	-0.672	0.459
Multicomponent foods or meal components	15	-0.082	0.177	-0.474	0.309
Produce (combined fresh and processed)	15	-0.150	0.187	-0.565	0.264
Raw and ready to cook combined category (meat and poultry products)	15	-0.171	0.230	-0.681	0.339
<b>All Categories</b>	<b>75</b>	<b>-0.135</b>	<b>0.216</b>	<b>-0.568</b>	<b>0.298</b>

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

The Bland-Altman difference plot for all the samples is given Figure 3g.

**Figure 3g – Bland-Altman difference plot for all the samples for 1ml pour plate at 36h incubation**



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 7.

**Table 7 - Data which are outside of the accepted limits for 1ml pour plate at 36h incubation**

Type	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference	Lower / Upper limits	Comments
sea bass fillets	T17	6.23	6.60	6.42	0.37	0.30	Naturally contaminated
battered haddock fillet goujons	T25	4.81	4.18	4.49	-0.64	-0.57	
butter milk	T69	5.15	4.34	4.74	-0.80	-0.78	

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 3 data points from a total of 75 data points which are within the expectation. The samples with values outside

of the accepted limits belonged to two out of the five food categories, indicating that they are random outliers in the analysis.

It was noted that the buttermilk sample has a high log difference (-0.80). To investigate the outlying buttermilk result, the effect of acidity on the alternative method was assessed. Other acidic products were tested in the study including soured cream (pasteurized dairy type), fresh cut fruit and heat-treated fruit. Large log differences between the reference and alternative methods were not observed in any other acidic sample tested in the study. After running an additional test on buttermilk, using MRD (pH adjusted and not pH adjusted) and BPW (pH adjusted and not pH adjusted) there was little difference between samples that were pH adjusted and not adjusted. The results do not suggest that acidity of the product effects the performance of the method.

Although acidic products had a minimal impact on the performance of One Plate TVC, it was noted that the log difference between the alternative and reference method was still relatively high. Additional root cause analysis was carried out to determine possible explanations for the high log difference between reference and alternative methods observed. Additional buttermilk samples sourced from other manufacturers were tested to determine the possibility of a trend with buttermilk samples producing high log differences between methods, the results are shown in Table 8.

**Table 8 - Additional buttermilk samples tested**

Sample code	Sample	Ingredients listed	Log difference (alternative – reference)			
			Spread 36h	Spread 48h	Pour 36h	Pour 48h
T76	Buttermilk 2	Buttermilk	0.01	0.01	-0.13	-0.11
T77	Buttermilk 3	Skimmed Cows' Milk (95%), Skimmed Cows' Milk Powder, Starter Culture	-0.09	-0.09	-0.12	-0.12

Table 8 shows that the additional buttermilk samples tested did not produce high log differences between the reference and alternative methods. The results of the additional buttermilk samples indicate that the original buttermilk tested was an outlier.

As buttermilk is a fermented product, the potential trend of the effect of fermented products on the performance of One Plate TVC was also investigated. It was noted that there were minimal fermented products tested in the MCS, therefore four additional fermented products were tested, and the results are shown in Table 9.

**Table 9 – Additional fermented products tested**

Sample code	Sample	Log difference (alternative – reference)			
		Spread 36h	Spread 48h	Pour 36h	Pour 48h
T69	Soured cream	-0.25	-0.25	0.11	0.11
T80	Salami slices	0.17	0.19	0.03	0.11
T81	Salami milano	-0.18	-0.08	-0.29	-0.12
T82	Kimchi	-0.10	-0.10	-0.14	-0.11

Table 9 shows that there are no significant log differences between the alternative and reference methods, and therefore no indication of a systematic bias with One Plate TVC and fermented products.

To summarise, the conclusions of the root cause analysis are:

- A high log difference of buttermilk sample was also observed on repeat
- Other buttermilk samples produced comparable counts between alternative and reference methods
- There is not a trend for acidic products effecting the OP TVC method
- There is not a trend for fermented products being incompatible with the OP TVC method
- The buttermilk sample originally tested is an outlier

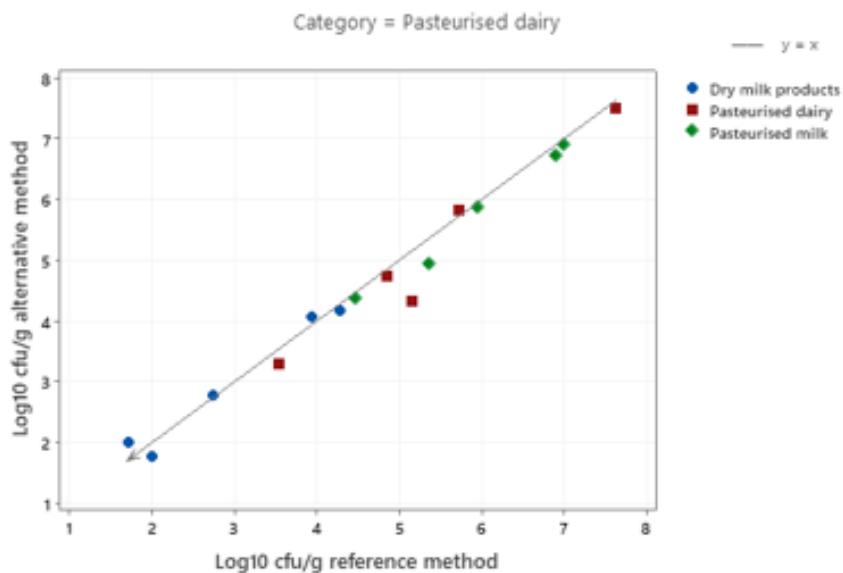
#### **Conclusion (RT study OPTVC 1ml pour at 36h incubation)**

The relative trueness of the Alternative method is satisfied as the expectation of not more than 1 in 20 data points outside of the acceptability limits is met. There is no trend indication of systematic bias regarding sample type or contamination procedures. The bias on each category is minimal.

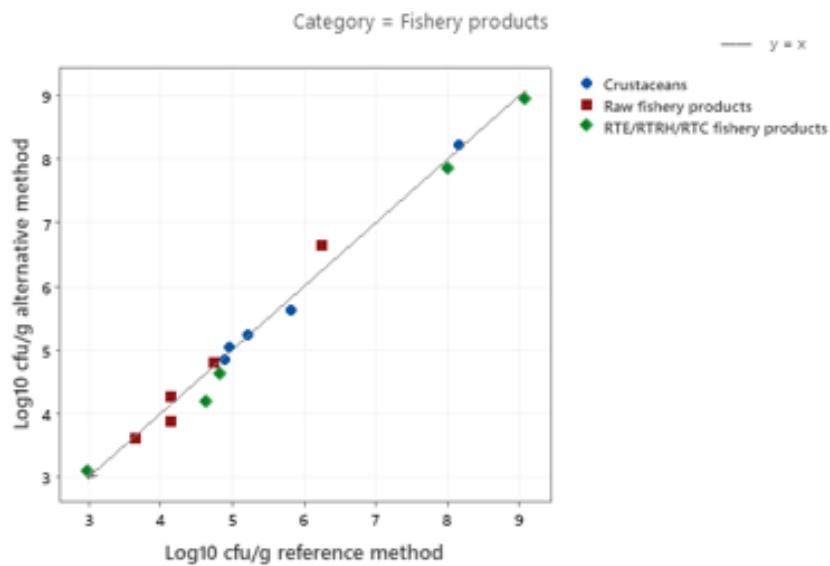
#### **1ml pour plates with 48h incubation**

The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ). Data for OPTVC 0.1ml pour plates incubated for 48h are given in figure 4a-e

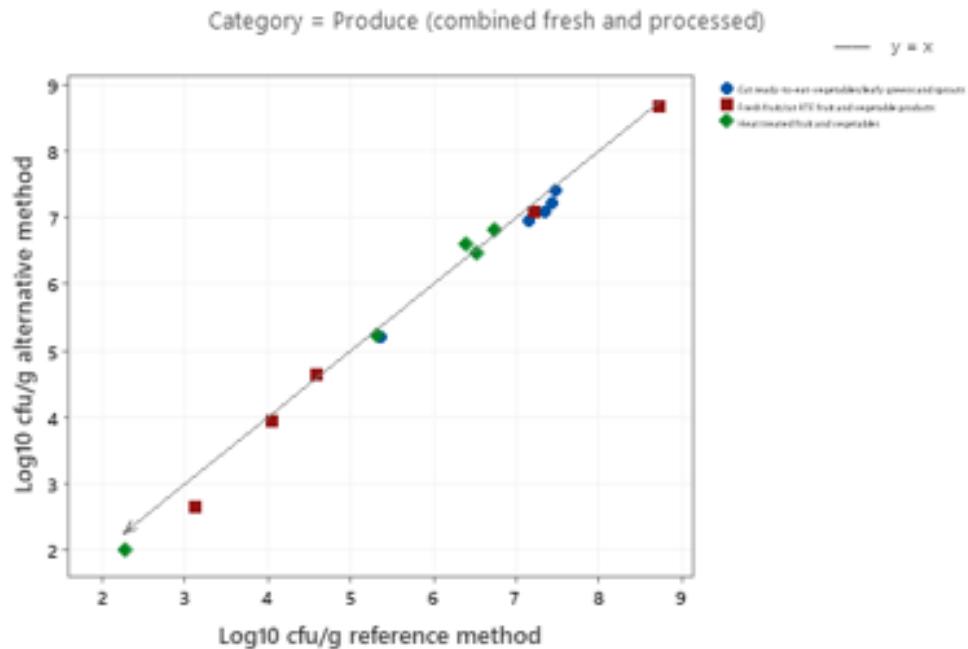
**Figure 4a - Scatter plot of the reference method versus OPTVC pour plate method results for the pasteurised Dairy products) 1ml pour plate at 48h incubation**



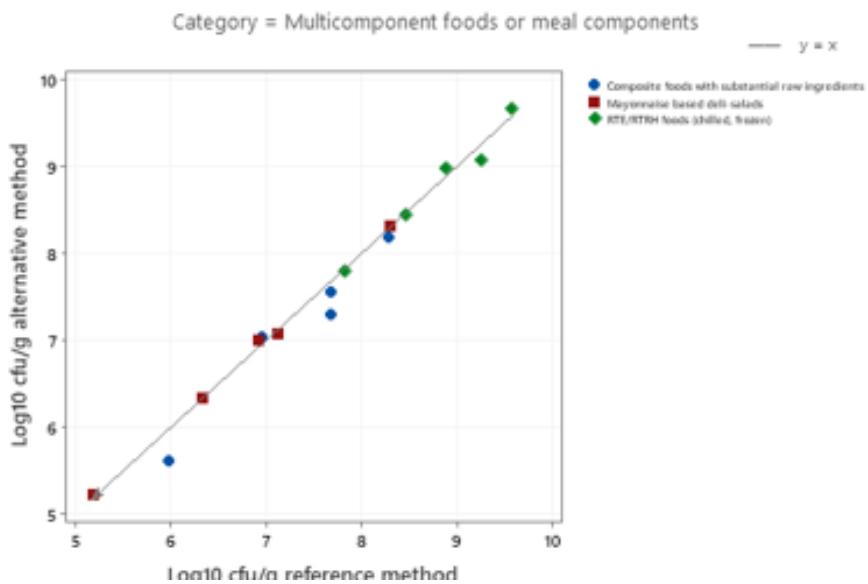
**Figure 4b - Scatter plot of the reference method versus OPTVC pour plate method results for the raw fishery products 1ml pour plate at 48h incubation**



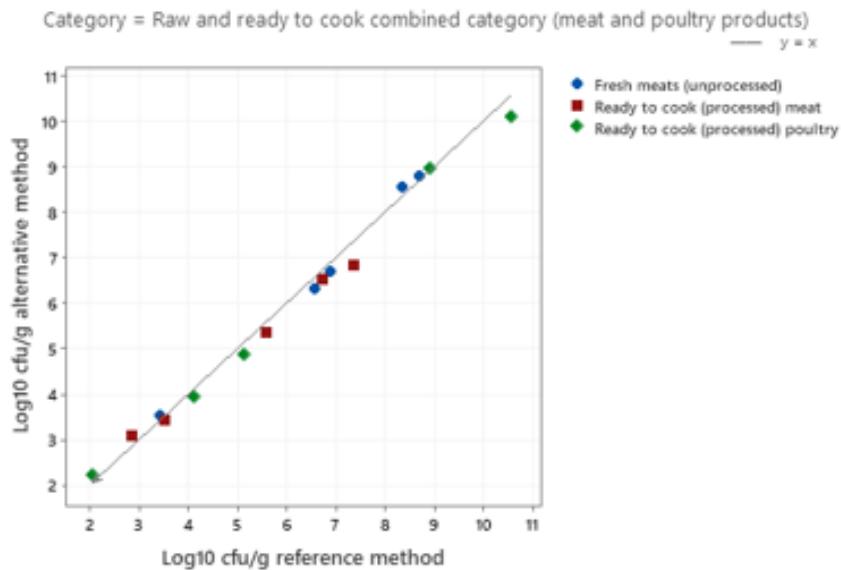
**Figure 4c - Scatter plot of the reference method versus OPTVC pour plate method results for the Produce and fruits (combined category fresh and processed) 1ml pour plate at 48h incubation**



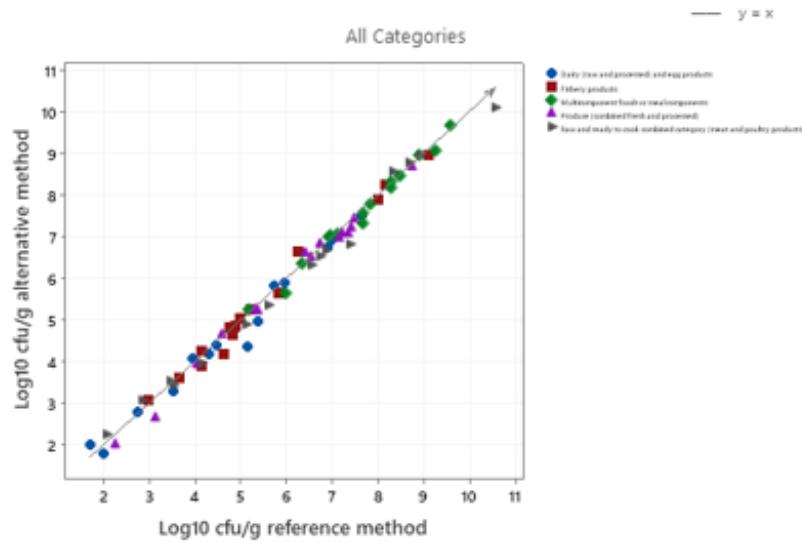
**Figure 4d - Scatter plot of the reference method versus OPTVC pour plate method results for the Multi-component foods or meal components 1ml pour plate at 48h incubation**



**Figure 4e - Scatter plot of the reference method versus OPTVC pour plate method results for the Raw and Ready to cook RTC Meat and poultry 1ml pour plate at 48h incubation**



**Figure 4f - Scatter plot of the reference method versus OPTVC pour plate method results for all the categories for 1ml pour plate at 48h incubation**



According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plot show good agreement between the reference and alternative methods with a small negative bias of -0.081 observed for the 5 food categories.

A summary of the calculated values per category is provided in table 10.

**Table 10 - Summary of the calculated values per category for 1ml pour plate at 48h incubation**

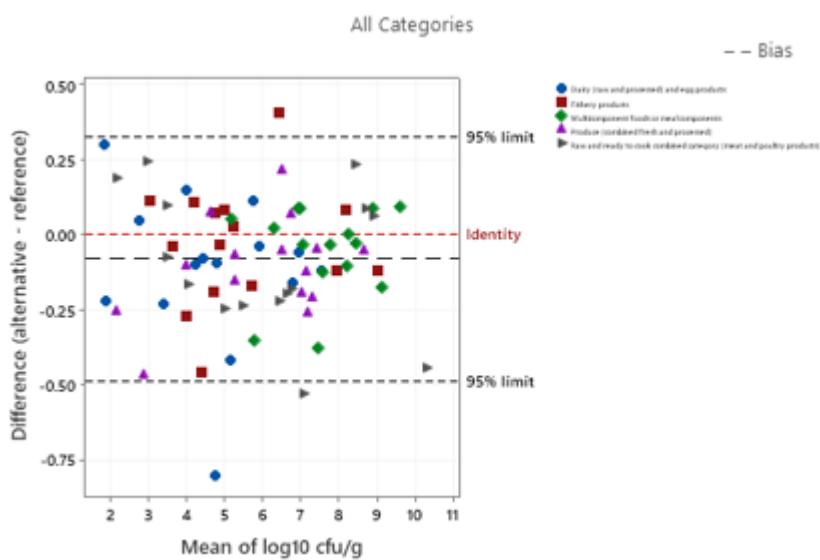
Category	n	Dbar	sD	95% Lower limit	95% Upper limit
Pasteurised dairy products	15	-0.115	0.257	-0.684	0.454
Raw fishery products	15	-0.035	0.202	-0.481	0.412
Multicomponent foods or meal components	15	-0.054	0.151	-0.390	0.281
Produce (combined fresh and processed)	15	-0.110	0.164	-0.474	0.253
Raw and ready to cook combined category (meat and poultry products)	15	-0.092	0.238	-0.619	0.436
<b>All Categories</b>	<b>75</b>	<b>-0.081</b>	<b>0.203</b>	<b>-0.489</b>	<b>0.327</b>

$\bar{D}$  : Average difference

SD: standard deviation of differences n: number of samples

The Bland-Altman difference plot for all the samples is given Figure 4g.

**Figure 4g – Bland-Altman difference plot for all the samples for 1ml pour plate at 48h incubation**



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 11.

**Table 11 - Data which are outside of the accepted limits for 1ml pour plate at 48h incubation**

Type	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Lower / Upper limits	Comments
sea bass fillets	T17	6.23	6.63	6.43	0.40	0.33	Naturally contaminated
bbq pork riblets	T6	7.36	6.83	7.10	-0.53	-0.49	
butter milk	T69	5.15	4.34	4.74	-0.80	-0.49	

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 3 data points from a total of 75 data points which are within the accepted limits. The samples with values outside of the accepted limits belonged to three out of the five food categories, indicating that they are random outliers in the analysis.

There is no indication of systematic bias in this study, with a minimal bias of -0.081 observed for the 5 food categories. As a result of the good agreement between the reference and alternative methods, the calculated acceptability limits are relatively narrow at -0.49 and 0.33 log.

It was noted that the buttermilk sample has a high log difference (-0.80). This was investigated in a root cause analysis on page 26. The conclusion was that this is an outlying sample and there is no indication of systematic bias of the One Plate TVC method with any of the following products or product types: buttermilk, acidic products, or fermented products.

### 3.2.8 Conclusion (RT study OPTVC 1ml pour plate with 48h incubation)

The relative trueness of the Alternative method is satisfied as the expectation of not more than 1 in 20 data points outside of the acceptability limits is met. There is no trend indication of systematic bias regarding sample type or contamination procedures. The bias on each category is minimal.

### 3.3 Accuracy profile study

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

**3.3.1 Categories, sample types and strains**

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 12.

**Table 12 - Categories, types, items, strains and inoculation levels for accuracy profile study**

Category	Types	inoculated Strain	Item
Pasteurised dairy products	Dry dairy products	<i>Bacillus cereus</i> CRA 1724 Isolated from Dried milk	Milk powder
			Dessert powder
Raw fishery products	RTC fish	<i>Pseudomonas fragi</i> CRA7222 Isolated from spoiled fish	Frozen white fish
			Chilled tuna steak
Produce and fruits (combined category fresh and processed)	Cut ready to eat	<i>E.coli</i> CRA3379 isoated from spinach	Lettuce
			Grated carrot
Raw and RTC Meat and poultry (Combined category)	Fresh meats	<i>Citrobacter freundii</i> CRA403 Isolated from chicken	Raw stir fry beef strips
			Chicken breast fillets
Multicomponent	Composite foods with raw /processed ingredients	<i>Hafnia alvei</i> CRA7417 Isolated from pate	Sandwich
			Pasta salad

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.

Five separate 10g test portions were removed from the bulk sample and mixed with 90ml MRD or appropriate diluent and enumerated on both methods as shown in Appendix A

### 3.3.2 Calculations and interpretation of accuracy profile study

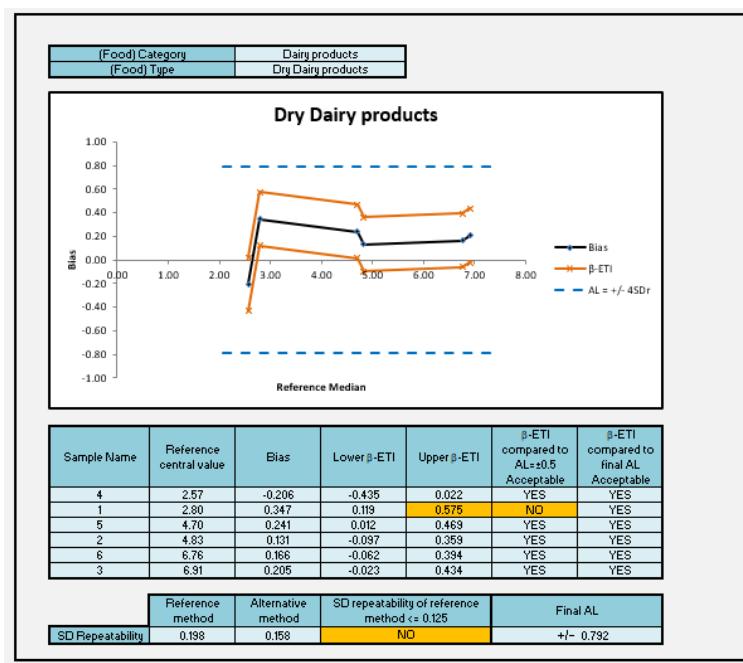
The summary tables (in log CFU/g) are provided Annex C. The statistical results and the accuracy profiles are provided Figures 5-8 a-e.

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>

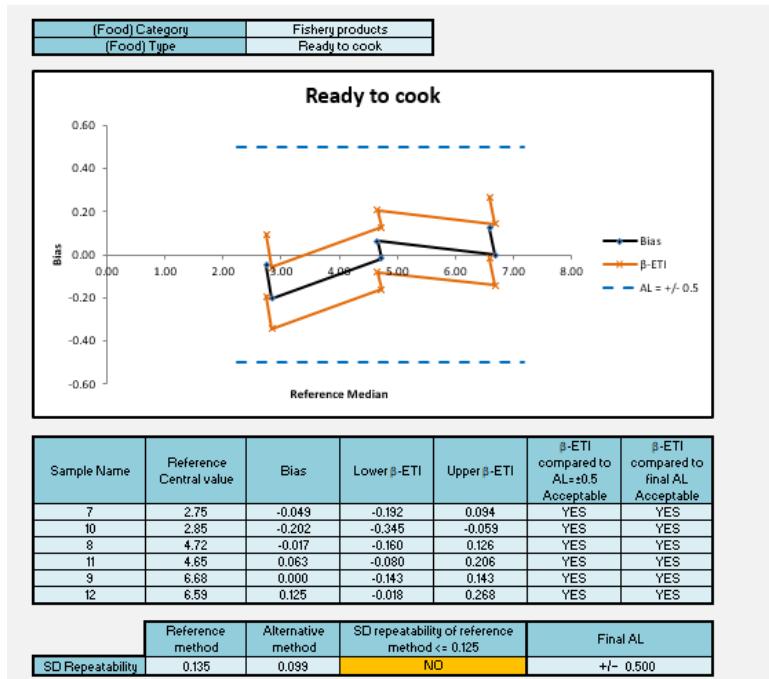
#### **0.1ml spread plate with 36h incubation**

The accuracy profile plots for 0.1ml spread plate 36h incubation are shown in Figures 5 a-e

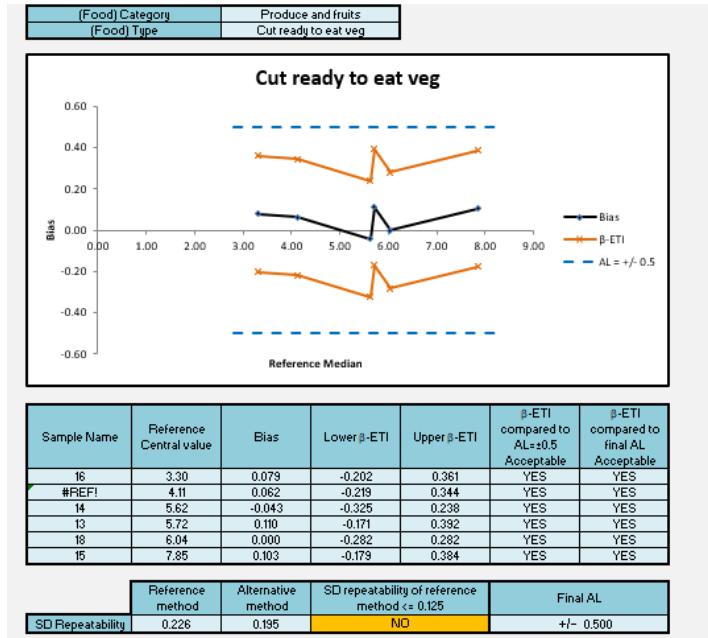
**Figure 5a - Accuracy profile of the reference method versus OPTVC results for the pasteurised dairy products 0.1ml spread plate with 36h incubation**



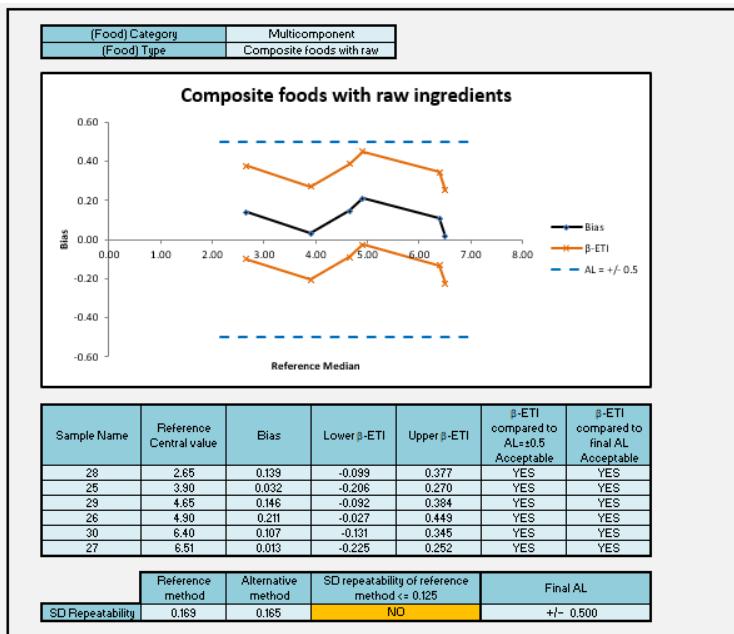
**Figure 5b - Accuracy profile of the reference method versus OPTVC results for the Fishery products 0.1ml spread plate with 36h incubation**



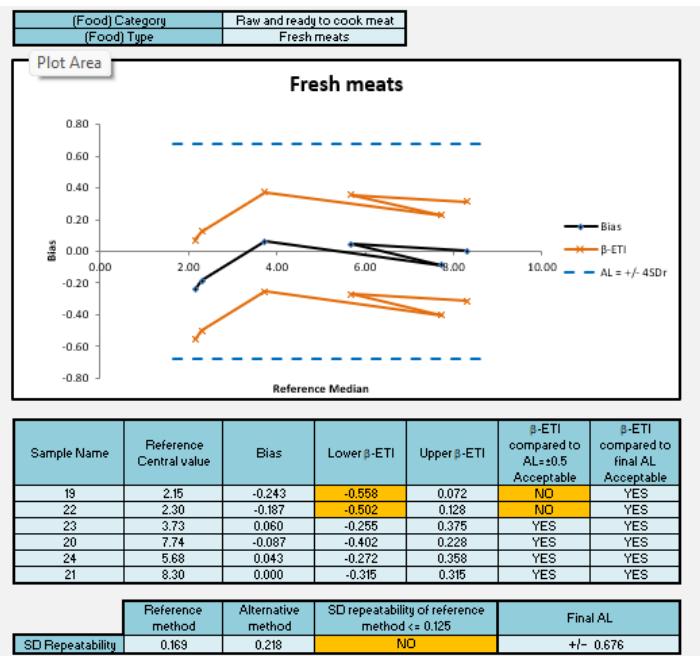
**Figure 5c - Accuracy profile of the reference method versus OPTVC results for the produce and fruits (combined category fresh and processed) 0.1ml spread plate with 36h incubation**



**Figure 5d - Accuracy profile of the reference method versus OPTVC results for the Multi-component foods or meal components 0.1ml spread plate with 36h incubation**



**Figure 5e - Accuracy profile of the reference method versus OPTVC results for the Raw and Ready to cook RTC Meat and poultry 0.1ml spread plate with 36h incubation**



In this study the following three categories met the AL of 0.5log Fishery products, fresh produce, and multicomponent foods.

In this study, the following categories required the new AL to be calculated fresh meats and dairy products. Both of these categories met the new AL value of 0.676 and 0.792 respectively.

The higher repeatability observed in the dairy category is likely due to variation in die off of the lyophilised culture in the drying process and during ambient storage of the sample.

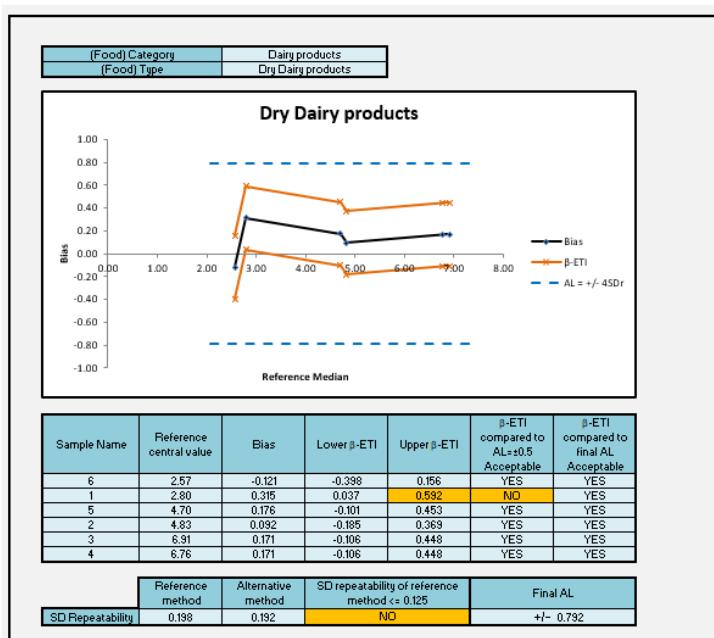
#### Conclusion (AP study) 0.1ml spread plate with 36h incubation.

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

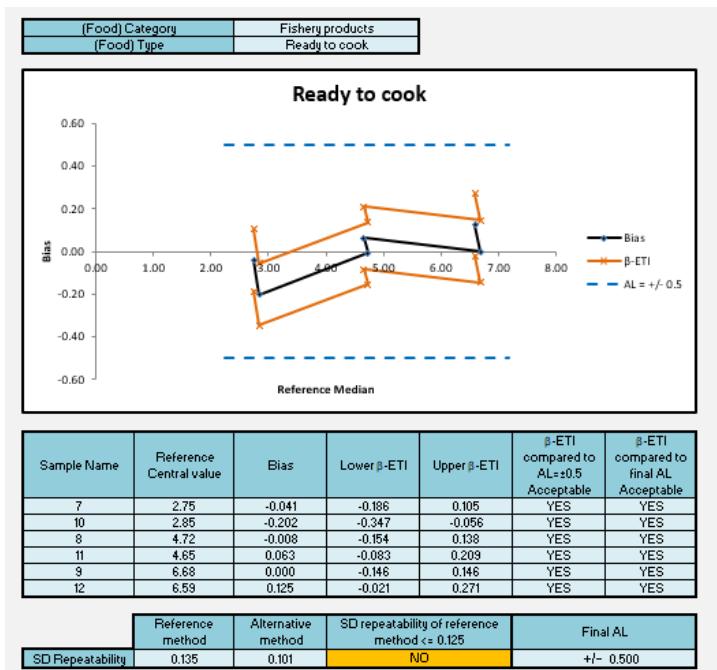
#### 0.1ml spread plate with 48h incubation

The accuracy profile plots for 0.1ml spread plate 48h incubation are shown in Figures 6 a-e

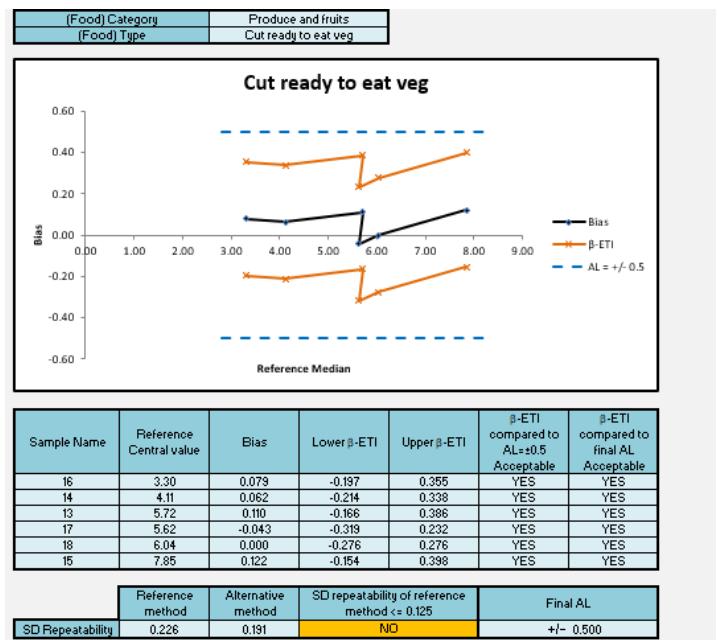
**Figure 6a - Accuracy profile of the reference method versus OPTVC results for the pasteurised dairy products 0.1ml spread plate with 48h incubation**



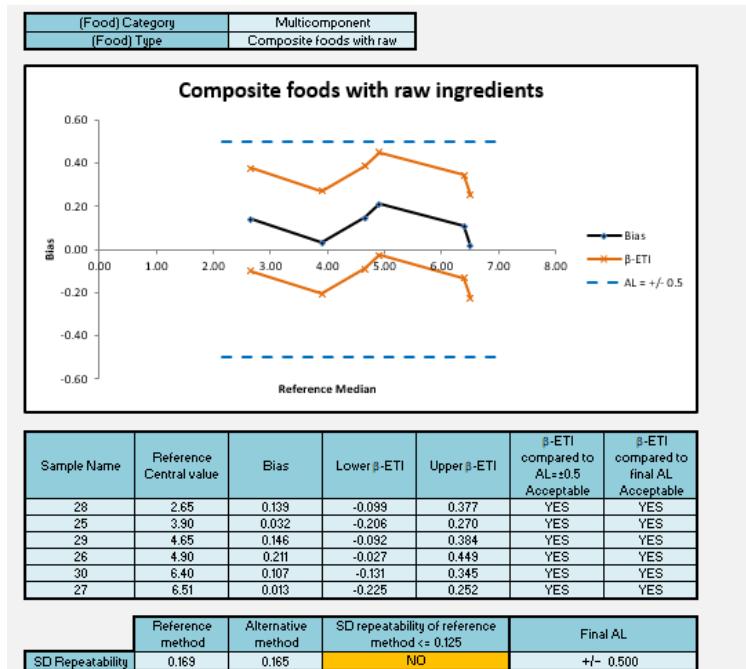
**Figure 6b - Accuracy profile of the reference method versus OPTVC results for the raw fishery products 0.1ml spread plate with 48h incubation**



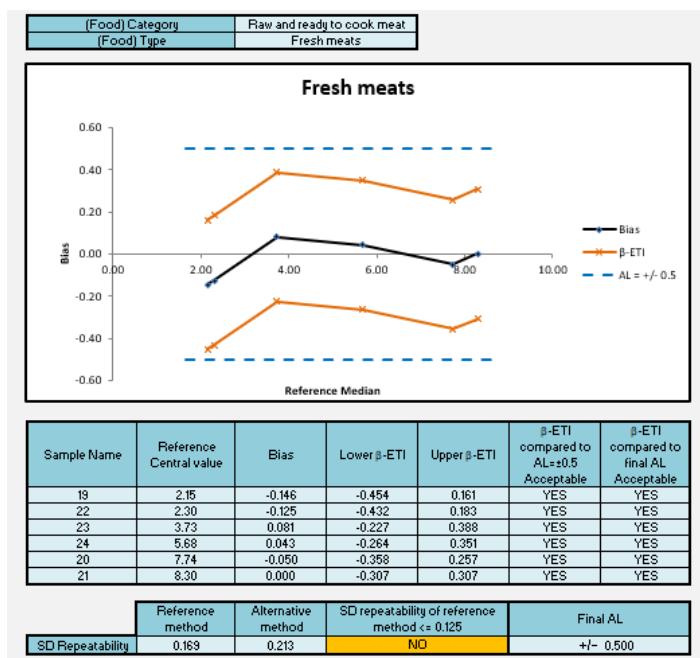
**Figure 6c - Accuracy profile of the reference method versus OPTVC results for the Produce and fruits (combined category fresh and processed) 0.1ml spread plate with 48h incubation**



**Figure 6d - Accuracy profile of the reference method versus OPTVC results for the Multi-component foods or meal components 0.1ml spread plate with 48h incubation**



**Figure 6e - Accuracy profile of the reference method versus OPTVC results for the Raw and Ready to cook RTC Meat and poultry 0.1ml spread plate with 48h incubation**



In this study for OP TVC 0.1ml spread plates with 48h incubation, four categories met the AL of 0.5log fishery products, fresh produce, multicomponent foods and raw and ready to eat meat.

In this study, the dairy products category required recalculation of the acceptability limits. This category met the new AL value of 0.792.

The higher repeatability observed in the dairy category is likely due to variation in die off of the lyophilised culture in the drying process and during ambient storage of the sample.

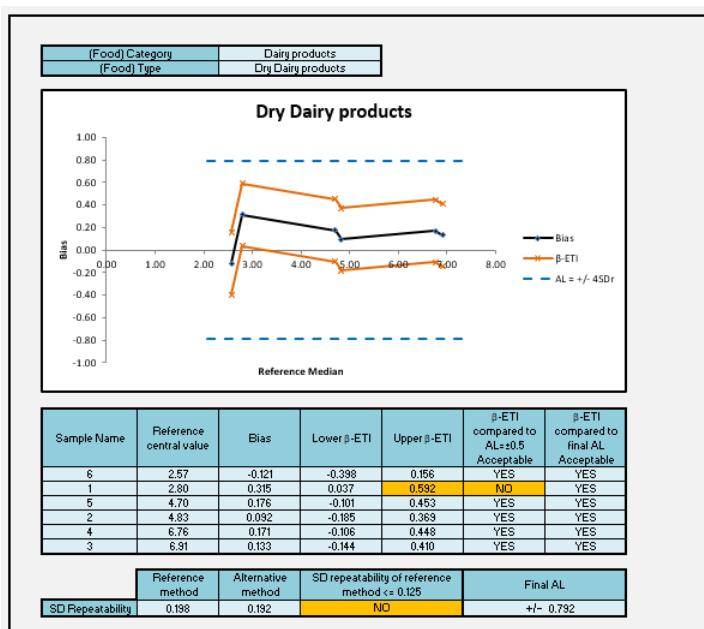
#### Conclusion (AP study) OP TVC 0.1ml spread plates with 48h incubation.

The accuracy of the Alternative method (OP TVC 0.1ml spread plates with 48h incubation) is satisfied as all categories met the 0.5log AL or the re-calculated AL

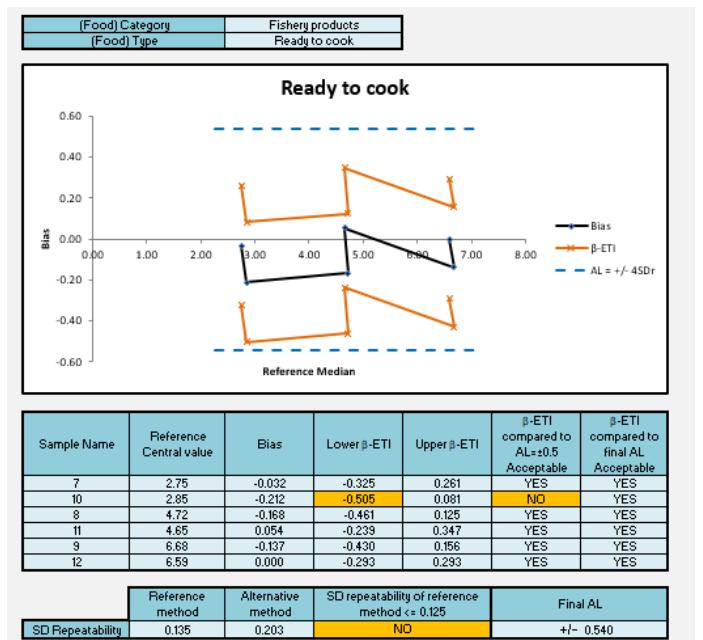
#### 1ml pour plate with 36h incubation

The accuracy profile plots for 0.1ml spread plate 36h incubation are shown in Figures 7 a-e

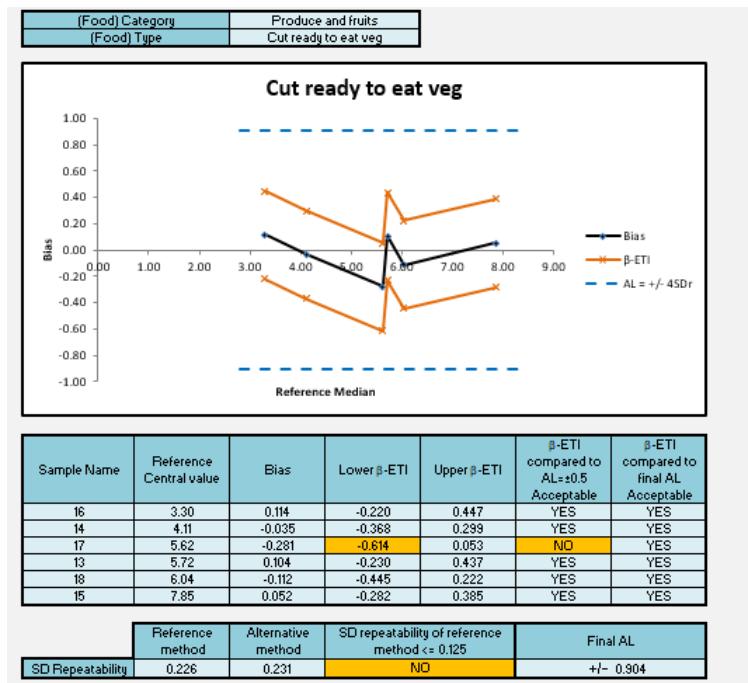
**Figure 7a - Accuracy profile of the reference method versus OPTVC pour plate method results for the pasteurised dairy products 1ml pour plate with 36h incubation**



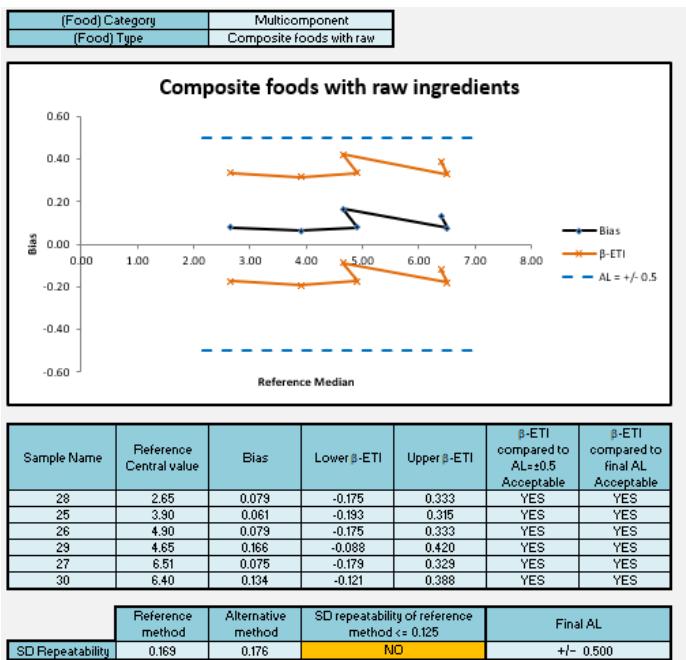
**Figure 7b - Accuracy profile of the reference method versus OPTVC pour plate method results for the raw fishery products 1ml pour plate with 36h incubation**



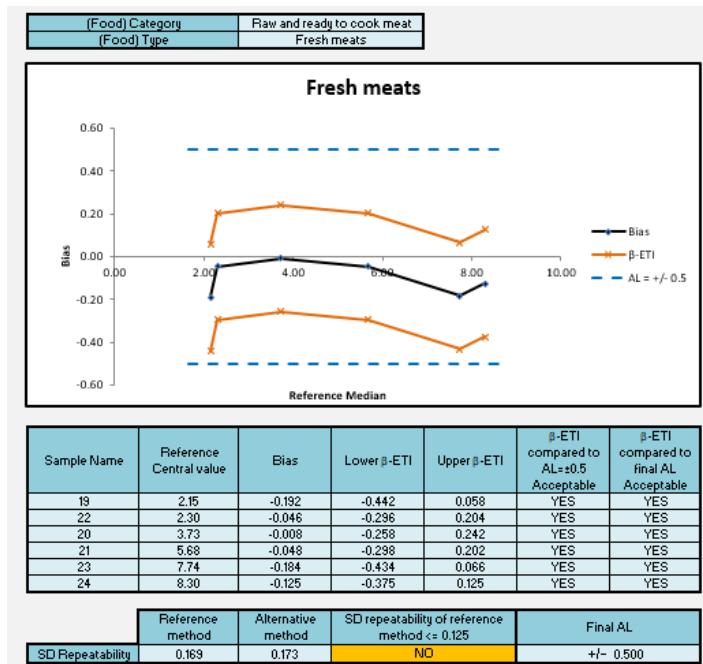
**Figure 7c - Accuracy profile of the reference method versus OPTVC pour plate method results for the Produce and fruits (combined category fresh and processed) 1ml pour plate with 36h incubation**



**Figure 7d - Accuracy profile of the reference method versus OPTVC pour plate method results for the Multi-component foods or meal components 1ml pour plate with 36h incubation**



**Figure 7e - Accuracy profile of the reference method versus OPTVC pour plate method results for the Raw and Ready to cook RTC Meat and poultry 1ml pour plate with 36h incubation**



In this study the following categories met the AL of 0.5log multicomponent foods and raw and ready to cook meat products

In this study, the following categories required the new AL to be calculated: dairy products, fresh produce, multicomponent and fishery products. All categories met the new AL value of 0.540, 0.904 and 0.792 respectively.

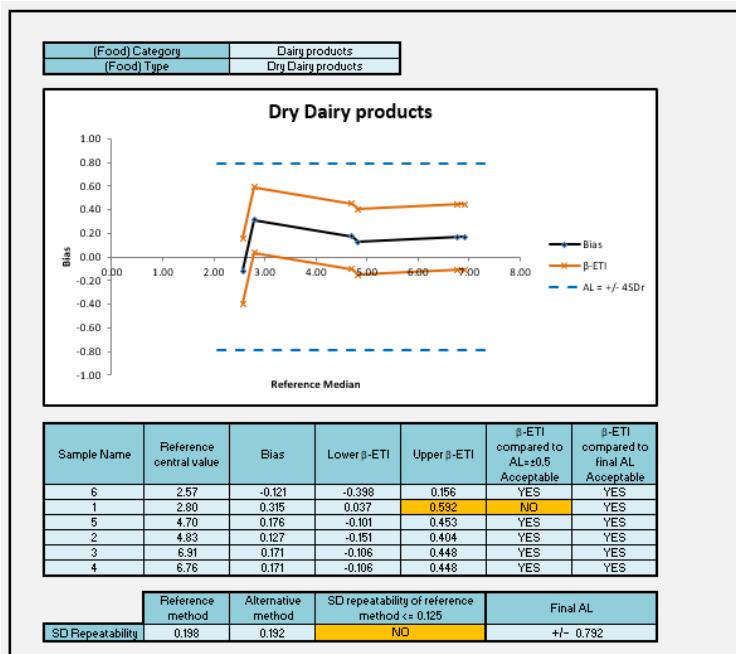
**Conclusion (AP study) OP TVC 1ml pour plates with 36h incubation.**

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

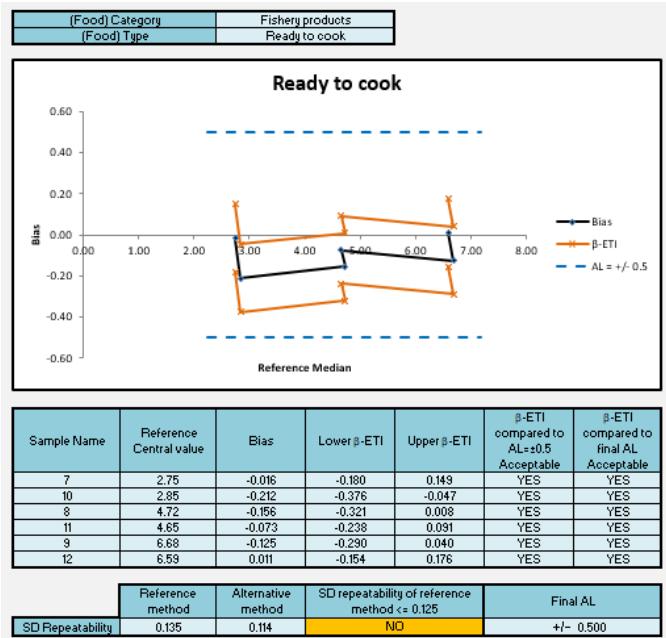
**1ml pour plate with 48h incubation**

The accuracy profile plots for 0.1ml spread plate 48h incubation are shown in Figures 8 a-e.

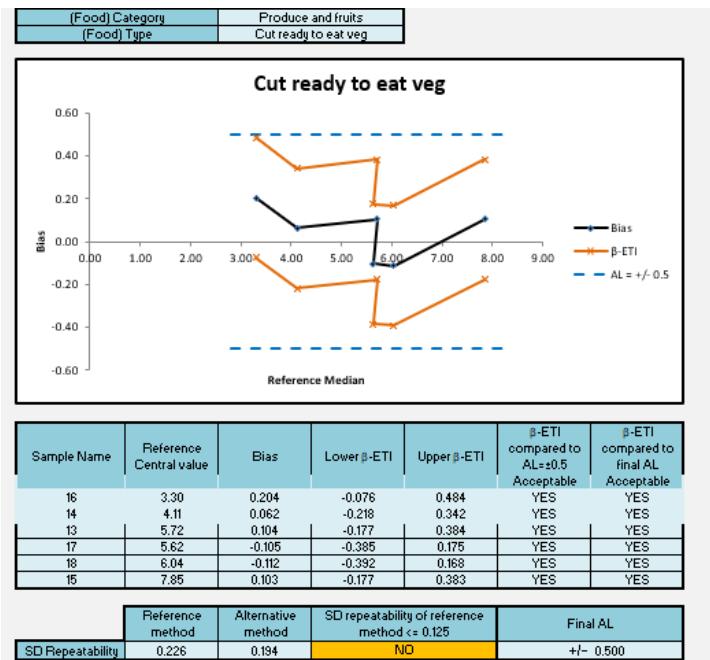
**Figure 8a - Accuracy profile of the reference method versus OPTVC pour plate method results for the pasteurised dairy products category 1ml pour plate with 48h incubation**



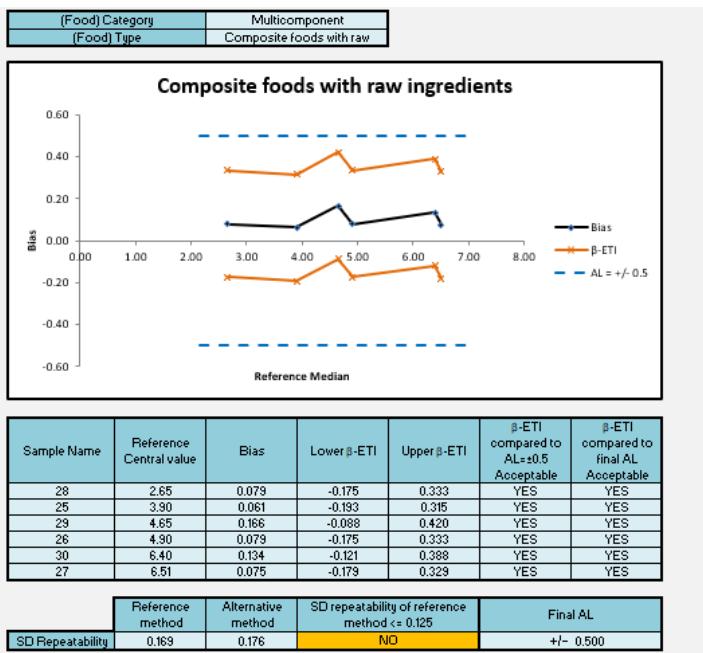
**Figure 8b - Accuracy profile of the reference method versus OPTVC pour plate method results for the Raw fishery products 1ml pour plate with 48h incubation**



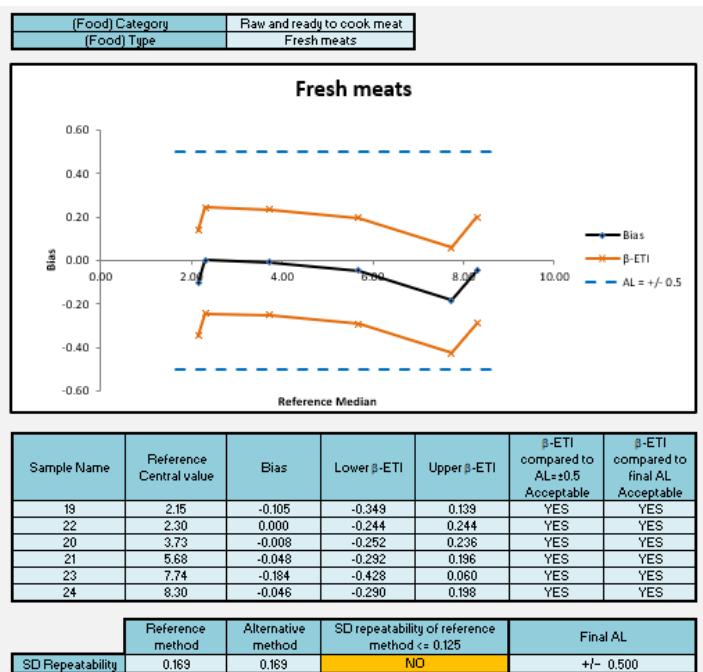
**Figure 8c - Accuracy profile of the reference method versus OPTVC pour plate method results for the Produce and fruits (combined category fresh and processed) 1ml pour plate with 48h incubation**



**Figure 8d - Accuracy profile of the reference method versus OPTVC pour plate method results for the Multi-component foods or meal components 1ml pour plate with 48h incubation**



**Figure 8e - Accuracy profile of the reference method versus OPTVC pour plate method results for the Raw and Ready to cook RTC Meat and poultry 1ml pour plate with 48h incubation**



In this study for OP TVC 1ml pour plates with 48h incubation, the four categories met the AL of 0.5log fishery products, fresh produce, multicomponent foods and raw and ready to eat meat.

In this study, the dairy products category required recalculation of the acceptability limits. This category met the new AL value of 0.792.

The higher repeatability observed in the dairy category is likely due to variation in die-off of the lyophilised culture in the drying process and during ambient storage of the sample.

#### **Conclusion (AP study) OP TVC 1ml pour plates with 48h incubation.**

The accuracy of the Alternative method (OP TVC 1ml pour plates with 48h incubation) is satisfied as all categories met the 0.5log AL or the re-calculated AL.

### **3.4 Inclusivity / exclusivity**

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

#### **3.4.1 Protocols**

- Inclusivity

50 strains were grown in NB medium at 30°C overnight. Each strain was tested once with the alternative method, the reference method and a non-selective agar.

#### **3.4.2 Results**

A summary of the results is given in Annex D.

- Inclusivity

A total of 50 strains were tested for inclusivity. All 50 isolates showed a positive result with the reference and alternative methods (OP TVC 0.1ml spread plate with 36h incubation, 0.1ml spread plate with 48h incubation, 1ml pour plate with 36h incubation and 1ml pour plate with 48h incubation).

#### **3.4.3 Conclusion inclusivity and exclusivity study**

The alternative Neogen OP TVC 1ml pour and 0.1ml spread plate method is selective and specific for the 36h and 48h time points.

### 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.

## 4 Overall conclusions of the validation MCS study

- The alternative method One Plate TVC for enumeration of aerobic plate count shows satisfactory results for relative trueness for both plating formats and incubation times
- The alternative One Plate TVC for enumeration of aerobic plate count shows satisfactory results for accuracy profile for both plating formats and incubation time.
- The alternative One Plate TVC for enumeration of aerobic plate count is selective and specific for both plating formats and incubation times

## 5 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.

The interlaboratory study has been carried out using the spread plate format for the alternative method, as agreed by the MVTC in 59th meeting.

### 5.1 Study organisation

#### 5.1.1 Collaborators

Samples were sent to 16 laboratories, each laboratory had one participant. The participants were from 5 countries: Ireland, England, Italy, Poland and Slovakia.

#### 5.1.2 Matrix and strain used

Canned spaghetti hoops was inoculated with *Lelliottia amnigena* ATCC 33072 strain, isolated from soil.

#### 5.1.3 Sample preparation

Samples were prepared and inoculated on 22nd January 2024 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 in Table 13.

**Table 13 - Contamination levels**

Contamination level	Sample code Collaborator 1
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

#### 5.1.4 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 24 h to 48 h to the involved laboratories. The temperature conditions were required to stay lower or equal to 8°C during transport, and ≤-18°C during storage in the laboratories.

#### 5.1.5 Analysis of Samples

Collaborative study laboratories and the expert laboratory carried out the analyses on 29th January 2024 with the alternative and reference methods. The analyses by the reference method and the alternative method were performed on the same day.

### 5.2 Experimental parameters controls

#### 5.2.1 Detection of *Lelliottia amnigena* the matrix before inoculation

In order to detect the presence of *Lelliottia amnigena*, the reference method was performed on five portions (10 g) before the inoculation. All the results were negative.

#### 5.2.2 Strain stability during transport

Duplicate samples inoculated at four levels ( $10^2$ ,  $10^3$ ,  $10^5$ ,  $10^6$  cfu/g) were tested for the enumeration of total viable count after 7 days of storage at ≤-18°C (Table 14). Frozen samples were thawed under controlled conditions prior to analysis.

**Table 14 - *Lelliottia amnigena* stability in the matrix**

Day	Storage conditions	Alternative method (log cfu/g) – 36 hour count One Plate Total Viable Count spread plate								Reference method (log cfu/g)							
		Level 1		Level 2		Level 3		Level 4		Level 1		Level 2		Level 3		Level 4	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	A	b
Day 0	N/A	2.2	2.1	3.2	3.3	4.7	5.1	6.3	6.5	2.3	2.3	3.1	3.1	5.1	5.1	6.4	6.3
Day 7	Storage at $\leq -18^{\circ}\text{C}$	1.5	1	2.6	2.6	4.5	4.6	5.9	5.6	1.7	1.7	2.5	2.4	4.6	4.6	6.0	6.0

No growth was observed during storage. A small amount of die off was observed at all levels after 7 days storage and this was taken into consideration when inoculating the samples.

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 15.

**Table 15 - Sample temperatures at receipt**

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
1	-2.5°C	-3.5°C	25/01/2024 11:20	29/01/2024
2	-9.2°C	0.0°C	26/01/2024 11:20	29/01/2024
3	-14.4°C	-1.2°C	25/01/2024 8:50	29/01/24
4	-17.6°C	-1.5°C	25/01/2024 8:50	29/01/2024
5	-16.6°C	-1.3°C	25/01/2024 10:50	29/01/2024
6	-16.5°C	-1.3°C	25/01/2024 15:00	29/01/2024 12:00

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
7	-18.8°C	-1.4°C	25/01/2024 11:00	29/01/2024 12:10
8	-16.4°C	-4.2°C	25/01/2024 13:30	29/01/2024
9	-17.8°C	-1.6°C	25/01/2024 10:15	29/01/2024
10	-9.2°C	-1.9°C	25/01/2024 15:00	29/01/2024 12:00
11	-0.4°C	0.9°C	25/01/24 11:00	29/01/2024
12	Did not carry out study due to temperature at sample receipt			
13	Did not receive the samples			
14	Did not receive the samples			
15	-1.1°C	2.0°C	25/01/24 16:00	29/01/2024
16	Not available	Temperature probe data not available. Water blank frozen on receipt	25/01/24 10:50	29/01/2024

No problem was encountered during the transport or at receipt for 13 collaborators. All the samples were delivered on time and in appropriate conditions. Labs 13 and 14 did not receive the samples due to issues with customs. Lab 12 received samples but the time in transit was 5 days and the temperature upon sample receipt were outside the set criteria ( $\leq 8^{\circ}\text{C}$ ). For the remaining laboratories, temperatures during shipment and at receipt were all correct.

### 5.3 Calculation and summary of data

The results for Lab 2 were not received and therefore not included in the analysis.

Lab 6 was excluded from analysis due to a high-level sample containing zero counts on both methods, indicating issues with plating samples.

Labs 5, 15 and 16 were excluded from the analysis for the low-level results due to the counts of  $<1$  log cfu/ml.

### 5.3.1 MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 16.

**Table 16 – Results obtained by the expert lab.**

Level	Reference method	Alternative method – 36-hour incubation	Alternative method – 48-hour incubation
Blank	<1	<1	<1
Low	2.5	2.3	2.3
Low	2.5	2.2	2.2
Medium	4.4	4.5	4.5
Medium	4.3	4.0	4.0
High	5.4	5.3	5.3
High	5.5	5.4	5.4

### 5.3.2 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 17.

The accuracy profile plot is shown in Figure 6 and the statistical analysis of the data shown in Table 18 and 19.

Table 17 - Summary of the results of the interlaboratory study per analyte level (k

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 36 hour		Alternative method (Log cfu/g) – 48 hour	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Low	20	25	50	40	50	40
3	Low	10	10	30	20	30	20
4	Low	30	50	80	150	130	140
7	Low	125	20	150	110	160	120
8	Low	30	40	100	70	100	70
9	Low	290	240	260	250	260	250
10	Low	40	60	40	180	40	180
11	Low	30	40	300	160	310	160
1	Medium	3800	1500	3600	3800	3700	3800
3	Medium	4000	1500	2600	1200	2800	1200
4	Medium	8000	13000	13000	20000	13000	20000
5	Medium	7200	16000	15000	35000	15000	35000
7	Medium	17000	7800	19000	6400	19000	6700
8	Medium	2500	2300	11000	5600	11000	5600
9	Medium	24000	23000	13000	25000	13000	25000
10	Medium	13000	10000	28000	19000	28000	19000
11	Medium	11000	3500	19000	15000	20000	15000
15	Medium	1500	360	8700	1200	8900	1300
16	Medium	2400	990	4500	670	5700	710
1	High	54000	17000	30000	28000	30000	28000
3	High	25000	55000	18000	70000	20000	7100
4	High	87000	120000	70000	190000	72000	190000
5	High	150000	210000	200000	260000	200000	260000
7	High	140000	57000	170000	110000	170000	110000
8	High	340000	31000	13000	11000	13000	11000
9	High	230000	210000	260000	240000	260000	240000
10	High	130000	490000	290000	210000	290000	210000
11	High	750000	28000	170000	98000	250000	100000
15	High	15000	4500	100000	18000	110000	18000
16	High	25000	4400	21000	2700	25000	4400
1	Blank	<1		<1		<1	
3	Blank	<1		<1		<1	
4	Blank	<1		<1		<1	
5	Blank	<1		<1		<1	
7	Blank	<1		<1		<1	
8	Blank	<1		<1		<1	
9	Blank	<1		<1		<1	
10	Blank	<1		<1		<1	

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 36 hour		Alternative method (Log cfu/g) – 48 hour	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
11	Blank	<1		<1		<1	
15	Blank	<1		<1		<1	
16	Blank	<1		<1		<1	

Figure 6. Accuracy profile of One Plate Total Viable Count from the ILS – 36h incubation

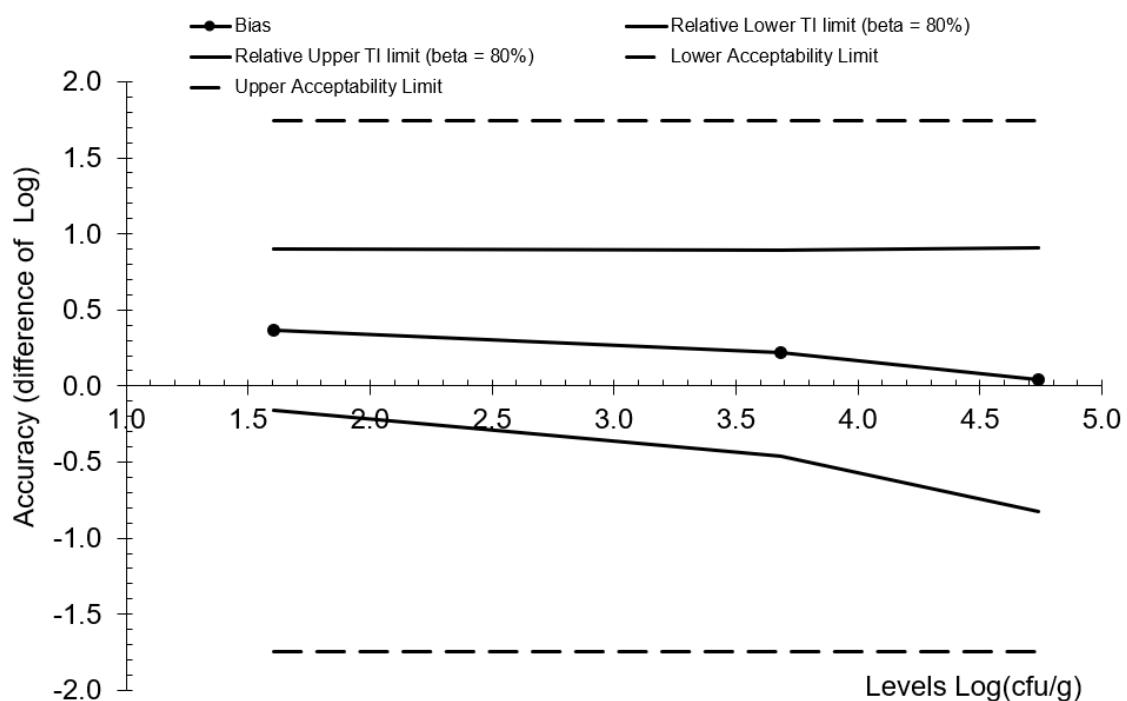


Figure 7. Accuracy profile of One Plate Total Viable Count from the ILS – 48h incubation

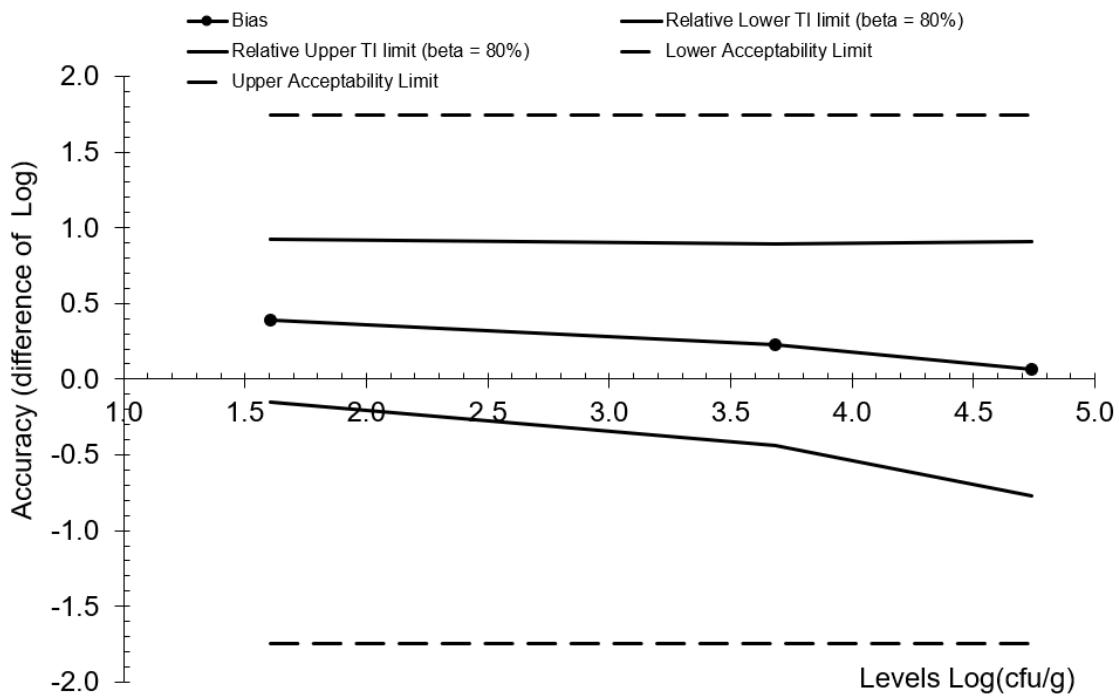


Table 18. Statistical analysis of the ILS data according to the ISO spreadsheet – 36-hour incubation of OP TVC

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	1.603	3.684	4.737			
Number of participants (K)	8	11	11	8	11	11
Average for alternative method	1.973	3.901	4.777	1.603	3.684	4.737
Repeatability standard deviation (sr)	0.203	0.312	0.289	0.218	0.254	0.431
Between-labs standard deviation (sl)	0.305	0.376	0.544	0.370	0.431	0.468
Reproducibility standard deviation (sR)	0.366	0.488	0.616	0.429	0.500	0.637
Corrected number of dof	9.493	14.882	12.457	9.056	12.919	15.590
Coverage factor	1.449	1.389	1.407			
Interpolated Student t	1.378	1.341	1.353			
Tolerance interval standard deviation	0.3852	0.5058	0.6400			
Lower TI limit	1.443	3.223	3.911			
Upper TI limit	2.504	4.580	5.644			
<b>Bias</b>	<b>0.370</b>	<b>0.218</b>	<b>0.040</b>			
Relative Lower TI limit (beta = 80%)	-0.161	-0.461	-0.826			
Relative Upper TI limit (beta = 80%)	0.901	0.896	0.906			
Lower Acceptability Limit	-1.75	-1.75	-1.75			
Upper Acceptability Limit	1.75	1.75	1.75			
<b>New acceptability limits may be based on reference method pooled variance</b>						
Pooled repro standard dev of reference	0.529					

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

Table 19. Statistical analysis of the ILS data according to the ISO spreadsheet – 48-hour incubation of OP TVC

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	1.603	3.684	4.737			
Number of participants (K)	8	11	11	8	11	11
Average for alternative method	1.990	3.913	4.803	1.603	3.684	4.737
Repeatability standard deviation (sr)	0.192	0.319	0.286	0.218	0.254	0.431
Between-labs standard deviation (sL)	0.316	0.357	0.524	0.370	0.431	0.468
Reproducibility standard deviation (sR)	0.370	0.479	0.597	0.429	0.500	0.637
Corrected number of dof	9.158	15.372	12.572	9.056	12.919	15.590
Coverage factor	1.454	1.386	1.406			
Interpolated Student t	1.381	1.339	1.353			
Tolerance interval standard deviation	0.3894	0.4957	0.6204			
Lower TI limit	1.452	3.249	3.964			
Upper TI limit	2.527	4.577	5.643			
<b>Bias</b>	<b>0.386</b>	<b>0.229</b>	<b>0.066</b>			
<b>Relative Lower TI limit (beta = 80%)</b>	<b>-0.152</b>	<b>-0.435</b>	<b>-0.773</b>			
<b>Relative Upper TI limit (beta = 80%)</b>	<b>0.924</b>	<b>0.893</b>	<b>0.905</b>			
<b>Lower Acceptability Limit</b>	<b>-1.75</b>	<b>-1.75</b>	<b>-1.75</b>			
<b>Upper Acceptability Limit</b>	<b>1.75</b>	<b>1.75</b>	<b>1.75</b>			
<b>New acceptability limits may be based on reference method pooled variance</b>						
Pooled repro standard dev of reference	0.529					

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

TRUE  
TRUE

There is little difference in the ILS results with increased incubation time of the alternative method, this is consistent with the results of the MCS. The accuracy profile and statistical analysis meet the ISO 16140-2 criteria and indicate that the alternative method has good agreement with the reference method.

A review of the accuracy profile and statistical analysis revealed that there was a high acceptability limit of  $\pm 1.75$  logs. To investigate the possible reasons for the high AL seen in the ILS, a root cause analysis was carried out.

### 5.3.3 Root cause analysis

The root cause analysis considered all factors that could have contributed to the relatively high level of variability observed between replicates. Analysis of the raw data showed that the same batches of media were used by all participants and that the incubation time and temperature were correct. In addition, the experimental data from the ILS was investigated during the root cause analysis.

Table 20 shows the repeatability of the reference and alternative methods. Table 21 shows the standard deviation between labs.

Table 20. Repeatability of the reference and alternative methods

Method	Low	Medium	High
Reference	0.218	0.254	0.431
36h alternative	0.203	0.312	0.289
48h alternative	0.192	0.319	0.286

Data indicates that the repeatability of both the reference and alternative methods is consistently high for all levels.

Table 21. Standard deviation between labs of the reference and alternative methods

Method	Low	Medium	High
Reference	0.370	0.431	0.468
36h alternative	0.316	0.357	0.524
48h alternative	0.305	0.376	0.544

The standard deviation observed between labs is high with both reference and alternative methods at all 3 levels of contamination analysed but is larger with the samples inoculated at the high level. High repeatability and standard deviation values are observed with both the reference and alternative methods.

The log differences between the reference and alternative methods given in Table 22 show good agreement between the counts, with most of the log differences being within 0.5log across the three levels of contamination analysed.

Table 22. Log differences between reference and alternative methods (reference-alternative)

Lab Number	Low level log differences				Medium level log differences				High level log differences			
	36h incubation of alternative method		48h incubation of alternative method		36h incubation of alternative method		48h incubation of alternative method		36h incubation of alternative method		48h incubation of alternative method	
	1	2	1	2	1	2	1	2	1	2	1	2
1	0.4	0.2	0.4	0.2	0.0	0.4	0.0	0.4	-0.3	0.2	-0.3	0.2
3	0.5	0.3	0.5	0.3	-0.2	-0.1	-0.2	-0.1	-0.1	0.1	-0.1	0.1
4	0.4	0.5	0.6	0.4	0.2	0.2	0.2	0.2	-0.1	0.2	-0.1	0.2
5	N/A	N/A	N/A	N/A	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
7	0.1	0.7	0.1	0.8	0.0	-0.1	0.0	-0.1	0.1	0.3	0.1	0.3
8	0.5	0.2	0.5	0.2	0.6	0.4	0.6	0.4	-0.4	-0.4	-0.4	-0.4
9	0.0	0.0	0.0	0.0	-0.3	0.0	-0.3	0.0	0.1	0.1	0.1	0.1
10	0.0	0.5	0.0	0.5	0.3	0.3	0.3	0.3	0.3	-0.4	0.3	-0.4
11	1.0	0.6	1.0	0.6	0.2	0.6	0.3	0.6	-0.6	0.5	-0.5	0.6
15	N/A	N/A	N/A	N/A	0.8	0.5	0.8	0.6	0.8	0.6	0.9	0.6
16	N/A	N/A	N/A	N/A	0.3	-0.2	0.4	-0.1	-0.1	-0.2	0.0	0.0

The matrix used in the study was a canned product and the uninoculated sample produced zero counts from all participants which indicates that natural contamination is an unlikely cause of the large variation in levels.

It is possible that the level of *Lelliottia amnigena* changed in transit, and this is likely to occur at different rates between labs. The results of the initial stability trial indicate that there was a small amount of die off in low level samples over 7 days. Some parcels were sent to labs in Europe and were in transit for 2 days, other parcels were sent within the UK and were in transit for 1 day. The storage conditions for all parcels were within the specified criteria although each parcel may be potentially subject to different stresses.

Analysis of the expert lab data shown in Table 14 revealed that there was little variation observed between replicate samples. The expert lab samples were not transported and therefore were not subject to the same stress as the participant sample sets. This interlaboratory study was run alongside an interlaboratory study for the One Plate EBAC study, 2022LR108, using the same matrix and inoculating strain. The results observed in the 2022LR108 study indicated stress of the organism: small colonies were observed on both selective media types and lower levels were observed than inoculated. Lower levels than expected were also observed in the results of the One Plate TVC interlaboratory study. An additional stability test was carried out for the One Plate EBAC study as part of a root cause analysis. This involved repeating the sample inoculation, but changing the conditions of storage to better mimic the realistic conditions of transport. The results of this test showed die-off of the strain in the matrix, consistent with the results of the interlaboratory study.

The conclusion of the root cause analysis is that the large acceptability limit is due to high variability between samples and results between labs. This is likely to be due to the stability of the strain in the matrix when subject to transport conditions. The impact of this conclusion on the validity of the dataset is minimal and in fact shows the performance of the media with stressed organisms.

The interlaboratory study data for the One Plate Total Viable Count meets the ISO 16140-2 criteria. The data shows that there is good agreement between the reference and the alternative methods, which is consistent with the results of the method comparison study.

## 6 Overall conclusions of the validation study

- The alternative method, One Plate Total Viable Count, for enumeration of aerobic plate count shows satisfactory results for relative trueness;
- The alternative method, One Plate Total Viable Count, for enumeration of aerobic plate count shows satisfactory results for accuracy profile;
- The alternative method, One Plate Total Viable Count, for enumeration of aerobic plate count is selective and specific.
- The alternative method, One Plate Total Viable Count, for enumeration of aerobic plate count shows satisfactory performance in the ILS
- The alternative method, One Plate Total Viable Count, for enumeration of aerobic plate count shows comparable performance to the reference method ISO 4833-1

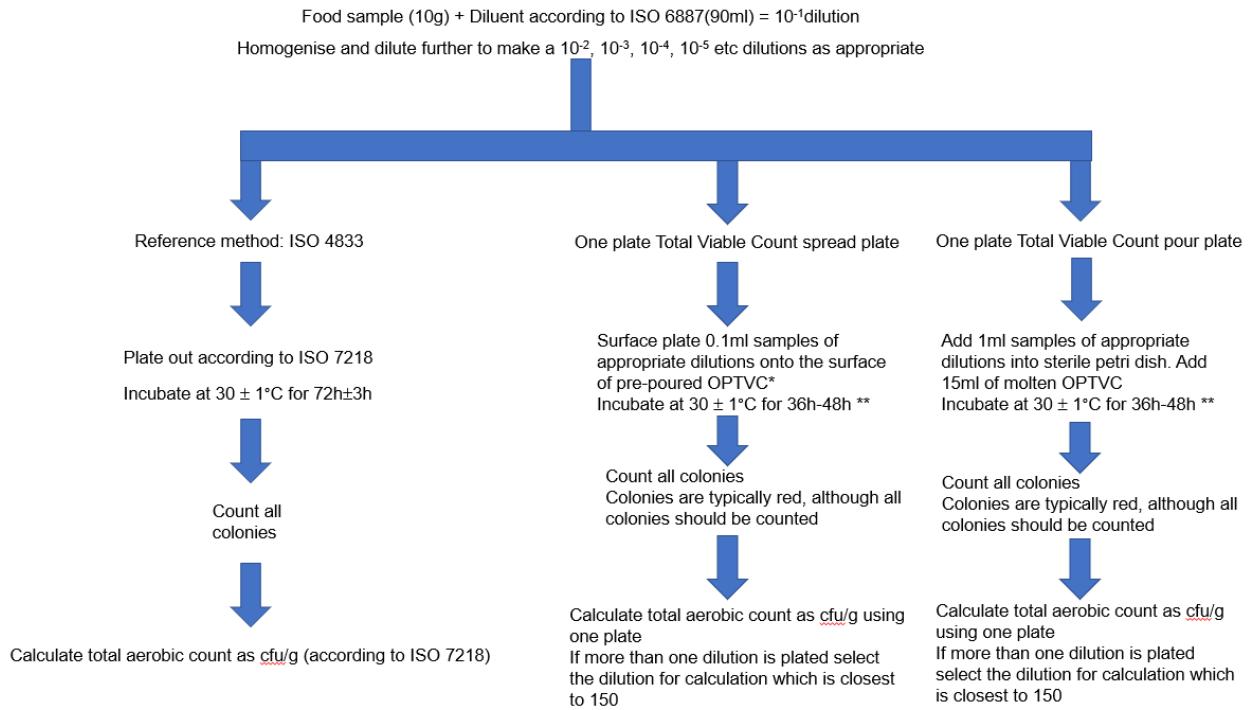
Date,

10 June 2024



Alice Foxall  
Project Manager – Method Validations

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



\*It is possible to spread 1ml of the initial suspension on 3 plates (90mm) for low number estimation.

\*\*Validation will be carried out at 36 hour and 48 hour incubation

**ANNEX B: Calculation and interpretation of relative trueness**

36 hour pour							
Type	Item	Sample Code	log(Ref)	log(Alt)	Mean	Difference	
<b>Raw and ready to cook (meat and poultry products)</b>							
Fresh meats (unprocessed)	british chicken breast fillets	T1	8.68	8.77	8.73	0.09	
Fresh meats (unprocessed)	lean diced beef	T2	8.32	8.56	8.44	0.23	
Fresh meats (unprocessed)	fresh lamb chops	T3	6.54	6.32	6.43	-0.22	
Fresh meats (unprocessed)	turkey thigh mince 7% fat	T4	6.86	6.51	6.68	-0.36	
Fresh meats (unprocessed)	pork loin steaks	T5	3.43	3.26	3.34	-0.18	
Ready to cook (processed) meat	bbq pork riblets	T6	7.36	6.83	7.10	-0.53	
Ready to cook (processed) meat	pork shoulder in a bbq sauce	T7	5.58	5.15	5.36	-0.43	
Ready to cook (processed) meat	fire pit sweet and smoky beef kebabs	T8	3.51	3.30	3.40	-0.20	
Ready to cook (processed) meat	fire pit beef burgers	T9	2.83	2.78	2.81	-0.05	
Ready to cook (processed) meat	pork sausages	T10	6.72	6.49	6.60	-0.22	
Ready to cook (processed) poultry	breaded chicken goujons	T11	5.11	4.84	4.98	-0.28	
Ready to cook (processed) poultry	british turkey meatballs	T12	4.11	3.92	4.02	-0.19	
Ready to cook (processed) poultry	ready to eat bbq roast chicken wings	T13	8.91	8.94	8.93	0.04	
Ready to cook (processed) poultry	chicken kiev bites	T14	2.04	2.23	2.14	0.19	
Ready to cook (processed) poultry	southern fried breaded chicken mini fillets	T15	10.56	10.11	10.34	-0.44	
<b>Raw fishery products</b>							
Raw fishery products	2 basa fillets	T16	4.15	4.15	4.15	0.00	
Raw fishery products	2 sea bass fillets	T17	6.23	6.60	6.42	0.37	
Raw fishery products	2 smoked basa fillets	T18	4.15	3.71	3.93	-0.44	

36 hour pour						
Type	Item	Sample Code	log(Ref)	log(Alt)	Mean	Difference
Raw fishery products	2 smoked Norwegian haddock fillets	T19	4.73	4.81	4.77	0.07
Raw fishery products	2 salmon fillets	T20	3.65	3.56	3.60	-0.10
RTE/RTRH/RTC fishery products	2 Thai Inspired Salmon & Cod F/Cakes 290G	T21	9.08	8.96	9.02	-0.12
RTE/RTRH/RTC fishery products	fish bites	T22	2.97	2.89	2.93	-0.08
RTE/RTRH/RTC fishery products	Extra Special 2 cod fishcakes with roasted tomato and mozzarella	T23	8.00	7.85	7.92	-0.15
RTE/RTRH/RTC fishery products	golden breaded cod fillets	T24	4.63	4.15	4.39	-0.49
RTE/RTRH/RTC fishery products	battered haddock fillet goujons	T25	4.81	4.18	4.49	-0.64
Crustaceans	extra special jumbo prawns	T26	5.20	5.23	5.22	0.03
Crustaceans	King Prawns 150G	T27	8.15	8.23	8.19	0.08
Crustaceans	Lemon and garlic king prawns	T28	4.96	5.04	5.00	0.08
Crustaceans	Jumbo king prawns	T29	4.88	4.85	4.86	-0.04
Crustaceans	Large raw peeled king prawns	T30	5.81	5.62	5.71	-0.18
Produce (combined category fresh and processed)						
Cut ready-to-eat-vegetables/leafy greens and sprouts	Italian wild rocket	T31	7.41	7.04	7.23	-0.37
Cut ready-to-eat-vegetables/leafy greens and sprouts	Babyleaf salad	T32	7.34	7.08	7.21	-0.26
Cut ready-to-eat-vegetables/leafy greens and sprouts	Fine beans & tenderstem broccoli	T33	5.36	5.15	5.25	-0.22
Cut ready-to-eat-vegetables/leafy greens and sprouts	mixed leaf salad	T34	7.46	7.40	7.43	-0.06
Cut ready-to-eat-vegetables/leafy greens and sprouts	Butterhead salad	T35	7.15	6.90	7.02	-0.25
Fresh fruit/cut RTE fruit and vegetable products	blueberries	T36	8.72	8.62	8.67	-0.10
Fresh fruit/cut RTE fruit and vegetable products	strawberries	T37	4.58	4.63	4.61	0.05

36 hour pour							
Type	Item	Sample Code	log(Ref)	log(Alt)	Mean	Difference	
Fresh fruit/cut RTE fruit and vegetable products	rainbow fruit platter	T38	7.20	7.08	7.14	-0.12	
Fresh fruit/cut RTE fruit and vegetable products	apple banana strawberry and grape	T39	4.04	3.79	3.91	-0.26	
Fresh fruit/cut RTE fruit and vegetable products	watermelon fingers	T40	3.11	2.58	2.85	-0.53	
Heat treated fruit and vegetables	Invigorate Super Smoothie 300MI	T41	6.53	6.48	6.50	-0.05	
Heat treated fruit and vegetables	Mixed Berries & Banana Smoothie 330MI	T42	6.40	6.61	6.51	0.21	
Heat treated fruit and vegetables	Tropical Defence Super Smoothie 300MI	T43	6.74	6.81	6.77	0.07	
Heat treated fruit and vegetables	Pineapples Bananas & Coconuts Smoothie 750ml	T44	2.26	2.00	2.13	-0.26	
Heat treated fruit and vegetables	pure orange and mango fruit juice	T45	5.30	5.20	5.25	-0.10	
Multicomponent foods and meal components							
Composite foods with substantial raw ingredients	Mango Passion Fruit & Orange Smoothie 330MI	T46	6.95	7.04	7.00	0.09	
Composite foods with substantial raw ingredients	chicken and sweetcorn sandwich	T47	7.68	7.28	7.48	-0.40	
Composite foods with substantial raw ingredients	cheese and pickle sandwich	T48	5.98	5.45	5.71	-0.53	
Composite foods with substantial raw ingredients	egg and cress sandwich	T49	7.68	7.54	7.61	-0.14	
Composite foods with substantial raw ingredients	cheese and onion sandwich	T50	8.28	8.15	8.21	-0.13	
RTE/RTRH foods (chilled, frozen)	lasagne	T51	7.82	7.76	7.79	-0.06	
RTE/RTRH foods (chilled, frozen)	tomato and mozzarella pasta bake	T52	8.48	8.41	8.45	-0.06	
RTE/RTRH foods (chilled, frozen)	macaroni cheese	T53	9.26	9.08	9.17	-0.18	
RTE/RTRH foods (chilled, frozen)	tikka masala and pilau rice	T54	8.89	8.98	8.93	0.09	
RTE/RTRH foods (chilled, frozen)	spicy chicken pasta	T55	9.58	9.60	9.59	0.02	
Mayonnaise based deli-salads	triple grain salad mayonnaise	T56	6.32	6.34	6.33	0.02	

36 hour pour							
Type	Item	Sample Code	log(Ref)	log(Alt)	Mean	Difference	
Mayonnaise based deli-salads	party salad mayonnaise	T57	7.11	7.08	7.10	-0.03	
Mayonnaise based deli-salads	Chicken & Bacon Caesar Salad 265G	T58	5.18	5.20	5.19	0.03	
Mayonnaise based deli-salads	ham egg and coleslaw salad	T59	6.91	6.97	6.94	0.05	
Mayonnaise based deli-salads	greek salad	T60	8.30	8.30	8.30	0.00	
Heat processed dairy							
Pasteurised milk	Semi skimmed milk 1	T61	5.36	4.94	5.15	-0.42	
Pasteurised milk	Semi skimmed milk 2	T62	5.94	5.83	5.88	-0.11	
Pasteurised milk	Jersey milk	T63	6.99	6.92	6.96	-0.06	
Pasteurised milk	Whole milk	T64	4.46	4.41	4.44	-0.05	
Pasteurised milk	Skimmed milk	T65	6.89	6.56	6.72	-0.34	
Pasteurised dairy products	fresh whipping cream	T66	3.53	3.30	3.42	-0.23	
Pasteurised dairy products	soured cream	T67	5.72	5.83	5.77	0.11	
Pasteurised dairy products	fat free cottage cheese, onion & chive	T68	7.62	7.45	7.54	-0.18	
Pasteurised dairy products	butter milk	T69	5.15	4.34	4.74	-0.80	
Pasteurised dairy products	light original soft cheese	T70	4.85	4.76	4.80	-0.10	
Dried dairy products	dried skim milk	T71	4.28	4.18	4.23	-0.10	
Dried dairy products	Strawberry milk shake powder	T72	2.00	1.70	1.85	-0.30	
Dried dairy products	skimmed milk powder	T73	3.93	4.08	4.00	0.15	
Dried dairy products	non fat skim milk	T74	1.70	1.60	1.65	-0.10	
Dried dairy products	non fat skim milk	T75	2.73	2.76	2.74	0.02	

**48 hour pour**

36 hour pour										
Type	Item			Sample Code		log(Ref)	log(Alt)	Mean	Difference	
Type	Item	Sample Code	log(Ref)	log(Alt)	Mean	Difference				
<b>Raw and ready to cook (meat and poultry products)</b>										
Fresh meats (unprocessed)	british chicken breast fillets	T1	8.68	8.77	8.73				0.09	
Fresh meats (unprocessed)	lean diced beef	T2	8.32	8.56	8.44				0.23	
Fresh meats (unprocessed)	fresh lamb chops	T3	6.54	6.32	6.43				-0.22	
Fresh meats (unprocessed)	turkey thigh mince 7% fat	T4	6.86	6.68	6.77				-0.18	
Fresh meats (unprocessed)	pork loin steaks	T5	3.43	3.53	3.48				0.10	
Ready to cook (processed) meat	bbq pork riblets	T6	7.36	6.83	7.10				-0.53	
Ready to cook (processed) meat	pork shoulder in a bbq sauce	T7	5.58	5.34	5.46				-0.24	
Ready to cook (processed) meat	fire pit sweet and smoky beef kebabs	T8	3.51	3.43	3.47				-0.07	
Ready to cook (processed) meat	fire pit beef burgers	T9	2.83	3.08	2.96				0.25	
Ready to cook (processed) meat	pork sausages	T10	6.72	6.52	6.62				-0.20	
Ready to cook (processed) poultry	breaded chicken goujons	T11	5.11	4.87	4.99				-0.24	
Ready to cook (processed) poultry	british turkey meatballs	T12	4.11	3.95	4.03				-0.16	
Ready to cook (processed) poultry	ready to eat bbq roast chicken wings	T13	8.91	8.97	8.94				0.06	
Ready to cook (processed) poultry	chicken kiev bites	T14	2.04	2.23	2.14				0.19	
Ready to cook (processed) poultry	southern fried breaded chicken mini fillets	T15	10.56	10.11	10.34				-0.44	
<b>Raw fishery products</b>										
Raw fishery products	2 basa fillets	T16	4.15	4.26	4.20				0.11	
Raw fishery products	2 sea bass fillets	T17	6.23	6.63	6.43				0.40	
Raw fishery products	2 smoked basa fillets	T18	4.15	3.88	4.01				-0.27	
Raw fishery products	2 smoked Norwegian haddock fillets	T19	4.73	4.81	4.77				0.07	

36 hour pour									
Type	Item			Sample Code		log(Ref)	log(Alt)	Mean	Difference
Raw fishery products	2 salmon fillets	T20	3.65	3.61	3.63				-0.04
RTE/RTRH/RTC fishery products	2 Thai Inspired Salmon & Cod F/Cakes 290G	T21	9.08	8.96	9.02				-0.12
RTE/RTRH/RTC fishery products	fish bites	T22	2.97	3.08	3.02				0.11
RTE/RTRH/RTC fishery products	Extra Special 2 cod fishcakes with roasted tomato and mozzarella	T23	8.00	7.88	7.94				-0.12
RTE/RTRH/RTC fishery products	golden breaded cod fillets	T24	4.63	4.18	4.40				-0.46
RTE/RTRH/RTC fishery products	battered haddock fillet goujons	T25	4.81	4.62	4.72				-0.19
Crustaceans	extra special jumbo prawns	T26	5.20	5.23	5.22				0.03
Crustaceans	King Prawns 150G	T27	8.15	8.23	8.19				0.08
Crustaceans	Lemon and garlic king prawns	T28	4.96	5.04	5.00				0.08
Crustaceans	Jumbo king prawns	T29	4.88	4.85	4.86				-0.04
Crustaceans	Large raw peeled king prawns	T30	5.81	5.63	5.72				-0.17
Produce (combined category fresh and processed)									
Cut ready-to-eat-vegetables/leafy greens and sprouts	Italian wild rocket	T31	7.41	7.20	7.31				-0.21
Cut ready-to-eat-vegetables/leafy greens and sprouts	Babyleaf salad	T32	7.34	7.08	7.21				-0.26
Cut ready-to-eat-vegetables/leafy greens and sprouts	Fine beans & tenderstem broccoli	T33	5.36	5.20	5.28				-0.16
Cut ready-to-eat-vegetables/leafy greens and sprouts	mixed leaf salad	T34	7.46	7.41	7.44				-0.05
Cut ready-to-eat-vegetables/leafy greens and sprouts	Butterhead salad	T35	7.15	6.95	7.05				-0.20
Fresh fruit/cut RTE fruit and vegetable products	blueberries	T36	8.72	8.67	8.70				-0.05
Fresh fruit/cut RTE fruit and vegetable products	strawberries	T37	4.58	4.65	4.62				0.07
Fresh fruit/cut RTE fruit and vegetable products	rainbow fruit platter	T38	7.20	7.08	7.14				-0.12

36 hour pour								
Type	Item	Sample Code			log(Ref)	log(Alt)	Mean	Difference
Fresh fruit/cut RTE fruit and vegetable products	apple banana strawberry and grape	T39	4.04	3.93	3.99			-0.11
Fresh fruit/cut RTE fruit and vegetable products	watermelon fingers	T40	3.11	2.64	2.88			-0.47
Heat treated fruit and vegetables	Invigorate Super Smoothie 300ML	T41	6.53	6.48	6.50			-0.05
Heat treated fruit and vegetables	Mixed Berries & Banana Smoothie 330ML	T42	6.40	6.61	6.51			0.21
Heat treated fruit and vegetables	Tropical Defence Super Smoothie 300ML	T43	6.74	6.81	6.77			0.07
Heat treated fruit and vegetables	Pineapples Bananas & Coconuts Smoothie 750ml	T44	2.26	2.00	2.13			-0.26
Heat treated fruit and vegetables	pure orange and mango fruit juice	T45	5.30	5.23	5.27			-0.07
Multicomponent foods and meal components								
Composite foods with substantial raw ingredients	Mango Passion Fruit & Orange Smoothie 330ML	T46	6.95	7.04	7.00			0.09
Composite foods with substantial raw ingredients	chicken and sweetcorn sandwich	T47	7.68	7.30	7.49			-0.38
Composite foods with substantial raw ingredients	cheese and pickle sandwich	T48	5.98	5.62	5.80			-0.35
Composite foods with substantial raw ingredients	egg and cress sandwich	T49	7.68	7.56	7.62			-0.12
Composite foods with substantial raw ingredients	cheese and onion sandwich	T50	8.28	8.18	8.23			-0.10
RTE/RTRH foods (chilled, frozen)	lasagne	T51	7.82	7.79	7.80			-0.03
RTE/RTRH foods (chilled, frozen)	tomato and mozzarella pasta bake	T52	8.48	8.45	8.46			-0.03
RTE/RTRH foods (chilled, frozen)	macaroni cheese	T53	9.26	9.08	9.17			-0.18
RTE/RTRH foods (chilled, frozen)	tikka masala and pilau rice	T54	8.89	8.98	8.93			0.09
RTE/RTRH foods (chilled, frozen)	spicy chicken pasta	T55	9.58	9.67	9.63			0.09
Mayonnaise based deli-salads	triple grain salad mayonnaise	T56	6.32	6.34	6.33			0.02
Mayonnaise based deli-salads	party salad mayonnaise	T57	7.11	7.08	7.10			-0.03

36 hour pour									
Type	Item			Sample Code		log(Ref)	log(Alt)	Mean	Difference
Mayonnaise based deli-salads	Chicken & Bacon Caesar Salad 265G	T58	5.18	5.23	5.20				0.05
Mayonnaise based deli-salads	ham egg and coleslaw salad	T59	6.91	7.00	6.96				0.09
Mayonnaise based deli-salads	greek salad	T60	8.30	8.30	8.30				0.00
Heat processed dairy									
Pasteurised milk	Semi skimmed milk 1	T61	5.36	4.94	5.15				-0.42
Pasteurised milk	Semi skimmed milk 2	T62	5.94	5.90	5.92				-0.04
Pasteurised milk	Jersey milk	T63	6.99	6.93	6.96				-0.06
Pasteurised milk	Whole milk	T64	4.46	4.38	4.42				-0.08
Pasteurised milk	Skimmed milk	T65	6.89	6.73	6.81				-0.16
Pasteurised dairy products	fresh whipping cream	T66	3.53	3.30	3.42				-0.23
Pasteurised dairy products	soured cream	T67	5.72	5.83	5.77				0.11
Pasteurised dairy products	fat free cottage cheese, onion & chive	T68	7.62	7.51	7.56				-0.12
Pasteurised dairy products	butter milk	T69	5.15	4.34	4.74				-0.80
Pasteurised dairy products	light original soft cheese	T70	4.85	4.76	4.80				-0.10
Dried dairy products	dried skim milk	T71	4.28	4.18	4.23				-0.10
Dried dairy products	Strawberry milk shake powder	T72	2.00	1.78	1.89				-0.22
Dried dairy products	skimmed milk powder	T73	3.93	4.08	4.00				0.15
Dried dairy products	non fat skim milk	T74	1.70	2.00	1.85				0.30
Dried dairy products	non fat skim milk	T75	2.73	2.78	2.76				0.05

36 hour spread

type	Item	Sample code	log(Ref)	log(Alt)	Mean	Difference
<b>Raw and ready to cook (meat and poultry products)</b>						
Fresh meats (unprocessed)	british chicken breast fillets	T1	8.68	8.85	8.77	0.17
Fresh meats (unprocessed)	lean diced beef	T2	8.32	8.61	8.47	0.29
Fresh meats (unprocessed)	fresh lamb chops	T3	6.54	6.43	6.49	-0.11
Fresh meats (unprocessed)	turkey thigh mince 7% fat	T4	6.86	6.63	6.75	-0.23
Fresh meats (unprocessed)	pork loin steaks	T5	3.43	3.51	3.47	0.07
Ready to cook (processed) meat	bbq pork riblets	T6	7.36	7.58	7.47	0.22
Ready to cook (processed) meat	pork shoulder in a bbq sauce	T7	5.58	5.59	5.59	0.01
Ready to cook (processed) meat	fire pit sweet and smoky beef kebabs	T8	3.51	3.38	3.44	-0.12
Ready to cook (processed) meat	fire pit beef burgers	T9	2.83	3.11	2.97	0.28
Ready to cook (processed) meat	pork sausages	T10	6.72	6.60	6.66	-0.11
Ready to cook (processed) poultry	breaded chicken goujons	T11	5.11	5.11	5.11	0.00
Ready to cook (processed) poultry	british turkey meatballs	T12	4.11	3.93	4.02	-0.18
Ready to cook (processed) poultry	ready to eat bbq roast chicken wings	T13	8.91	9.04	8.97	0.13
Ready to cook (processed) poultry	chicken kiev bites	T14	2.04	2.41	2.23	0.37
Ready to cook (processed) poultry	southern fried breaded chicken mini fillets	T15	10.56	10.43	10.49	-0.12
<b>Raw fishery products</b>						
Raw fishery products	2 basa fillets	T16	4.15	4.32	4.23	0.18
Raw fishery products	2 sea bass fillets	T17	6.23	6.72	6.48	0.49
Raw fishery products	2 smoked basa fillets	T18	4.15	3.84	3.99	-0.31
Raw fishery products	2 smoked Norwegian haddock fillets	T19	4.73	4.88	4.80	0.14
Raw fishery products	2 salmon fillets	T20	3.65	3.45	3.55	-0.21
RTE/RTRH/RTC fishery products	2 Thai Inspired Salmon & Cod F/Cakes 290G	T21	9.08	9.11	9.10	0.03

RTE/RTRH/RTC fishery products	fish bites	T22	2.97	3.20	3.09		0.23
RTE/RTRH/RTC fishery products	Extra Special 2 cod fishcakes with roasted tomato and mozzarella	T23	8.00	7.82	7.91		-0.18
RTE/RTRH/RTC fishery products	golden breaded cod fillets	T24	4.63	4.36	4.50		-0.27
RTE/RTRH/RTC fishery products	battered haddock fillet goujons	T25	4.81	4.41	4.61		-0.40
Crustaceans	extra special jumbo prawns	T26	5.20	5.15	5.18		-0.06
Crustaceans	King Prawns 150G	T27	8.15	8.60	8.37		0.46
Crustaceans	Lemon and garlic king prawns	T28	4.96	5.08	5.02		0.12
Crustaceans	Jumbo king prawns	T29	4.88	4.79	4.84		-0.09
Crustaceans	Large raw peeled king prawns	T30	5.81	5.78	5.79		-0.03
<b>Produce (combined category fresh and processed)</b>							
Cut ready-to-eat-vegetables/leafy greens and sprouts	Italian wild rocket	T31	7.41	7.20	7.31		-0.21
Cut ready-to-eat-vegetables/leafy greens and sprouts	Babyleaf salad	T32	7.34	7.20	7.27		-0.14
Cut ready-to-eat-vegetables/leafy greens and sprouts	Fine beans & tenderstem broccoli	T33	5.36	5.18	5.27		-0.19
Cut ready-to-eat-vegetables/leafy greens and sprouts	mixed leaf salad	T34	7.46	7.00	7.23		-0.46
Cut ready-to-eat-vegetables/leafy greens and sprouts	Butterhead salad	T35	7.15	6.97	7.06		-0.18
Fresh fruit/cut RTE fruit and vegetable products	blueberries	T36	8.72	8.62	8.67		-0.10
Fresh fruit/cut RTE fruit and vegetable products	strawberries	T37	4.58	4.83	4.70		0.25
Fresh fruit/cut RTE fruit and vegetable products	rainbow fruit platter	T38	7.20	7.32	7.26		0.12
Fresh fruit/cut RTE fruit and vegetable products	apple banana strawberry and grape	T39	4.04	4.04	4.04		0.00
Fresh fruit/cut RTE fruit and vegetable products	watermelon fingers	T40	3.11	3.04	3.08		-0.07
Heat treated fruit and vegetables	Invigorate Super Smoothie 300ML	T41	6.53	6.59	6.56		0.06
Heat treated fruit and vegetables	Mixed Berries & Banana Smoothie 330ML	T42	6.40	6.73	6.57		0.33
Heat treated fruit and vegetables	Tropical Defence Super Smoothie 300ML	T43	6.74	6.54	6.64		-0.20
Heat treated fruit and vegetables	Pineapples Bananas & Coconuts Smoothie 750ml	T44	2.26	2.08	2.17		-0.18

Heat treated fruit and vegetables	pure orange and mango fruit juice	T45	5.30	5.32	5.31		0.02
<b>Multicomponent foods and meal components</b>							
Composite foods with substantial raw ingredients	Mango Passion Fruit & Orange Smoothie 330ML	T46	6.95	7.18	7.07		0.22
Composite foods with substantial raw ingredients	chicken and sweetcorn sandwich	T47	7.68	7.48	7.58		-0.20
Composite foods with substantial raw ingredients	cheese and pickle sandwich	T48	5.98	5.73	5.86		-0.25
Composite foods with substantial raw ingredients	egg and cress sandwich	T49	7.68	7.68	7.68		0.00
Composite foods with substantial raw ingredients	cheese and onion sandwich	T50	8.28	8.20	8.24		-0.07
RTE/RTRH foods (chilled, frozen)	lasagne	T51	7.82	7.76	7.79		-0.06
RTE/RTRH foods (chilled, frozen)	tomato and mozzarella pasta bake	T52	8.48	8.48	8.48		0.00
RTE/RTRH foods (chilled, frozen)	macaroni cheese	T53	9.26	9.30	9.28		0.05
RTE/RTRH foods (chilled, frozen)	tikka masala and pilau rice	T54	8.89	9.04	8.97		0.15
RTE/RTRH foods (chilled, frozen)	spicy chicken pasta	T55	9.58	9.79	9.68		0.21
Mayonnaise based deli-salads	triple grain salad mayonnaise	T56	6.32	6.28	6.30		-0.04
Mayonnaise based deli-salads	party salad mayonnaise	T57	7.11	7.23	7.17		0.12
Mayonnaise based deli-salads	Chicken & Bacon Caesar Salad 265G	T58	5.18	5.30	5.24		0.12
Mayonnaise based deli-salads	ham egg and coleslaw salad	T59	6.91	7.08	7.00		0.17
Mayonnaise based deli-salads	greek salad	T60	8.30	8.30	8.30		0.00
<b>Heat processed dairy</b>							
Pasteurised milk	Semi skimmed milk 1	T61	5.36	5.30	5.33		-0.06
Pasteurised milk	Semi skimmed milk 2	T62	5.94	5.81	5.87		-0.13
Pasteurised milk	Jersey milk	T63	6.99	7.08	7.03		0.09
Pasteurised milk	Whole milk	T64	4.46	4.30	4.38		-0.16
Pasteurised milk	Skimmed milk	T65	6.89	6.95	6.92		0.06
Pasteurised dairy products	fresh whipping cream	T66	3.53	3.78	3.65		0.25

Pasteurised dairy products	soured cream	T67	5.72	5.46	5.59		-0.25
Pasteurised dairy products	fat free cottage cheese, onion & chive	T68	7.62	7.59	7.61		-0.03
Pasteurised dairy products	butter milk	T69	5.15	5.00	5.07		-0.15
Pasteurised dairy products	light original soft cheese	T70	4.85	4.92	4.89		0.07
Dried dairy products	dried skim milk	T71	4.28	4.08	4.18		-0.20
Dried dairy products	Strawberry milk shake powder	T72	2.00	1.70	1.85		-0.30
Dried dairy products	skimmed milk powder	T73	3.93	4.08	4.00		0.15
Dried dairy products	non fat skim milk	T74	1.70	1.78	1.74		0.08
Dried dairy products	non fat skim milk	T75	2.73	2.28	2.51		-0.45

48 hour spread							
type	Item	Sample code	log(Ref)	log(Alt)	Mean	Difference	
<b>Raw and ready to cook (meat and poultry products)</b>							
Fresh meats (unprocessed)	british chicken breast fillets	T1	8.68	8.85	8.77		0.17
Fresh meats (unprocessed)	lean diced beef	T2	8.32	8.61	8.47		0.29
Fresh meats (unprocessed)	fresh lamb chops	T3	6.54	6.43	6.49		-0.11
Fresh meats (unprocessed)	turkey thigh mince 7% fat	T4	6.86	6.63	6.75		-0.23
Fresh meats (unprocessed)	pork loin steaks	T5	3.43	3.53	3.48		0.10
Ready to cook (processed) meat	bbq pork riblets	T6	7.36	7.58	7.47		0.22
Ready to cook (processed) meat	pork shoulder in a bbq sauce	T7	5.58	5.59	5.59		0.01
Ready to cook (processed) meat	fire pit sweet and smoky beef kebabs	T8	3.51	3.38	3.44		-0.12
Ready to cook (processed) meat	fire pit beef burgers	T9	2.83	3.30	3.07		0.47
Ready to cook (processed) meat	pork sausages	T10	6.72	6.62	6.67		-0.09

Ready to cook (processed) poultry	breaded chicken goujons	T11	5.11	5.11	5.11		0.00
Ready to cook (processed) poultry	British turkey meatballs	T12	4.11	3.94	4.03		-0.17
Ready to cook (processed) poultry	ready to eat bbq roast chicken wings	T13	8.91	9.04	8.97		0.13
Ready to cook (processed) poultry	chicken kiev bites	T14	2.04	2.41	2.23		0.37
Ready to cook (processed) poultry	southern fried breaded chicken mini fillets	T15	10.56	10.43	10.49		-0.12
<b>Raw fishery products</b>							
Raw fishery products	2 basa fillets	T16	4.15	4.67	4.41		0.53
Raw fishery products	2 sea bass fillets	T17	6.23	6.73	6.48		0.50
Raw fishery products	2 smoked basa fillets	T18	4.15	3.92	4.04		-0.22
Raw fishery products	2 smoked Norwegian haddock fillets	T19	4.73	4.88	4.80		0.14
Raw fishery products	2 salmon fillets	T20	3.65	3.46	3.56		-0.19
RTE/RTRH/RTC fishery products	2 Thai Inspired Salmon & Cod F/Cakes 290G	T21	9.08	9.11	9.10		0.03
RTE/RTRH/RTC fishery products	fish bites	T22	2.97	3.34	3.16		0.37
RTE/RTRH/RTC fishery products	Extra Special 2 cod fishcakes with roasted tomato and mozzarella	T23	8.00	7.83	7.91		-0.16
RTE/RTRH/RTC fishery products	golden breaded cod fillets	T24	4.63	4.40	4.52		-0.24
RTE/RTRH/RTC fishery products	battered haddock fillet goujons	T25	4.81	4.59	4.70		-0.22
Crustaceans	extra special jumbo prawns	T26	5.20	5.18	5.19		-0.03
Crustaceans	King Prawns 150G	T27	8.15	8.60	8.37		0.46
Crustaceans	Lemon and garlic king prawns	T28	4.96	5.08	5.02		0.12
Crustaceans	Jumbo king prawns	T29	4.88	4.81	4.85		-0.07
Crustaceans	Large raw peeled king prawns	T30	5.81	5.79	5.80		-0.01
<b>Produce (combined category fresh and processed)</b>							
Cut ready-to-eat-vegetables/leafy greens and sprouts	Italian wild rocket	T31	7.41	7.36	7.39		-0.05
Cut ready-to-eat-vegetables/leafy greens and sprouts	Babyleaf salad	T32	7.34	7.20	7.27		-0.14

Cut ready-to-eat-vegetables/leafy greens and sprouts	Fine beans & tenderstem broccoli	T33	5.36	5.23	5.30		-0.13
Cut ready-to-eat-vegetables/leafy greens and sprouts	mixed leaf salad	T34	7.46	7.04	7.25		-0.42
Cut ready-to-eat-vegetables/leafy greens and sprouts	Butterhead salad	T35	7.15	6.98	7.06		-0.17
Fresh fruit/cut RTE fruit and vegetable products	blueberries	T36	8.72	8.62	8.67		-0.10
Fresh fruit/cut RTE fruit and vegetable products	strawberries	T37	4.58	4.83	4.70		0.25
Fresh fruit/cut RTE fruit and vegetable products	rainbow fruit platter	T38	7.20	7.32	7.26		0.12
Fresh fruit/cut RTE fruit and vegetable products	apple banana strawberry and grape	T39	4.04	4.04	4.04		0.00
Fresh fruit/cut RTE fruit and vegetable products	watermelon fingers	T40	3.11	3.08	3.10		-0.03
Heat treated fruit and vegetables	Invigorate Super Smoothie 300ML	T41	6.53	6.59	6.56		0.06
Heat treated fruit and vegetables	Mixed Berries & Banana Smoothie 330ML	T42	6.40	6.73	6.57		0.33
Heat treated fruit and vegetables	Tropical Defence Super Smoothie 300ML	T43	6.74	6.54	6.64		-0.20
Heat treated fruit and vegetables	Pineapples Bananas & Coconuts Smoothie 750ml	T44	2.26	2.08	2.17		-0.18
Heat treated fruit and vegetables	pure orange and mango fruit juice	T45	5.30	5.32	5.31		0.02
<b>Multicomponent foods and meal components</b>							
Composite foods with substantial raw ingredients	Mango Passion Fruit & Orange Smoothie 330ML	T46	6.95	7.18	7.07		0.22
Composite foods with substantial raw ingredients	chicken and sweetcorn sandwich	T47	7.68	7.48	7.58		-0.20
Composite foods with substantial raw ingredients	cheese and pickle sandwich	T48	5.98	5.76	5.87		-0.21
Composite foods with substantial raw ingredients	egg and cress sandwich	T49	7.68	7.68	7.68		0.00
Composite foods with substantial raw ingredients	cheese and onion sandwich	T50	8.28	8.23	8.25		-0.05
RTE/RTRH foods (chilled, frozen)	lasagne	T51	7.82	7.76	7.79		-0.06
RTE/RTRH foods (chilled, frozen)	tomato and mozzarella pasta bake	T52	8.48	8.48	8.48		0.00
RTE/RTRH foods (chilled, frozen)	macaroni cheese	T53	9.26	9.30	9.28		0.05
RTE/RTRH foods (chilled, frozen)	tikka masala and pilau rice	T54	8.89	9.04	8.97		0.15
RTE/RTRH foods (chilled, frozen)	spicy chicken pasta	T55	9.58	9.79	9.68		0.21

Mayonnaise based deli-salads	triple grain salad mayonnaise	T56	6.32	6.28	6.30		-0.04
Mayonnaise based deli-salads	party salad mayonnaise	T57	7.11	7.23	7.17		0.12
Mayonnaise based deli-salads	Chicken & Bacon Caesar Salad 265G	T58	5.18	5.30	5.24		0.12
Mayonnaise based deli-salads	ham egg and coleslaw salad	T59	6.91	7.08	7.00		0.17
Mayonnaise based deli-salads	greek salad	T60	8.30	8.30	8.30		0.00
<b>Heat processed dairy</b>							
Pasteurised milk	Semi skimmed milk 1	T61	5.36	5.30	5.33		-0.06
Pasteurised milk	Semi skimmed milk 2	T62	5.94	5.90	5.92		-0.04
Pasteurised milk	Jersey milk	T63	6.99	7.08	7.03		0.09
Pasteurised milk	Whole milk	T64	4.46	4.30	4.38		-0.16
Pasteurised milk	Skimmed milk	T65	6.89	6.97	6.93		0.08
Pasteurised dairy products	fresh whipping cream	T66	3.53	3.78	3.65		0.25
Pasteurised dairy products	soured cream	T67	5.72	5.46	5.59		-0.25
Pasteurised dairy products	fat free cottage cheese, onion & chive	T68	7.62	7.62	7.62		0.00
Pasteurised dairy products	butter milk	T69	5.15	5.00	5.07		-0.15
Pasteurised dairy products	light original soft cheese	T70	4.85	4.92	4.89		0.07
Dried dairy products	dried skim milk	T71	4.28	4.08	4.18		-0.20
Dried dairy products	Strawberry milk shake powder	T72	2.00	1.70	1.85		-0.30
Dried dairy products	skimmed milk powder	T73	3.93	4.08	4.00		0.15
Dried dairy products	non fat skim milk	T74	1.70	1.78	1.74		0.08
Dried dairy products	non fat skim milk	T75	2.73	2.28	2.51		-0.45

**ANNEX C: Summary tables accuracy profile study**

**0.1ml spread plate with 36h incubation**

(Food) Category 1		Dairy products											
(Food) Type 1		Dry Dairy products											
			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	
4	Dessert powder	low	350	370	540	390	310	390	210	260	230	230	
1	Milk powder	low	630	610	600	750	660	1400	1400	1100	1100	1400	
5	Dessert powder	intermediate	56000	50000	46000	140000	41000	80000	88000	87000	92000	78000	
2	Milk powder	intermediate	61000	55000	68000	74000	82000	92000	92000	95000	15000	100000	
6	Dessert powder	high	6500000	5500000	8500000	5800000	5800000	8800000	8500000	7700000	7900000	9100000	
3	Milk powder	high	11000000	9500000	8100000	1100000	7100000	16000000	11000000	13000000	13000000	11000000	

(Food) Category 2		Fishery products											
(Food) Type 2		Ready to cook		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	
7	White fish	low	560	700	610	540	540	510	550	460	500	360	
10	Tuna steak	low	660	970	830	580	700	230	430	490	530	440	
8	White fish	intermediate	38000	53000	16000	63000	69000	50000	62000	51000	46000	52000	
11	Tuna steak	intermediate	41000	68000	45000	35000	46000	83000	61000	47000	52000	42000	
9	White fish	high	5100000	4000000	4800000	6600000	4600000	3200000	3300000	4800000	4800000	5100000	
12	Tuna steak	high	4300000	3100000	5700000	2800000	3900000	4400000	7400000	5200000	5000000	6500000	

(Food) Category 3		Produce and fruits											
(Food) Type 3		Cut ready to eat veg		Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5	
16	Grated carrot	low	2000	4400	1500	4200	1300	2400	4700	1600	3600	1100	
14	Lettuce	low	23000	10000	13000	16000	10000	28000	10000	19000	15000	11000	
17	Grated carrot	intermediate	430000	450000	330000	420000	310000	510000	450000	370000	380000	360000	
13	Lettuce	intermediate	470000	460000	720000	520000	810000	800000	570000	670000	610000	1000000	
18	Grated carrot	high	1300000	900000	800000	1900000	1100000	1900000	1100000	1200000	600000	500000	
15	Lettuce	high	44000000	71000000	12000000	10000000	90000000	50000000	70000000	90000000	19000000	16000000	

(Food) Category 4		Raw and ready to cook 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	Raw stir fry beef strips	low	400	90	110	140	170	80	130	90	50	70	
22	Chicken breast fillets	low	240	200	200	200	220	190	130	130	90	230	
23	Chicken breast fillets	intermediate	5400	4500	8100	5700	4400	6200	5100	9900	8400	3500	
20	Raw stir fry beef strips	intermediate	41000000	91000000	39000000	55000000	96000000	26000000	97000000	14000000	45000000	73000000	
24	Chicken breast fillets	high	620000	660000	250000	450000	480000	530000	1000000	200000	480000	540000	
21	Raw stir fry beef strips	high	210000000	200000000	160000000	480000000	200000000	200000000	190000000	160000000	430000000	200000000	

(Food) Category 5		Multicomponent											
(Food) Type 5		Composite foods with raw ingredients											
			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	
28	Pasta salad	low	670	450	420	670	360	620	620	640	790	590	
25	Sandwich	low	9700	2300	8500	7900	6400	12000	3200	8500	9200	6900	
29	Pasta salad	intermediate	48000	25000	50000	45000	45000	70000	37000	75000	63000	50000	
26	Sandwich	intermediate	100000	61000	47000	100000	80000	150000	63000	51000	140000	130000	
30	Pasta salad	high	2500000	2800000	2100000	3500000	1500000	3700000	2800000	3900000	2900000	3200000	
27	Sandwich	high	8300000	3400000	3100000	2700000	3200000	9400000	2800000	4000000	3000000	3300000	

**0.1ml spread plate with 48h incubation**

(Food) Category 1		Dairy products											
(Food) Type 1		Dry Dairy products											
			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	
6	Dessert powder	low	350	370	540	390	310	280	280	420	270	260	
1	Milk powder	low	630	610	600	750	660	1300	12000	1100	1000	1400	
5	Dessert powder	intermediate	56000	50000	46000	140000	41000	72000	65000	83000	75000	76000	
2	Milk powder	intermediate	61000	55000	68000	74000	82000	100000	91000	84000	73000	74000	
3	Milk powder	high	11000000	9500000	8100000	1100000	7100000	12000000	9700000	13000000	12000000	10000000	
4	Dessert powder	high	6500000	5500000	8500000	5800000	5800000	7500000	8900000	10000000	8200000	8600000	

(Food) Category 2		Fishery products											
(Food) Type 2		Ready to cook											
			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	
7	White fish	low	560	700	610	540	540	510	550	470	560	360	
10	Tuna steak	low	660	970	830	580	700	230	430	490	560	440	
8	White fish	intermediate	38000	53000	16000	63000	69000	52000	62000	51000	48000	56000	
11	Tuna steak	intermediate	41000	68000	45000	35000	46000	83000	61000	47000	52000	42000	
9	White fish	high	5100000	4000000	4800000	6600000	4600000	3200000	3300000	4800000	4800000	5100000	
12	Tuna steak	high	4300000	3100000	5700000	2800000	3900000	4400000	7400000	5200000	5000000	6500000	

(Food) Category 3		Produce and fruits											
(Food) Type 3		Cut ready to eat veg		Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5	
16	Grated carrot	low	2000	4400	1500	4200	1300	2400	4700	1600	3600	1100	
14	Lettuce	intermediate	23000	10000	13000	16000	10000	28000	10000	19000	15000	11000	
13	Lettuce	low	470000	460000	720000	520000	810000	800000	570000	670000	610000	1000000	
17	Grated carrot	intermediate	430000	450000	330000	420000	310000	510000	450000	370000	380000	360000	
18	Grated carrot	high	1300000	900000	800000	1900000	1100000	1900000	1100000	1200000	600000	500000	
15	Lettuce	high	44000000	71000000	120000000	100000000	90000000	55000000	72000000	94000000	190000000	160000000	

(Food) Category 4		Raw and ready to cook 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	Raw stir fry beef strips	low	400	90	110	140	170	100	130	100	50	80		
22	Chicken breast fillets	low	240	200	200	200	220	200	150	140	90	230		
23	Chicken breast fillets	intermediate	5400	4500	8100	5700	4400	6500	5100	10000	8600	4000		
24	Chicken breast fillets	high	620000	660000	250000	450000	480000	530000	1000000	200000	480000	540000		
20	Raw stir fry beef strips	intermediate	41000000	91000000	39000000	55000000	96000000	26000000	97000000	16000000	49000000	77000000		
21	Raw stir fry beef strips	high	21000000	20000000	16000000	48000000	20000000	20000000	20000000	16000000	44000000	22000000		

(Food) Category 5		Multicomponent											
(Food) Type 5		Composite foods with raw ingredients		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	
28	Pasta salad	low	670	450	420	670	360	620	620	640	790	590	
25	Sandwich	low	9700	2300	8500	7900	6400	12000	3200	8500	9200	6900	
29	Pasta salad	intermediate	48000	25000	50000	45000	45000	70000	37000	75000	63000	50000	
26	Sandwich	intermediate	100000	61000	47000	100000	80000	150000	63000	51000	140000	130000	
30	Pasta salad	high	2500000	2800000	2100000	3500000	1500000	3700000	2800000	3900000	2900000	3200000	
27	Sandwich	high	8300000	3400000	3100000	2700000	3200000	9400000	2800000	4000000	3000000	3300000	

**1ml pour plate with 36h incubation**

(Food) Category 1		Dairy products											
(Food) Type 1		Dry Dairy products											
			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	
6	Dessert powder	low	350	370	540	390	310	280	280	400	270	260	
1	Milk powder	low	630	610	600	750	660	1300	12000	1100	1000	1400	
5	Dessert powder	intermediate	56000	50000	46000	140000	41000	71000	65000	83000	75000	76000	
2	Milk powder	intermediate	61000	55000	68000	74000	82000	100000	91000	84000	73000	74000	
4	Dessert powder	high	6500000	5500000	8500000	5800000	5800000	7500000	8900000	10000000	8200000	8600000	
3	Milk powder	high	11000000	9500000	8100000	1100000	7100000	11000000	9200000	13000000	12000000	10000000	

(Food) Category 2		Fishery products											
(Food) Type 2		Ready to cook		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	
7	White fish	low	560	700	610	540	540	540	670	480	520	410	
10	Tuna steak	low	660	970	830	580	700	650	620	430	360	370	
8	White fish	intermediate	38000	53000	16000	63000	69000	35000	60000	47000	29000	36000	
11	Tuna steak	intermediate	41000	68000	45000	35000	46000	31000	59000	51000	35000	350000	
9	White fish	high	5100000	4000000	4800000	6600000	4600000	3100000	3600000	3400000	4400000	3500000	
12	Tuna steak	high	4300000	3100000	5700000	2800000	3900000	5200000	3800000	4900000	2000000	3900000	

(Food) Category 3		Produce and fruits											
(Food) Type 3		Cut ready to eat veg		Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5	
16	Grated carrot	low	2000	4400	1500	4200	1300	1700	3200	980	2600	7700	
14	Lettuce	intermediate	23000	10000	13000	16000	10000	20000	11000	12000	7000	14000	
17	Grated carrot	intermediate	430000	450000	330000	420000	310000	82000	280000	220000	480000	100000	
13	Lettuce	low	470000	460000	720000	520000	810000	540000	460000	750000	660000	920000	
18	Grated carrot	high	1300000	900000	800000	1900000	1100000	1100000	550000	850000	980000	800000	
15	Lettuce	high	44000000	71000000	120000000	100000000	90000000	190000000	65000000	45000000	80000000	100000000	

(Food) Category 4		Raw and ready to cook 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	Raw stir fry beef strips	low	400	90	110	140	170	70	50	90	100	150
22	Chicken breast fillets	low	240	200	200	200	220	200	180	220	180	140
20	Raw stir fry beef strips	intermediate	5400	4500	8100	5700	4400	5300	5300	12000	8300	5100
21	Raw stir fry beef strips	high	620000	660000	250000	450000	480000	540000	780000	230000	370000	430000
23	Chicken breast fillets	intermediate	41000000	91000000	39000000	55000000	96000000	23000000	95000000	30000000	36000000	45000000
24	Chicken breast fillets	high	21000000	20000000	16000000	48000000	20000000	16000000	15000000	14000000	32000000	15000000

(Food) Category 5		Multicomponent										
(Food) Type 5		Composite foods with raw ingredients										
			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
28	Pasta salad	low	670	450	420	670	360	660	520	590	540	460
25	Sandwich	low	9700	2300	8500	7900	6400	11000	3900	9100	14000	8000
26	Sandwich	intermediate	100000	61000	47000	100000	80000	98000	65000	40000	96000	100000
29	Pasta salad	intermediate	48000	25000	50000	45000	45000	32000	19000	71000	67000	66000
27	Sandwich	high	8300000	3400000	3100000	2700000	3200000	8900000	3800000	2600000	3400000	4200000
30	Pasta salad	high	2500000	2800000	2100000	3500000	1500000	2800000	4100000	3700000	3300000	3400000

**1ml pour plate with 48h incubation**

(Food) Category 1		Dairy products														
(Food) Type 1		Dry Dairy products														
			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				
6	Dessert powder	low	350	370	540	390	310	280	280	420	270	260				
1	Milk powder	low	630	610	600	750	660	1300	12000	1100	1000	1400				
5	Dessert powder	intermediate	56000	50000	46000	140000	41000	72000	65000	83000	75000	76000				
2	Milk powder	intermediate	61000	55000	68000	74000	82000	110000	91000	92000	76000	84000				
3	Milk powder	high	11000000	9500000	8100000	1100000	7100000	12000000	9700000	13000000	12000000	10000000				
4	Dessert powder	high	6500000	5500000	8500000	5800000	5800000	7500000	8900000	10000000	8200000	8600000				

(Food) Category 2		Fishery products											
(Food) Type 2		Ready to cook		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	
7	White fish	low	560	700	610	540	540	540	670	480	540	410	
10	Tuna steak	low	660	970	830	580	700	650	630	430	360	370	
8	White fish	intermediate	38000	53000	16000	63000	69000	37000	60000	47000	29000	36000	
11	Tuna steak	intermediate	41000	68000	45000	35000	46000	31000	59000	51000	38000	35000	
9	White fish	high	5100000	4000000	4800000	6600000	4600000	3100000	3600000	3600000	4400000	3500000	
12	Tuna steak	high	4300000	3100000	5700000	2800000	3900000	5300000	3800000	4900000	2100000	4000000	

(Food) Category 3		Produce and fruits										
(Food) Type 3		Cut ready to eat veg		Reference method result					Alternative method result			
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
16	Grated carrot	low	2000	4400	1500	4200	1300	1900	3300	1300	3200	8800
14	Lettuce	intermediate	23000	10000	13000	16000	10000	24000	11000	15000	7000	16000
13	Lettuce	low	470000	460000	720000	520000	810000	550000	460000	750000	660000	920000
17	Grated carrot	intermediate	430000	450000	330000	420000	310000	330000	280000	330000	480000	280000
18	Grated carrot	high	1300000	900000	800000	1900000	1100000	1100000	550000	850000	980000	800000
15	Lettuce	high	44000000	71000000	120000000	100000000	90000000	190000000	70000000	45000000	90000000	100000000

(Food) Category 4		Raw and ready to cook 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	Raw stir fry beef strips	low	400	90	110	140	170	90	70	110	150	160	
22	Chicken breast fillets	low	240	200	200	200	220	240	200	220	190	140	
20	Raw stir fry beef strips	intermediate	5400	4500	8100	5700	4400	5300	5300	12000	8300	5100	
21	Raw stir fry beef strips	high	620000	660000	250000	450000	480000	540000	780000	230000	370000	430000	
23	Chicken breast fillets	intermediate	41000000	91000000	39000000	55000000	96000000	23000000	95000000	30000000	36000000	45000000	
24	Chicken breast fillets	high	21000000	20000000	16000000	48000000	20000000	19000000	18000000	16000000	37000000	18000000	

(Food) Category 5		Multicomponent											
(Food) Type 5		Composite foods with raw ingredients		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	
28	Pasta salad	low	670	450	420	670	360	660	520	590	540	460	
25	Sandwich	low	9700	2300	8500	7900	6400	11000	3900	9100	14000	8000	
29	Pasta salad	intermediate	48000	25000	50000	45000	45000	32000	19000	71000	67000	66000	
26	Sandwich	intermediate	100000	61000	47000	100000	80000	98000	65000	40000	96000	100000	
30	Pasta salad	high	2500000	2800000	2100000	3500000	1500000	2800000	4100000	3700000	3300000	3400000	
27	Sandwich	high	8300000	3400000	3100000	2700000	3200000	8900000	3800000	2600000	3400000	4200000	

**ANNEX D: Summary data for inclusivity study**

Code	Genus	species	CRA Number	Origin	OP TVC spread 36h (log cfu/ml)	OP TVC spread 48h (log cfu/ml)	OP TVC pour 36h (log cfu/ml)	OP TVC pour 48h (log cfu/ml)	Reference PCA (log cfu/ml)
1	<i>Raoultella</i>	<i>terrigena</i>	17343	raw milk	8.0	8.0	8.1	8.1	8.1
2	<i>Enterobacter</i>	<i>cloacae</i>	1472	dried milk	9.0	9.1	9.0	9.0	9.0
3	<i>Klebsiella</i>	<i>oxytoca</i>	8387	Water	8.5	8.5	8.4	8.4	8.4
4	<i>Kluyvera</i>	<i>ascorbata</i>	17126	industrial	8.2	8.2	8.4	8.4	7.8
5	<i>Escherichia</i>	<i>adecarboxylata</i>	5501	Skim milk powder	8.4	8.4	8.2	8.2	8.4
6	<i>Klebsiella</i>	<i>trevisanii</i>	NCIMB 8606	Ropy cream	8.0	8.0	8.1	8.1	8.1
7	<i>Pantoea</i>	<i>agglomerans</i>	17030, NCIMB 702072	Pasteurised milk	7.7	7.7	7.8	7.9	7.8
8	<i>Aeromonas</i>	<i>salmonicida</i>	8388, NCTC 8049	tin of milk with a fishy odour	6.8	6.8	6.9	6.9	7.0
9	<i>Escherichia</i>	<i>coli</i>	1476	Dried milk	8.7	8.7	8.8	8.8	8.6
10	<i>Rahnella</i>	<i>aqualtilis</i>	16911	drinking water	8.6	8.6	8.6	8.7	8.7
11	<i>Bacillus</i>	<i>coagulans</i>	16586	Sterilised milk	8.7	8.7	8.5	8.5	8.7
12	<i>Bacillus</i>	<i>weihenstephanensis</i>	16578	Pasteurised milk	7.5	7.4	7.4	7.4	7.6
13	<i>Lysinibacillus</i>	<i>sphaericus</i>	7746	unknown	7.7	7.9	7.6	7.6	7.7
14	<i>Brevibacillus</i>	<i>aigii</i>	7749	LMG15103	9.8	9.8	9.6	9.6	9.9
15	<i>Staphylococcus</i>	<i>cohnii</i>	272	skin	7.6	7.6	7.6	7.6	7.8
16	<i>Escherichia</i>	<i>hermanii</i>	7460	mixed seeds	8.9	8.9	8.8	8.8	8.7
17	<i>Buttiauxella</i>	<i>agrestis</i>	17110	Pond water	8.9	8.9	8.8	8.9	8.9
18	<i>Citrobacter</i>	<i>youngae</i>	NCTC 13709	Meat scraps	9.0	9.0	9.0	9.0	8.9
19	<i>Moraxella</i>	<i>osloensis</i>	17043	milk	7.7	7.7	7.5	7.5	7.7

Code	Genus	species	CRA Number	Origin	OP TVC spread 36h (log cfu/ml)	OP TVC spread 48h (log cfu/ml)	OP TVC pour 36h (log cfu/ml)	OP TVC pour 48h (log cfu/ml)	Reference PCA (log cfu/ml)
20	<i>Salmonella</i>	<i>Stanley</i>	1057	Boiled Ham	9.0	8.1	8.9	8.9	8.9
21	<i>Staphylococcus</i>	<i>carnosus</i>	284	goat's milk	7.7	7.7	7.3	7.3	7.5
22	<i>Listeria</i>	<i>ivanovii</i>	1123	soft cheese	8.2	8.2	8.1	8.1	8.1
23	<i>Streptococcus</i>	<i>thermophilus</i>	16045, NCIMB 8510	Pasteurised milk	7.7	7.7	7.6	7.6	7.4
24	<i>Lactobacillus</i>	<i>acidophilus</i>	7675	Dairy product	7.6	6.6	7.5	7.5	7.5
25	<i>Carnobacterium</i>	<i>divergens</i>	3910	Brie	8.2	8.2	8.2	8.2	8.1
26	<i>Staphylococcus</i>	<i>saprophyticus</i>	8999	distilled water environmental	7.7	7.7	8.6	7.6	7.5
27	<i>Klebsiella</i>	<i>ozaene</i>	4273	industrial isolate	8.1	7.1	8.1	8.1	7.6
28	<i>Enterococcus</i>	<i>faecalis</i>	1513	Dried milk powder	7.6	7.6	7.7	7.7	7.7
29	<i>Staphylococcus</i>	<i>cohnii</i>	272	skin	7.3	7.3	7.0	7.0	6.8
30	<i>Enterococcus</i>	<i>faecium</i>	16866	Uncooked Sausage	8.0	8.0	7.9	7.9	8.1
31	<i>Staphylococcus</i>	<i>aureus</i>	409/3026	Slow cheese	8.4	8.4	7.4	7.4	8.1
32	<i>Staphylococcus</i>	<i>epidermidis</i>	314	runway & can seam	7.9	7.9	7.7	7.7	7.7
33	<i>Pediococcus</i>	<i>pentosaceus</i>	16030	Brine	8.7	8.7	8.3	8.7	9.0
34	<i>Listeria</i>	<i>monocytogenes</i> 1/2a	1100	Stilton	8.4	8.5	8.1	8.1	8.7
35	<i>Listeria</i>	<i>innocua</i>	3130	Cheese factory	8.8	8.8	8.6	8.6	8.6
36	<i>Listeria</i>	<i>fleischmanii</i> subspecies <i>fleischmanii</i>	16876	Swiss hard cheese	9.1	9.1	9.0	9.0	9.0
37	<i>Siccibacter</i>	<i>turicensis</i>	17681	fruit powder	8.7	8.7	8.3	8.3	8.5
38	<i>Staphylococcus</i>	<i>hominis</i>	16828	unknown	8.0	7.8	7.8	7.8	7.7
39	<i>Staphylococcus</i>	<i>warneri</i>	3198	Dry sausage	8.0	8.0	7.9	8.0	7.9

Code	Genus	species	CRA Number	Origin	OP TVC spread 36h (log cfu/ml)	OP TVC spread 48h (log cfu/ml)	OP TVC pour 36h (log cfu/ml)	OP TVC pour 48h (log cfu/ml)	Reference PCA (log cfu/ml)
40	<i>Lactococcus</i>	<i>lactis</i>	16029	Green ham	7.4	7.4	7.4	7.4	7.5
41	<i>Lactobacillus</i>	<i>paracasei</i>	16659	Emmental cheese	8.8	8.9	8.0	8.4	8.7
42	<i>Micrococcus</i>	<i>roseus</i>	7775	water	8.2	8.2	8.1	8.2	8.1
43	<i>Streptococcus</i>	<i>lactis</i>	1511	dried milk powder	8.2	8.2	8.1	8.1	8.0
44	<i>Enterococcus</i>	<i>malodoratus</i>	16860	Gouda cheese	7.5	7.5	7.6	7.6	7.6
45	<i>Enterococcus</i>	<i>pseudoavium</i>	16852	Cow udder - bovine mastitis	8.4	8.4	8.2	8.2	8.3
46	<i>Byssochlamys</i>	<i>fulva</i>	16668; CBS113245	Pasteurised fruit juice	6.0	6.5	6.4	6.5	6.5
47	<i>Candida</i>	<i>krussei</i>	629	Yogurt base	7.1	7.1	7.0	7.1	7.1
48	<i>Dekkera</i>	<i>sp</i>	16678	unkown	7.6	7.5	7.8	7.8	7.8
49	<i>Geochium</i>	<i>conidium</i>	14398	factory isolate	7.8	8.1	7.2	8.1	8.1
50	<i>Fusarium</i>	<i>solani</i>	16976	factory isolate	6.3	6.1	6.2	6.2	6.2