Method Comparison Study for the ISO 16140-2:2016 validation of Kikkoman Biochemifa Company Easy Plate YM-R, for the enumeration of yeast and moulds in broad range of foods, petfoods and animal feeds and environmental samples

MicroVal study number: 2022LR109

Method/Kit name: Easy Plate YM-R

Report version: Summary Report

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Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v2.5

Company: Kikkoman Biochemifa Company

Expert Laboratory: Campden BRI

Method/Kit name: Easy Plate YM-R

Validation standard: Microbiology of the food chain— Method validation

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

Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)
Reference method: ISO 21527:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds

Part 1: Colony count technique in products with water activity greater than 0.95

Part 2: Colony count technique in products with water activity less than or equal to 0.95

Scope of validation: broad range of foods (7 categories) for Aw >0.95 and Aw ≤ 0.95 plus 2 additional categories

5 categories Aw >0.95:

- Heat processed milk and dairy products
- Bakery products
- Fruits and vegetables
- RTE/RTRH meat and poultry products
- Multi component foods or meal components

2 categories Aw ≤0.95

- Dried cereals fruits nut seeds and vegetables
- Chocolate and confectionary

Additional categories

- Pet food and animal feed
- Environmental samples

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
- DRBCA Dichloran Rose Bengal Chloramphenicol Agar
- DG18 Dichloran-Glycerol Agar

Contents

1 Introduction

In this project a harmonised MicroVal, NordVal and AOAC PTM validation study, based on ISO 16140-2:2016 and AOAC Appendix J, of the alternative method for the enumeration of yeasts and moulds in a broad range of foods, petfood and animal feed and environmental surfaces was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method:

Easy Plate YM-R is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate YM-R method is intended to indicate the level of yeast and mould in food and beverage products. It is compact and easy to use and reduces the total amount of waste produced during testing. 1ml volumes of appropriately diluted samples are plated onto the YM-R plates, allowed to soak in and then incubated at 25 ± 1°C for 72h-76h for all samples and 46h for selected categories (multicomponent foods, fresh produce, and environmental samples).

The alternative method can distinguish between yeasts and moulds (yeast colonies are small circles with defined edges and mould colonies are large with diffuse edges) however a combined yeasts and moulds was done for this study to align with the reference method.

The validation study was performed with both incubation times, 46h and 72h, for all samples. The data produced in the validation study was used to determine the incubation time. Based on the validation study data, an incubation time of 46h was selected for three categories and 72h incubation for all categories. Table 1 summarises the appropriate incubation time for each category.

Table 1. Incubation times for categories

Category	Meets ISO 16140-2 criteria at 46h incubation	Meets ISO 16140-2 criteria at 72h incubation
Heat processed milk and dairy products	✗	✓
Bakery products	✗	✓
Fresh produce and fruits	✓	✓
Ready to eat/ready to reheat meat and poultry products	✗	✓
Multicomponent foods or meal components	✓	✓
Dried cereals, fruits, nuts, seeds and vegetables	✗	✓
Chocolate and confectionery	✗	✓
Pet food and animal feed	✗	✓
Environmental samples	✓	✓

The raw data for the 6 additional categories at 46h incubation is available in the Annex for reference.

Reference methods:

ISO 21527 -2008: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds

Part 1: Colony count technique in products with water activity greater than 0.95

Part 2: Colony count technique in products with water activity equal to or less than 0.95

Scope of the validation study is a broad range of foods (7 categories) for Aw >0.95 and Aw ≤ 0.95 plus an additional 2 further categories :

- Heat processed milk and dairy products
- Bakery products
- Fruits and vegetables
- RTE/RTRH meat and poultry products
- Multi component foods or meal components
- Dried cereals fruits nut seeds and vegetables
- Chocolate and confectionary
- Pet food and animal feed
- Environmental samples

Criteria evaluated during the study:

- Method Comparison Study (MCS)
  - Relative Trueness study
  - Accuracy profile study
  - Limit of Quantification study(LOQ)
  - Inclusivity and exclusivity study

- Interlaboratory Study (ILS)

1.1.1 Conclusion (MCS)

Overall, the conclusions for the Method Comparison at 46 hours incubation are:

- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness for 3 specific categories; multicomponent foods, fresh produce and environmental samples
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile for 3 specific categories; multicomponent foods, fresh produce and environmental samples
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific

Overall, the conclusions for the Method Comparison at 72 hours incubation for a broad range of foods as well as two additional categories: pet food and animal feed and environmental samples:

- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific.

2 Method protocols

The Method Comparison Study was carried out using 50g portions of sample material. ISO 6887-1:2017 section 9.1 states that a minimum 10g or 10ml should be used, however, using larger test portions than the minimum above increased the sensitivity. In this study, a 50g sample was used in order to harmonise with the AOAC requirements.

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 and B for reference and alternative method.

Sample preparations used in the reference method were done using 0.1% peptone water broth as the diluent. This is quoted in ISO 21527 parts 1 and 2 to enable harmonisation with the BAM method. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution may also be used to improve reliability. If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results. Depending on the sample being tested and the expected contamination level, single or multiple dilutions was used with single or duplicate plates if considered necessary to improve the reliability of the calculated result and ensure at least two relevant plates were available for use in calculations. For samples where a low level of contamination is suspected, 1ml from the initial dilution was plated over 3 plates.

2.2 Alternative method

See the flow diagram in Annex A for reference and alternative method.

Easy plate YM-R is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate YM-R method is intended to indicate the level of yeast and mould in food and beverage products. It is compact and easy to use and reduces the total amount of waste produced during testing. 1ml volumes of appropriately diluted samples are plated onto the YM-R plates, allowed to soak in and then incubated at 25 ± 1°C for 72h-76h for all 9 categories included in the study and 46h for 3 selected categories (multicomponent foods and fresh produce and environmental samples). Typical colonies are purple on Easy Plate YM-R.

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 homonegised 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 Relative trueness study

The trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study was conducted using naturally or artificially contaminated samples. Different categories, types and items were tested for this.

A total of 8 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.

3.1.1 Number of samples
The categories, the types and the number of samples analyzed are presented in Table 2.

Table 2 – Categories, types and number of samples analyzed

Category	Types/Items	Preparation
Heat processed milk and dairy products	Pasteurised dairy products e.g. milk-based dessert, drinks, creams	0.1% peptone water broth @ room temperature
	Cheese e.g. hard and semi hard cheese	
	Fermented and acidified pasteurised milk and yogurt e.g. Yogurts with fruit, fermented milk drinks	
Fresh produce and fruits	Fresh fruit salad and fruit purees	0.1% peptone water broth @ room temperature
	Chilled fruit juices	
	Fermented vegetables e.g. sauerkraut, olives	
Multi component foods or meal components	Composite foods with raw ingredients e.g. tuna and cucumber sandwich, pasta salads.	0.1% peptone water broth @ room temperature
	Mayonnaise based chilled salads e.g. prawn layered salad	
	Ambient stable acidified foods e.g. ketchup, fish sauce	
Bakery products	Bakery products with custard	0.1% peptone water broth @ room temperature
	Baked chill products without additives e.g. chilled quiches	
	Par baked bread products Aw >0.95	
RTE/RTRH meat and poultry products	Cooked meat and poultry e.g. cooked hams, pate, cooked poultry	0.1% peptone water broth @ room temperature
	Fermented or dried products e.g. Salami, chicken sausage	
	Raw cured products e.g. dry cured hams, smoked turkey	

Category	Types/Items	Preparation
Dried cereals fruits nuts seeds and vegetables	Dried cereals	PSD @ room temperature. Allow to resuscitate for 20-30 minutes and hand 'mash' for 1 minute
	Nuts and seeds, nut butters	
	Low and IF fruits aw <0.85	
Chocolate, and confectionary	Dry and sugared low moisture aw<0.85 e.g. praline	ISO 6887-4 Chocolate: melt chocolate between 42°C and 47°C before taking test portion Dry powders: PSD @ room temperature. Allow to soak for up to 1 hr before testing.
	Dry and sugared low moisture aw<0.65 e.g. syrups	
	Dry powders e.g. cake mixes	
Pet food and animal feed	Dry Food	0.1% peptone water broth @ room temperature. Allow to resuscitate for 20-30 minutes and hand 'mash' for 1 minute
	Wet food (raw and canned)	0.1% peptone water broth @ room temperature.
	Animal feeds (poultry and fish)	0.1% peptone water broth @ room temperature. Allow to resuscitate for 20-30 minutes and hand 'mash' for 1 minute
Environmental samples (food or feed production)	Surfaces (sponges, swabs) Equipment, floors, walls	0.1% peptone water broth @ room temperature.
	Wash water, cooling water	
	Dusts - Bakery and food manufacturing environment	

135 samples were analyzed, leading to 135 exploitable results.

3.1.2 Test sample preparation

Naturally contaminated samples were preferentially analyzed. 55 samples were screened for the presence of the target organism. From these samples 66% were positive for the target organism and these samples were used in the data analysis. The distribution of naturally contaminated samples between categories is shown in Table 3. The remaining 34% were negative for the target organism. It was therefore necessary to use artificial contamination procedures.

Table 3 – Screening of samples for target organism

Category	**Samples screened**	**Natural contamination – positive for target**	**% of category naturally contaminated**
Heat pasteurised milk and dairy products	1/15	1/15	7
Confectionery bakery and eggs	13/15	11/15	73
Fruits and vegetables	13/15	11/15	73
Ready to eat foods	4/15	1/15	7
Multi component foods or meal components	15/15	8/15	53
Dried cereals fruits nut seeds and vegetables	3/15	0/15	0
Chocolate and confectionary	1/15	0/.15	0
Pet food	5/15	4/15	27
Environmental	0/15	0/15	0

Artificial contaminations were obtained using a seeding or spiking protocol.

Seeded samples with Aw >0.95 were inoculated with yeast isolates and mould spores. After inoculation, the samples were stored under defined conditions to stress the cells, e.g. frozen foods were stored for at least 2 weeks at -20 °C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature. Food samples with an Aw<0.95 were inoculated with lyophilised cultures prior to storage for at least 2 weeks at room temperature.

All strains used to spike samples were stressed following relevant injury protocols for the product type. The injury efficiency was evaluated by comparing enumeration results onto an appropriate selective agar (DRBCA or MEA) and non-selective agars.

All strains used for artificial inoculations preferably originated from comparable sample types as the ones to be inoculated. Each particular strain was used to contaminate up to 5 different items.

27 % of the samples were naturally contaminated.

3.1.3 Protocols applied during the validation study
Incubation time

An incubation time of 46 and 72 hours was used for the study for the alternative method. The 72h time point was included for all foods however at the clients request, we are presenting the data using 46 hours for three selected categories: multicomponent foods, fresh produce and environmental samples.

3.1.4 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.

3.1.5 Calculation and interpretation of relative trueness study
The calculations are provided in Annex C.

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

Figure 1 to Figure 9 shows the scatter plots for all nine categories tested following 72 hours of incubation.

The Figure 10 shows the scatter plot for all the categories at 72 hours of incubation.

72-hour incubation results – all categories

Figure 1 - Scatter plot of the reference method versus alternative method results for the Heat processed milk and dairy products category

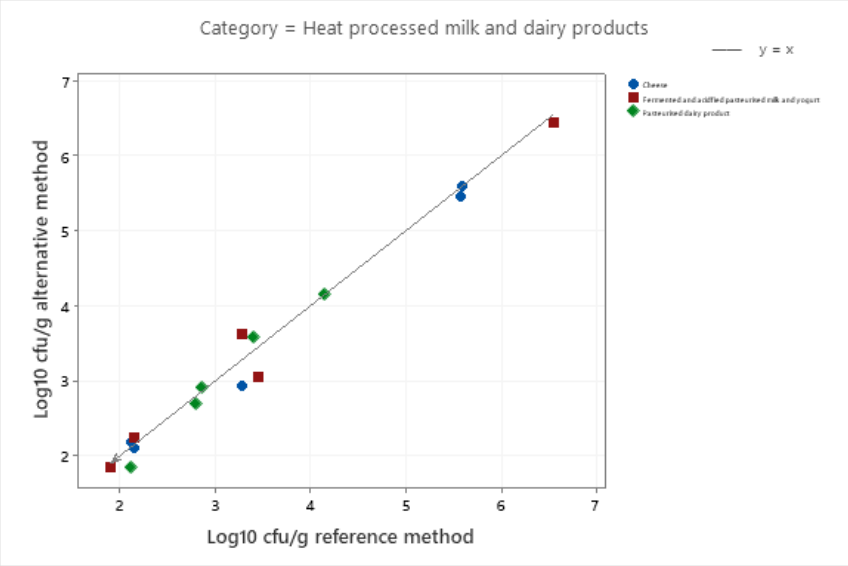


Figure 2- Scatter plot of the reference method versus alternative method results for the Fresh produce and fruits category

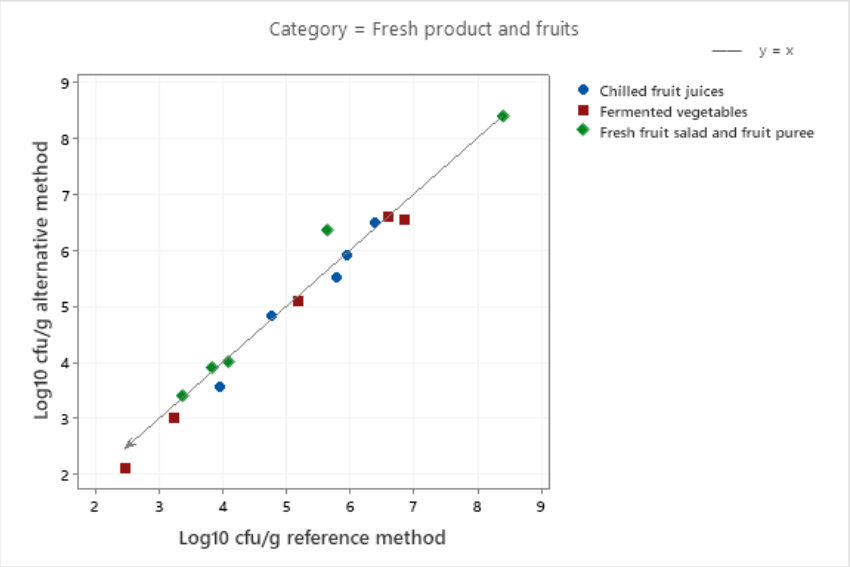


Figure 3- Scatter plot of the reference method versus alternative method results for the multicompoent foods category

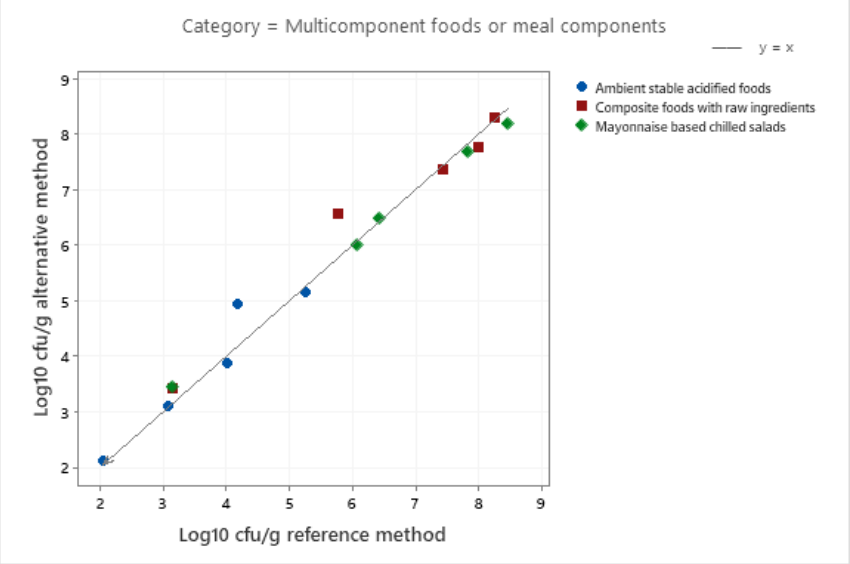


Figure 4- Scatter plot of the reference method versus alternative method results for the ready to eat/ready to reheat meat and poultry category

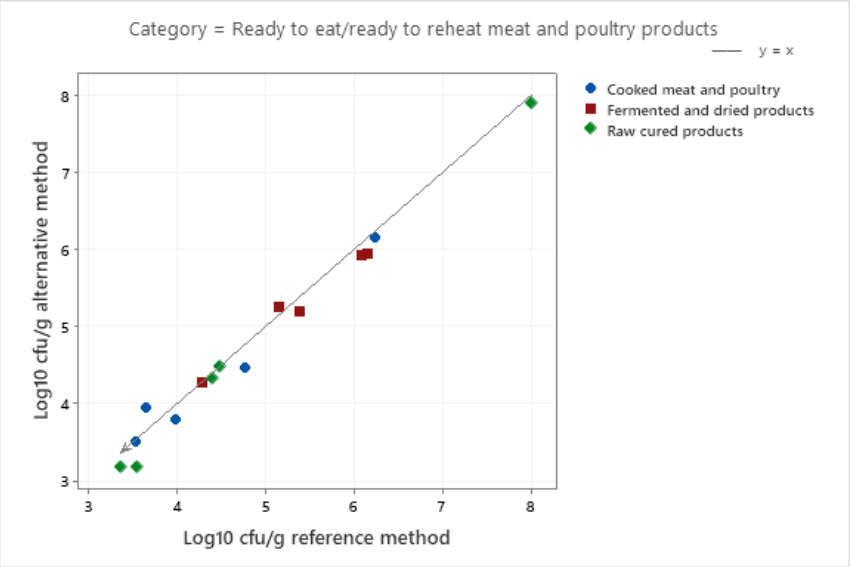


Figure 5- Scatter plot of the reference method versus alternative method results for the confectionery, bakery and eggs category

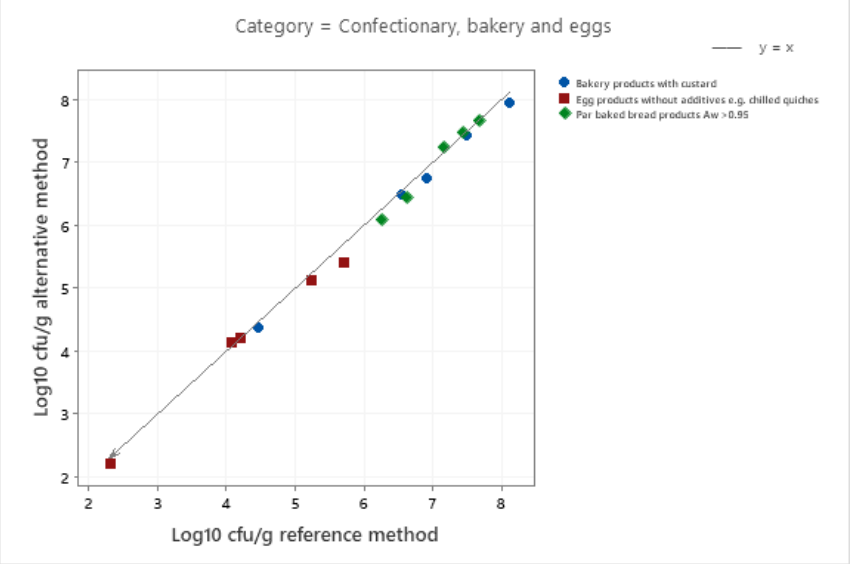


Figure 6- Scatter plot of the reference method versus alternative method results for the chocolate and confectionery category

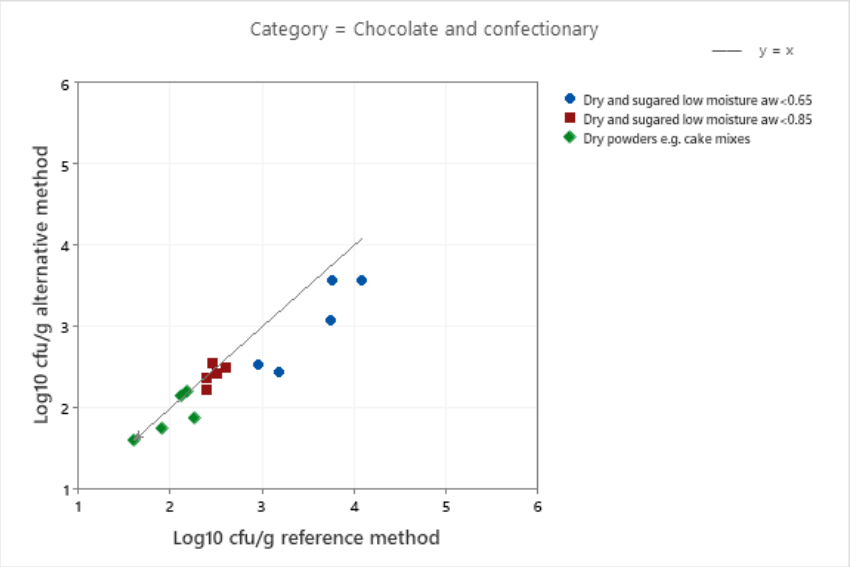


Figure 7- Scatter plot of the reference method versus alternative method results for the dried cereals, fruits and vegetables category

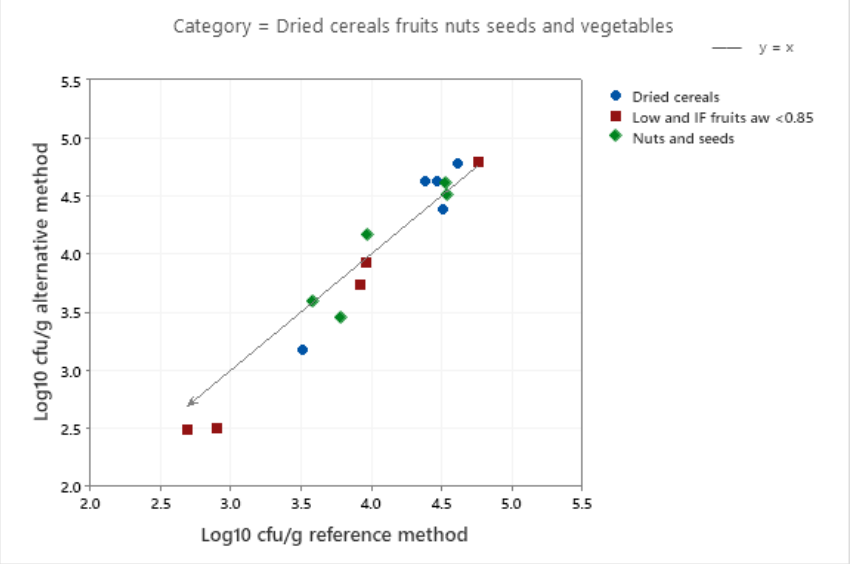


Figure 8- Scatter plot of the reference method versus alternative method results for the pet food and animal feed category

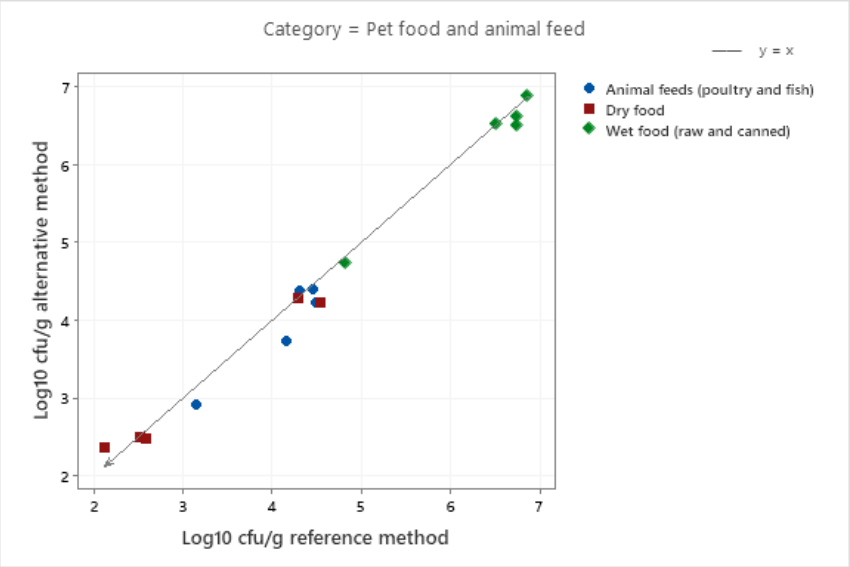


Figure 9 – Scatter plot of the reference method versus alternative method results for the environmental category

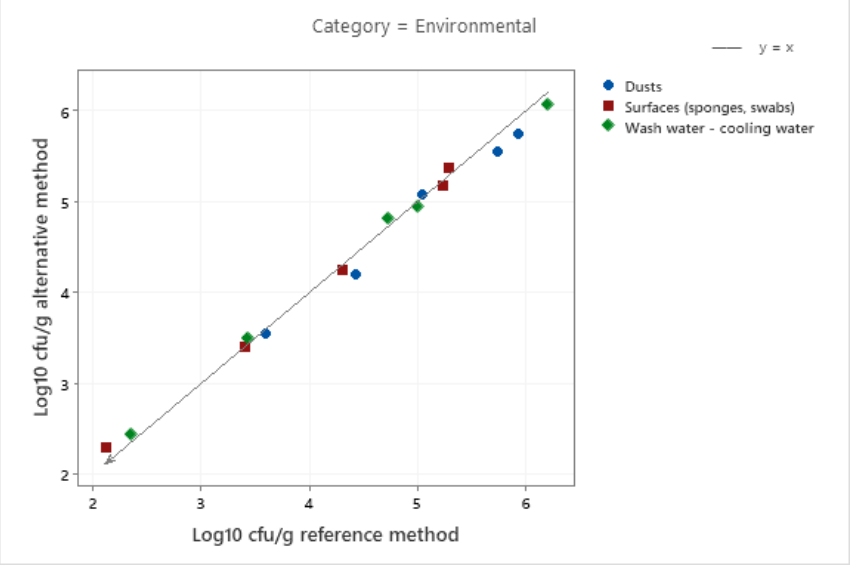
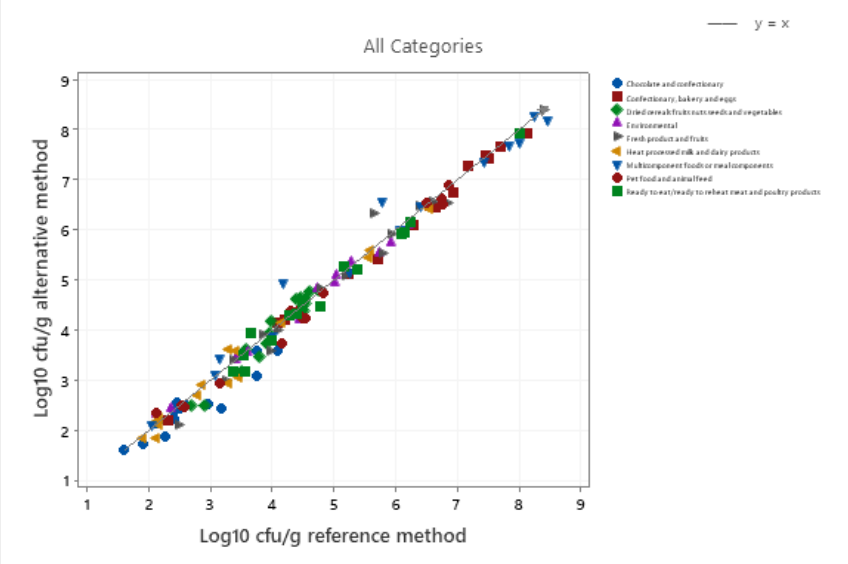


Figure 10 - Scatter plot of the reference method versus alternative method results for all categories



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 no obivous diagreement for 7 out of the 9 categories. There is some signs of negative bias in the chocolate and confectionary category. Although, the range of contamination in samples is between 10^2 cfu/g and 10^4 cfu/g so this affects the visual interpretation of the graph.

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

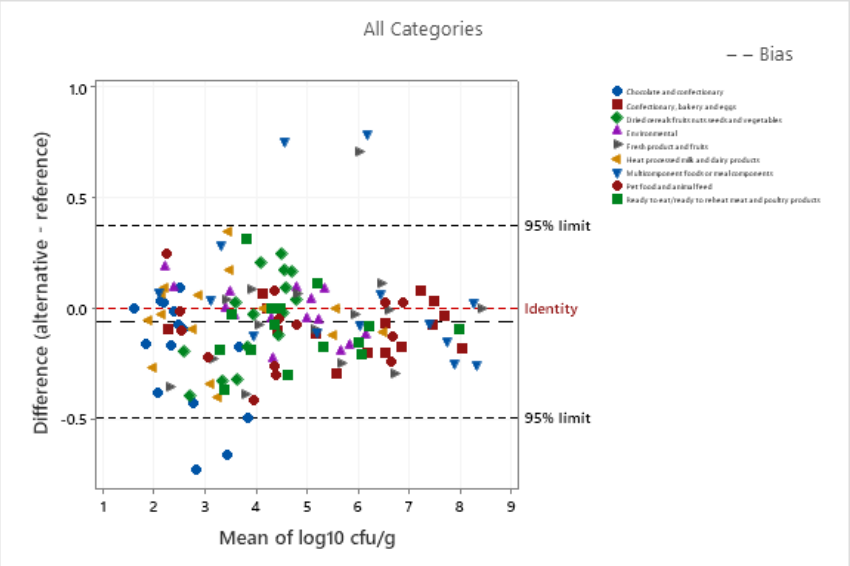
Table 4 - Summary of the calculated values per category

Category	n	$\bar{D}$	SD	95 % low limit	95 % upper limit
Chocolate and confectionary	15	-0.22	0.26	-0.80	0.36
Confectionary, bakery and eggs	15	-0.09	0.11	-0.33	0.15
Dried cereals fruits nuts seeds and vegetables	15	-0.04	0.21	-0.50	0.41
Environmental	15	-0.02	0.12	-0.29	0.24
Fresh product and fruits	15	-0.05	0.27	-0.64	0.54
Heat processed milk and dairy products	15	-0.05	0.19	-0.48	0.38
Multicomponent foods or meal components	15	0.08	0.32	-0.63	0.80
Pet food and animal feed	15	-0.10	0.17	-0.47	0.28
Ready to eat/ready to reheat meat and poultry products	15	-0.10	0.17	-0.46	0.27
All Categories	135	-0.06	0.22	-0.50	0.37

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

The Bland-Altman difference plot for all the samples is given Figure 11.

Figure 11 – Bland-Altman difference plot for all the samples



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in the Table 5.

Table 5 - Data which are outside of the accepted limits

Category	**Type**	**N° Sample**	**Reference method Log cfu/g**	**Alternat-ive method Log cfu/g**	**Mean Log cfu/g**	**Difference Alternative – reference)**	**Lower / Upper limits**	**Comments**
Fresh produce and fruits	Fresh fruit salad and fruit puree	R20	5.63	6.34	5.99	0.71	Upper limit 0.37	Naturally contaminated
Multicomponent foods	Composite foods with raw ingredients	R33	5.78	6.57	6.17	0.79	Upper limit 0.37	Naturally contaminated
Multicomponent foods	Ambient stable acidified foods	R44	4.18	4.93	4.55	0.75	Upper limit 0.37	Artificially inoculated with *Penicillium verrucosum*. Culture was acid adapted in 0.6% acetic acid and then inoculated into sauce and stored for 2 weeks at ambient.
Chocolate and confectionary	Dry and sugared low moisture aw <0.65	R100	3.18	2.45	2.81	-0.73	Lower limit -0.50	Inoculated with lyophilised culture, *Paecilomyces variotti*, and stored for >2 weeks at ambient
		R97	3.74	3.08	3.41	-0.66	Lower limit -0.50	Inoculated with lyophilised culture, *Paecilomyces variotti*, and stored for >2 weeks at ambient

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 135 data points which were outside of the accepted limits. This meets the expectation.

The five food items with differences outside of the limits belonged to 3 out of the 8 categories included in the study, suggesting that they are random outliers. Three out of the five food items showed positive bias and the remaining three food items gave negative bias. In addition, there was no impact on the type of sample contamination observed, with equal numbers of food items being naturally contaminated and artificially contaminated.

The two food items showing a negative bias belonged to chocolate and confectionary category. Therefore, a root cause analysis was carried out to determine potential trends in the data and reasons for the negative bias for this category.

An overall negative bias of -0.22 was observed in the chocolate and confectionery category. All Items in this category were artificially inoculated with a lyophilized mould isolate and stored for >2 weeks at ambient. Moulds have tendency to be slower growing which could possibly explain the greater negative bias seen between the reference and alternative methods. Limited bias was observed in the accuracy profile study of this category, of which 3 samples were inoculated with a xerophilic yeast and 3 samples were inoculated with the xerophilic mould *Paecilomyces variotti* CRA 16670. There was an overall bias of -0.07 observed in the accuracy profile study. The food type tested within the accuracy profile portion of the study was dry and sugared low moisture aw <0.85. Data from the accuracy profile does not indicate a trend towards negative bias for the product type dry and sugared low moisture aw <0.85.

The log differences between the reference and alternative methods for the chocolate and confectionary category obtained in the relative trueness study are shown in Table 6 below. Additional analysis of the data revealed a trend for higher log differences within the dry and sugared low moisture aw <0.65. Three out of the five items in this type have a log difference of ≥-0.50. All items of this type were inoculated with *Paecilomyces variotti* CRA 16670, isolated from fruit concentrate. This strain was also used for the accuracy profile study and no bias was observed. The results suggest that the negative bias observed in the chocolate and confectionery category is a result of the higher log differences achieved for the dry and sugared low moisture aw <0.65 type only.

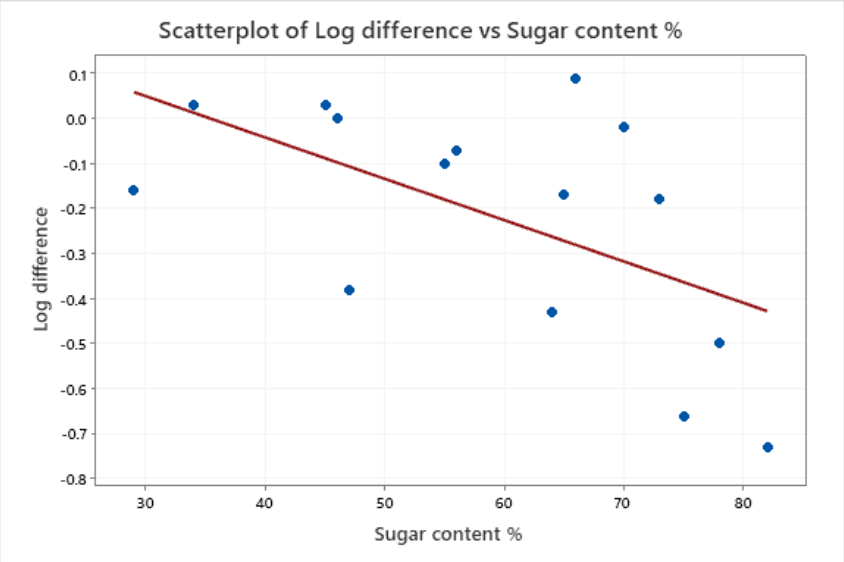
Table 6. Log differences between the reference and alternative methods for the chocolate and confectionery category

Type	Sample code	Sample	Sugar content %	Strain	CRA code	Log (Ref)	Log (Alt)	Difference
Dry and sugared low moisture aw<0.85	R91	milk chocolate	*56*	*Eurotium chevaleri*	16902	2.51	2.43	-0.07
	R92	marzipan	*65*			2.40	2.23	-0.17
	R93	vanilla frosting	*70*			2.40	2.38	-0.02
	R94	chocolate frosting	*55*			2.60	2.51	-0.10
	R95	lemon icing	*66*			2.46	2.56	0.09
Dry and sugared low moisture aw<0.65	R96	maple syrup	*73*	*Paecilomyces variotti*	16670	3.76	3.58	-0.18
	R97	golden syrup	*75*			3.74	3.08	-0.66
	R98	raspberry syrup	*78*			4.08	3.58	-0.50
	R99	black treacle	*64*			2.96	2.53	-0.43
	R100	honey	*82*			3.18	2.45	-0.73
Dry powders e.g. cake mixes	R101	choc chip cookie mix	*45*	*Eurotium repens*	17174	2.11	2.15	0.03
	R102	choc cake mix	*29*			1.90	1.74	-0.16
	R103	vanilla cupcake mix	*47*			2.26	1.88	-0.38
	R104	lemon drizzle mix	*34*			2.18	2.20	0.03
	R105	Victoria sponge mix	*46*			1.60	1.60	0.00

Two further categories contained samples that were low water activity: dried cereals, fruits, nuts, seeds and vegetables and pet food and animal feed were also included in this study. The bias observed in both of these categories is limited at -0.04 and -0.10 respectively. The higher log differences observed in the chocolate and confectionery category do not appear to be linked to the water activity of the food.

Additional statistics were carried out for the samples tested in the chocolate and confectionery category to determine whether there was a link between sugar content and performance of the alternative method. The log difference achieved was plotted against the % sugar content for each sample, shown in Figure 12. The plot revealed that there was a slight negative correlation between log difference and sugar content. Further analysis of the negative correlation showed a weak correlation (R-squared value of 32%). The additional analysis suggests that the performance of the method is not linked to sugar content of the samples analysed in the study.

Figure 12. Scatterplot showing log difference and sugar content for the chocolate and confectionery category



Although there is an indication of negative bias for dry and sugared low moisture (<0.65) foods, the results for the chocolate and confectionery category are within the acceptable performance metrics. It is recommended that end users should perform verification studies to show comparable results with their usual reference method. A root cause analysis investigated several possible trends associated with the negative bias of this sample type including sugar content, strain used and water activity. The root cause analysis could not find any reason to explain the negative bias. In conclusion, the samples with higher log differences between the reference and alternative methods are likely to be random outliers in the analysis.

All log differences between reference and alternative methods in the relative trueness study at 72h incubation were ≤-0.8 log. There is minimal bias between reference and alternative methods for the remaining 8 categories tested.

46h incubation – selected categories (multicomponent foods, fresh produce and environmental)

Figure 13 – Scatter plot of the reference method versus alternative method results for Fresh produce and fruits at 46h incubation

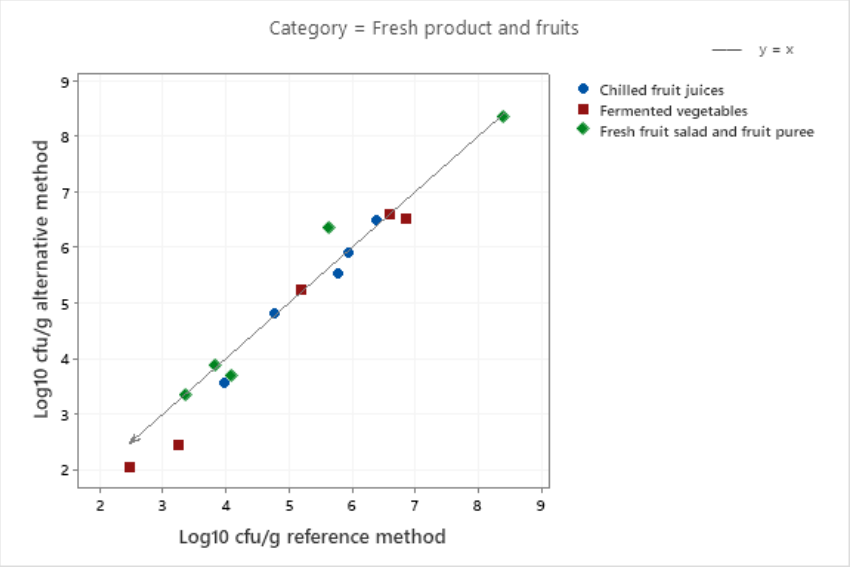


Figure 14 – Scatter plot of the reference method versus alternative method results for multicomponent foods and meal components at 46h incubation

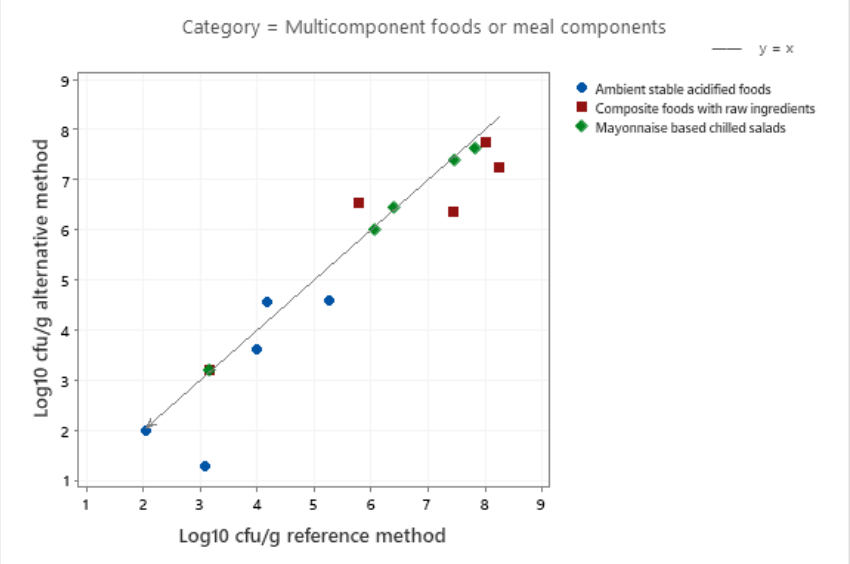


Figure 15 – Scatter plot of the reference method versus alternative method results for environmental samples at 46h incubation

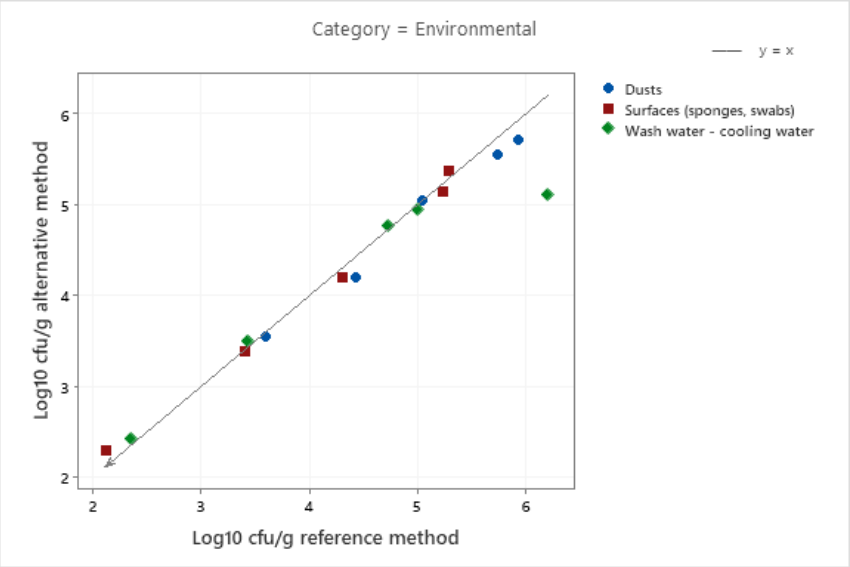
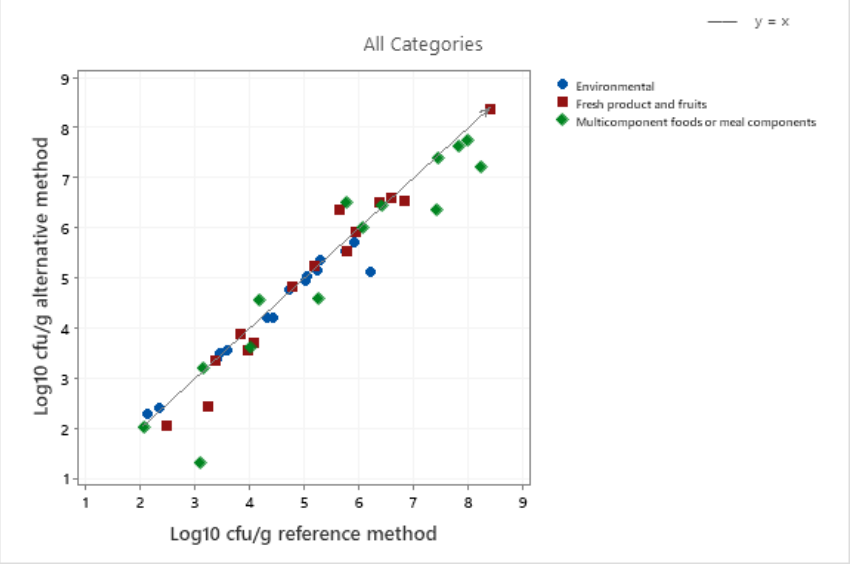


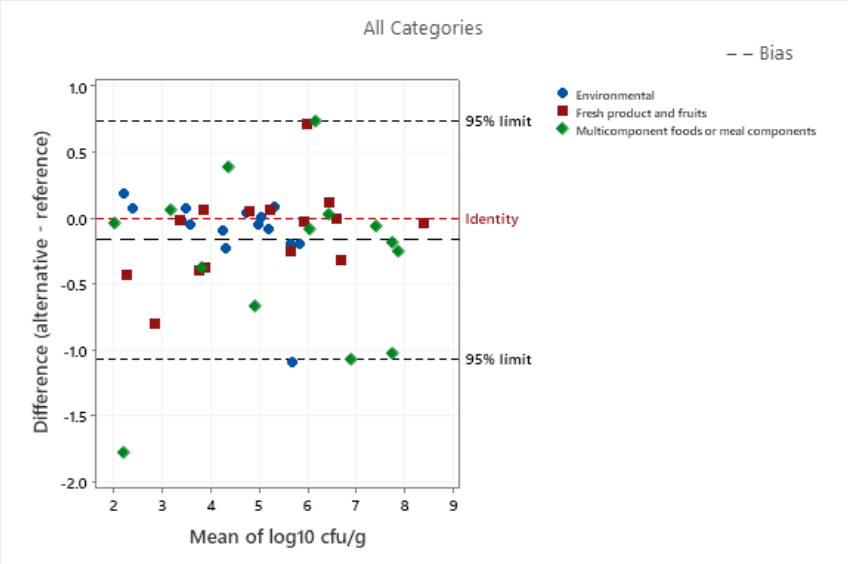
Figure 16 – Scatter plot of the reference method versus alternative method results for three categories at 46h incubation



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 no obivous diagreement or bias for the selected categories.

The Bland-Altman difference plot for the selected categories at 46h incubation is given Figure 17.

Figure 17 – Bland-altman difference plot for all samples at 46h 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

Category and Type	**Sample**	**N° Sample**	**Reference method** **Log cfu/g**	**Alternative method** **Log cfu/g**	**Mean** **Log cfu/g**	**Difference** **Alternative – reference)**	**Lower / Upper limits**	**Contamination**
Multicomponent foods – ambient stable acidified foods	Sweet chilli sauce	R42	3.08	1.30	2.19	-1.78	Lower -1.07	Artificially inoculated with *Penicillium verrucosum*. Culture was acid adapted in 0.6% acetic acid and then inoculated into

Category and Type	Sample	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Lower / Upper limits	Contamination
								sauce and stored for 2 weeks at ambient.

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there was 1 data point from a total of 30 data points which were outside of the accepted limits. This meets the expectation.

The sample outside of the acceptability limits, was R42 a sweet chilli sauce. 4 additional samples from this food type were inoculated following the same seeding protocol and had log differences ranging from -0.66 to 0.62. This suggests that this is not an issue with a specific strain or artificial inoculation procedure. Data from this food type suggests that the sweet chilli sauce is a random outlier in the analysis.

The ingredients in sample R42 are: Water, Sugar, Chilli Paste (7%) (Red Chilli, Salt, Acidity Regulator: Acetic Acid), Garlic Paste (5%) (Garlic, Sugar, Salt, Acidity Regulator: Acetic Acid), Modified Tapioca Starch, Acidity Regulator: Acetic Acid; Salt. The other items in this category are comparable although, there is a larger proportion of Acetic Acid and Chilli in this sample compared to the other samples tested in this product type.

The count for R42 improved with increased incubation time to 72 hours, with a 0.03 log difference between methods.

The alternative method results for sample R42 is below the quantification limit, the datapoints are included in the Bland-Altman analysis and were excluded in the calculated values in Table 8. To meet the ISO 16140-2 statistical analysis, the sample was repeated to investigate the possible reasons for the low counts obatined during the initial analysis.

For further root cause analysis, additional samples were tested to investigate further a potential link between sweet chilli sauce and higher log differences at 46h incubation. Two different sweet chilli sauces and two inoculating strains were used. The repeat analysis followed the same artificial contamination protocol.

Table 8. Sweet chilli sauce - troubleshooting samples

Sample code	Sample	Inoculating strain	Reference method (log cfu/g)	Alternative method - 46h incubation (log cfu/g)	Log difference (alternative 46h – reference)	Log difference 46h	Log difference (alternative 72h – reference)
C1	Sweet chilli sauce 1	*Penicillium verrucosum CRA 16059*	5.88	5.71	-0.17	5.71	-0.17
C2	Sweet chilli sauce 2	*Penicillium verrucosum CRA 16059*	6.00	6.11	0.11	6.11	0.11
C3	Sweet chilli sauce 1	*Candida sojae* CRA 15969	6.46	6.40	-0.06	6.40	-0.06
C4	Sweet chilli sauce 2	*Candida sojae* CRA 15969	6.04	6.15	0.10	6.15	0.10

C1 was used to replace R42 in the statistical analysis as it is a direct repeat of R42, it is also the worst-case result.

A summary of the calculated values per category is provided in Table 9.

Table 9 - Summary of the calculated values per category

Category	n	Dbar	sD	95% Lower limit	95% Upper limit
Fresh product and fruits	15	-0.11	0.30	-0.76	0.55
Multicomponent foods or meal components	15	-0.15	0.34	-0.87	0.64
Environmental	15	-0.10	0.48	-1.22	0.92
All Categories	**45**	**-0.12**	**0.37**	**-0.89**	**0.64**

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

3.1.6 Conclusion (RT study)

72-hour incubation – all categories

The relative trueness of the Alternative method is satisfied as there is good agreement between the reference and alternative methods based on the interpretation of the scatter plots. The overall bias is minimal (-0.07).

There is an indication of negative bias for dry and sugared low moisture (<0.65) foods, however the results for the chocolate and confectionery category are within the acceptable performance metrics. End users should perform verification studies to show comparable results with their usual reference method.

46-hour incubation – selected categories (multicomponent foods, fresh produce and fruits and environmental samples)

The relative trueness of the Alternative method is satisfied as there is good agreement between the reference and alternative methods based on the interpretation of the scatter plots and there is a minimal negative bias (-0.12).

3.2 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 was conducted using artificially contaminated samples. One type per category was tested with 2 items per type.

3.2.1 Categories, sample types and strains
Eight categories were tested with a single batch of two different food items using 6 samples per item.

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. Table 10 shows the food type/strain pairs were studied.

Preparation of samples was done as a bulk inoculation. A 300-400g bulk sample was inoculated with a sutable dilution of the inoculating strain and homogenised by hand massaging or stomaching to evenly distribute the inoculum. For all chilled stored matrices, the bulk samples was inoculated and stored at 2-8°C for 48-72h prior to analysis. For ambient stored products, lyophilised cells was used. The inoculation of samples with moulds was carried out using mould spores.

Five separate 50g test portions was removed from the bulk sample and mixed with 450ml 0.1% peptone water and enumerated on both methods.

For environmental samples, inoculum were applied to surfaces which were allowed to dry at room temperature (18-25°C) for 16-24 hours before sampling. Surfaces were sampled with sponges or swabs pre-moistened in 10ml Letheen broth. Sponges and swabs were held for 2 hours at room temperature before addition of diluent.

Table 10. Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Types	Inoculated Strain	Item
Heat processed milk and dairy products	Pasteurised dairy products	*Saccharomyces cerevisiae* CRA 15968 isolated from frozen fruit juice	Fermented yogurt drink
		Kluyveromyces marxianus CRA 6749 Dairy isolate	Cream cheese
Fruits and vegetables	Fresh produce	*Debaryomyces hansenii CRA 15969* Factory isolate	Vegetable juice
		Pichia galeiformis CRA16015 isolated from spoiled tomato juice	Beetroot salad
Multi component foods or meal components	Composite foods with raw ingredients e.g. sandwiches, pasta salads.	*Geotrichum conidium* CRA 14398, Factory isolate	Raw vegetable salad with dressing
		Saccharomyces exiguous CRA16017 isolated from spoiled mayonnaise	Frozen ready to reheat pizza
Confectionery bakery and eggs	Chilled RTE foods	*Aspergillus niger* CRA 16667 isolated from grapes	Quiche
		Kloeckera apiculata CRA6412 bakery isolate	Custard tart
RTE/RTRH foods	Ready to eat meat and poultry	*Penicillium chrysogeum* DSM 848 source unknown	Cooked breaded chicken patties
		Metschenikowia pulcherrina CRA16167 food spoilage isolate	Deli turkey
Dried cereals fruits nut seeds and vegetables	Nuts and seeds	*Eurotium amstelodami CRA* 8155 isolated from a mouldy kernel	Dried apricots
		Zygosaccharomy-ces rouxii CRA16128 isolated from spoiled fruit	Almond butter
Chocolate, bakery and confectionary	Dry and sugared low moisture aw<0.85	*Zygosaccharomyces bailli* CRA16125 isolated from nougat	Milk chocolate
		Paecilomyces variotii CRA16670 isolated from fruit concentrate	Cream puff
Environmental surfaces	Stainless steel (4” x 4”)	*Candia sojae* CRA16138 isolated from the soft drinks environment	Stainless steel sampled with sponges
	Sealed concrete (1” x 1”)	*Cryptococcus laurenti* CRA16139 isolated from a soft drinks factory	Sealed concrete sampled with swabs

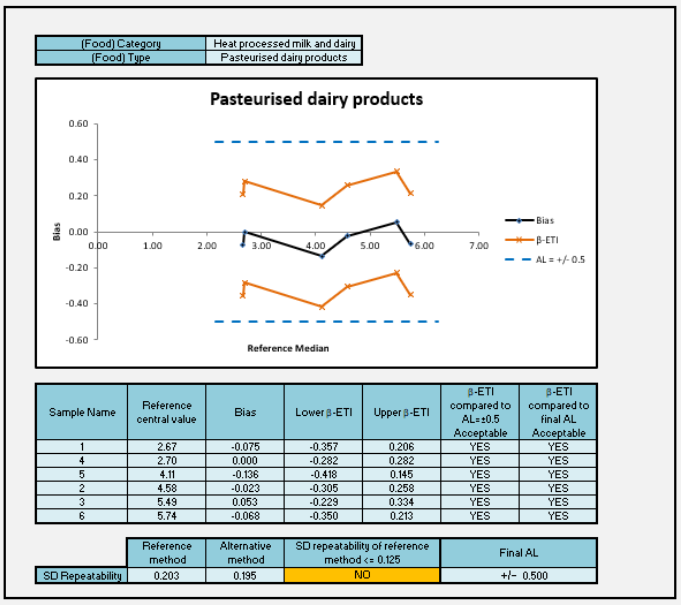
Category	Types	Inoculated Strain	Item
Pet food and animal feed	Dry dog food	*Trichosporon coremiiforme* CRA15962 *isolated from powder*	Kibbles
	Animal origin	*Yarrowia lipolytica* CRA16146 isolated from a factory	Meat and bone meal

3.2.2 Calculations and interpretation of accuracy profile study
The summary tables (in log CFU/g) in Annex D. Statistical results and the accuracy profiles for all categories at 72-hour incubation are provided in Figures 18-29.

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

72 hour incubation – all categories

Figure 18 – Accuracy profile for the heat processed milk and dairy products category

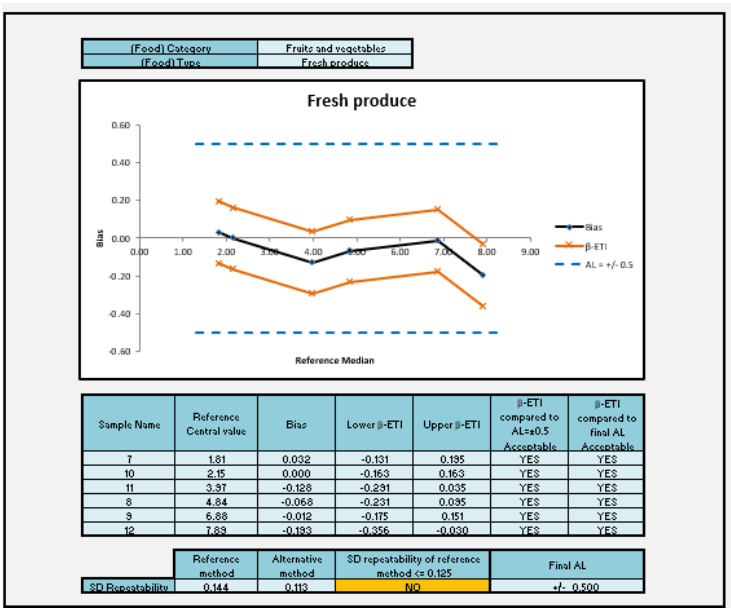


(Food) Category	Heat processed milk and dairy
(Food) Type	Pasteurised dairy products

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
1	2.67	-0.075	-0.357	0.206	YES	YES
4	2.70	0.000	-0.282	0.282	YES	YES
5	4.11	-0.136	-0.418	0.145	YES	YES
2	4.58	-0.023	-0.305	0.258	YES	YES
3	5.49	0.053	-0.229	0.334	YES	YES
6	5.74	-0.068	-0.350	0.213	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.203	0.195	NO	+/- 0.500

Figure 19 – Accuracy profile for the fresh produce and fruits category

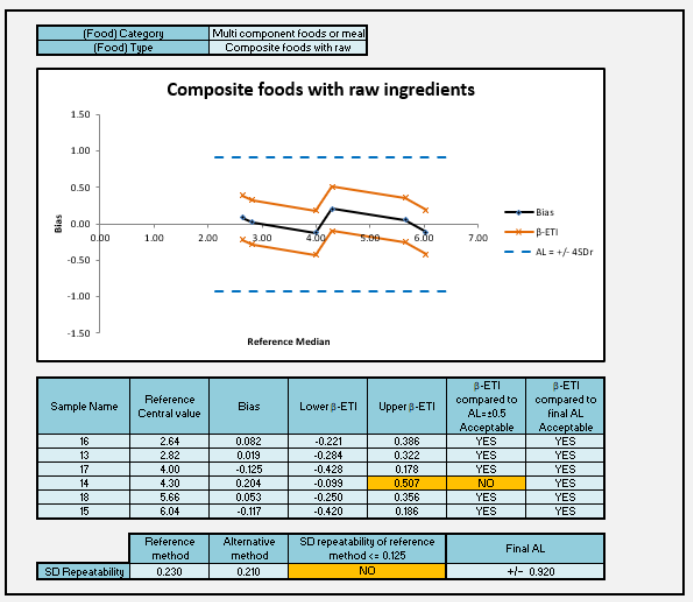


(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7	1.81	0.032	-0.131	0.195	YES	YES
10	2.15	0.000	-0.163	0.163	YES	YES
11	3.97	-0.128	-0.231	0.035	YES	YES
8	4.64	-0.068	-0.231	0.095	YES	YES
9	6.88	-0.012	-0.175	0.151	YES	YES
12	7.89	-0.193	-0.356	-0.030	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.113	NO	+/- 0.500

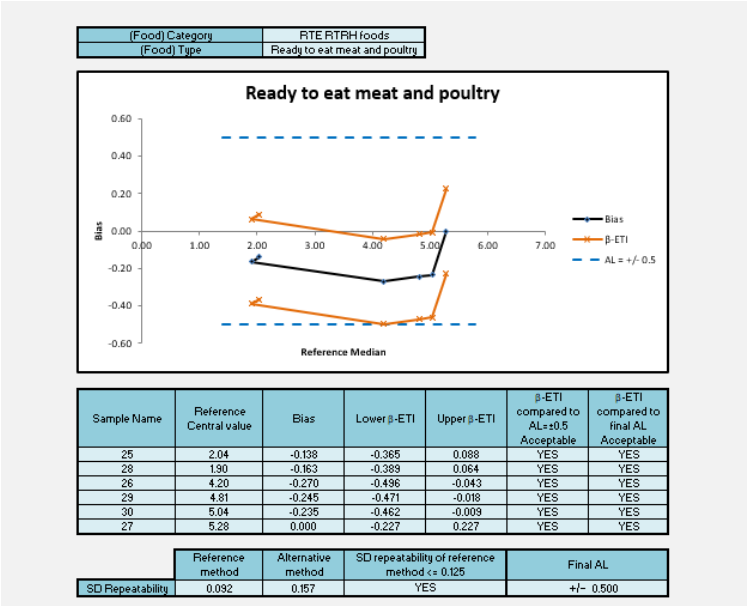
Figure 20 – Accuracy profile for the multicomponent foods category



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16	2.64	0.082	-0.221	0.386	YES	YES
13	2.82	0.019	-0.284	0.322	YES	YES
17	4.00	-0.125	-0.428	0.178	YES	YES
14	4.30	0.204	-0.099	0.507	NO	YES
18	5.66	0.053	-0.250	0.356	YES	YES
15	6.04	-0.117	-0.420	0.186	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.230	0.210	NO	+/- 0.920

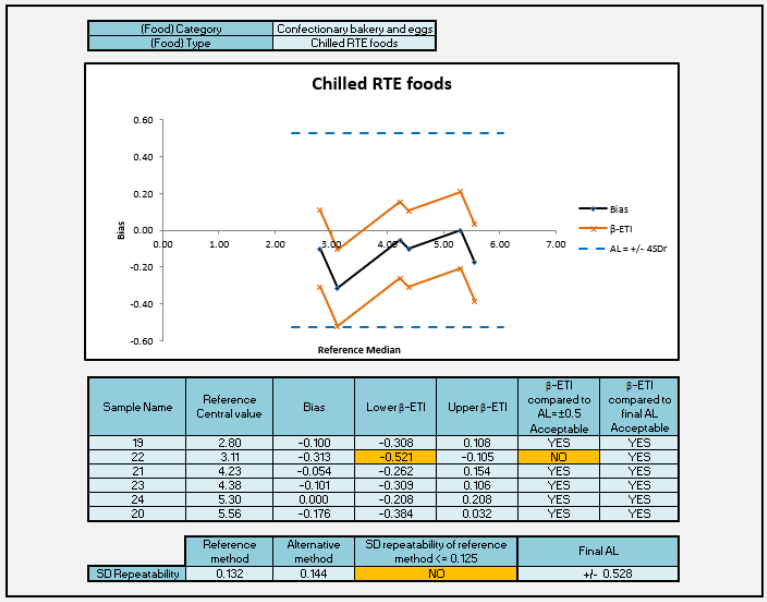
Figure 21 – Accuracy profile for the ready to eat/ready to reheat meat and poultry



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25	2.04	-0.138	-0.365	0.088	YES	YES
28	1.90	-0.163	-0.389	0.064	YES	YES
26	4.20	-0.270	-0.496	-0.043	YES	YES
29	4.81	-0.245	-0.471	-0.018	YES	YES
30	5.04	-0.235	-0.462	-0.009	YES	YES
27	5.28	0.000	-0.227	0.227	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.092	0.157	YES	+/- 0.500

Figure 22 – Accuracy profile for the the confectionery, bakery and eggs category

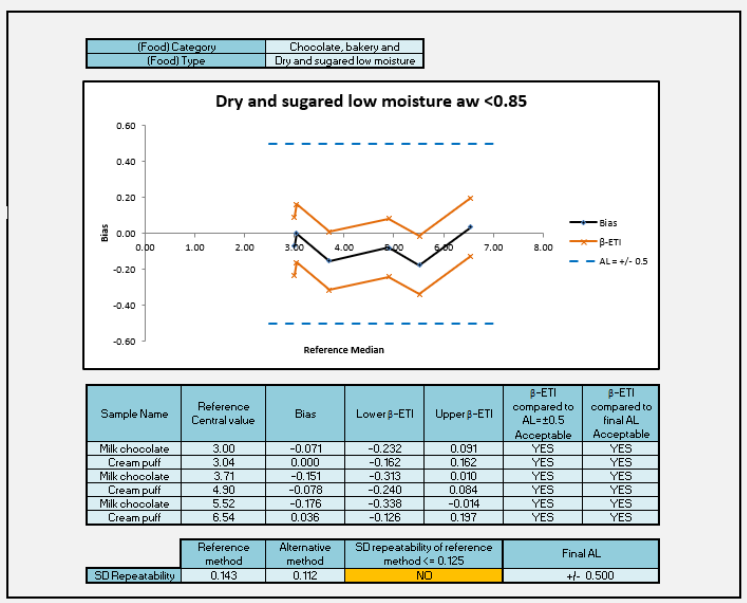


(Food) Category	Confectionary bakery and eggs
(Food) Type	Chilled RTE foods

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19	2.80	-0.100	-0.308	0.108	YES	YES
22	3.11	-0.313	-0.521	-0.105	NO	YES
21	4.23	-0.054	-0.262	0.154	YES	YES
23	4.38	-0.101	-0.309	0.106	YES	YES
24	5.30	0.000	-0.208	0.208	YES	YES
20	5.56	-0.176	-0.384	0.032	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.132	0.144	NO	+/- 0.528

Figure 23 – Accuracy profile for the chocolate and confectionery category

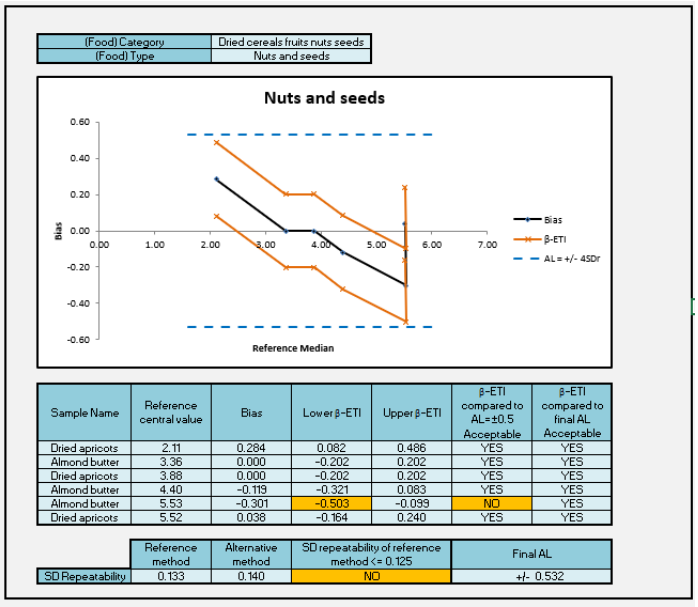


(Food) Category	Chocolate, bakery and
(Food) Type	Dry and sugared low moisture

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Milk chocolate	3.00	-0.071	-0.232	0.091	YES	YES
Cream puff	3.04	0.000	-0.162	0.162	YES	YES
Milk chocolate	3.71	-0.151	-0.313	0.010	YES	YES
Cream puff	4.30	-0.078	-0.240	0.084	YES	YES
Milk chocolate	5.52	-0.176	-0.338	-0.014	YES	YES
Cream puff	6.54	0.036	-0.126	0.197	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.143	0.112	NO	+/- 0.500

Figure 24 – Accuracy profile for the dried cereals, fruits and vegetables category

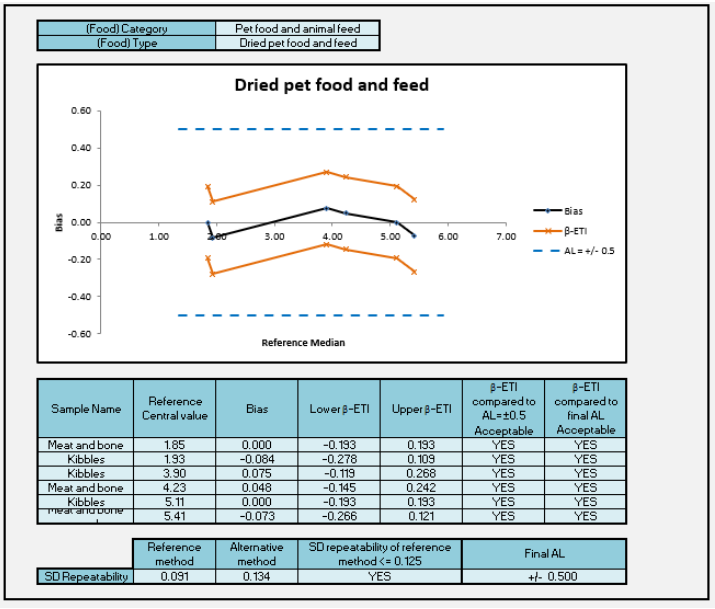


(Food) Category	Dried cereals fruits nuts seeds
(Food) Type	Nuts and seeds

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Dried apricots	2.11	0.284	0.082	0.486	YES	YES
Almond butter	3.36	0.000	-0.202	0.202	YES	YES
Dried apricots	3.88	0.000	-0.202	0.202	YES	YES
Almond butter	4.40	-0.119	-0.321	0.083	YES	YES
Almond butter	5.53	-0.301	-0.503	-0.099	NO	YES
Dried apricots	5.52	0.038	-0.164	0.240	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.133	0.140	NO	+/- 0.532

Figure 25 – Accuracy profile for the pet food and animal feed category

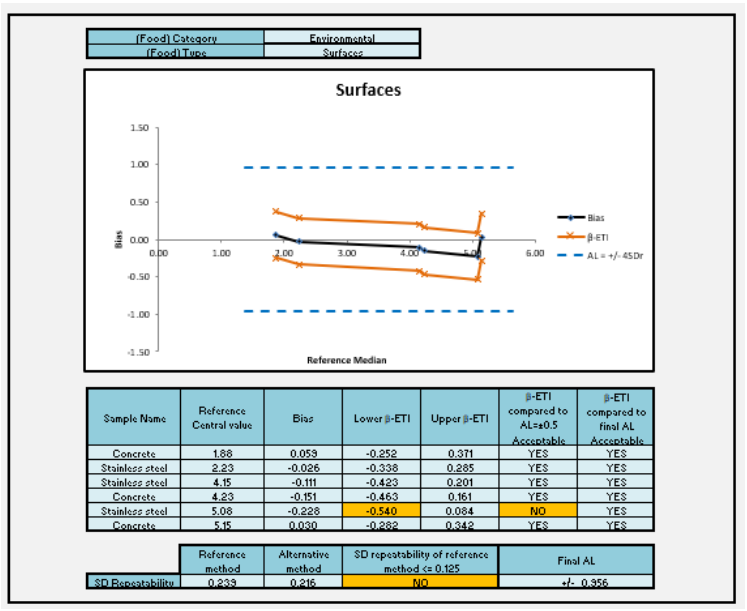


(Food) Category	Pet food and animal feed
(Food) Type	Dried pet food and feed

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Meat and bone	1.85	0.000	-0.193	0.193	YES	YES
Kibbles	1.93	-0.084	-0.278	0.109	YES	YES
Kibbles	3.90	0.075	-0.119	0.268	YES	YES
Meat and bone	4.23	0.048	-0.145	0.242	YES	YES
Kibbles	5.11	0.000	-0.193	0.193	YES	YES
Meat and bone	5.41	-0.073	-0.266	0.121	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.091	0.134	YES	+/- 0.500

Figure 26 – Accuracy profile for the environmental category



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Concrete	1.88	0.059	-0.252	0.371	YES	YES
Stainless steel	2.23	-0.026	-0.338	0.285	YES	YES
Stainless steel	4.15	-0.111	-0.423	0.201	YES	YES
Concrete	4.23	-0.151	-0.463	0.161	YES	YES
Stainless steel	5.08	-0.228	-0.540	0.084	NO	YES
Concrete	5.15	0.030	-0.282	0.342	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.233	0.216	NO	+/- 0.356

Comments – 72h incubation for all categories

In this study the following categories met the AL of 0.5log: pasteurised dairy products, fresh produce, pet food, dried cereals, RTE/RTRH meat and poultry, chocolate and confectionary.

In this study, the following categories required the new AL to be calculated Multicomponent foods, environmental samples and Confectionary bakery and eggs. All of these categories met the new AL value of 0.920, 0.956 and 0.528 respectively.

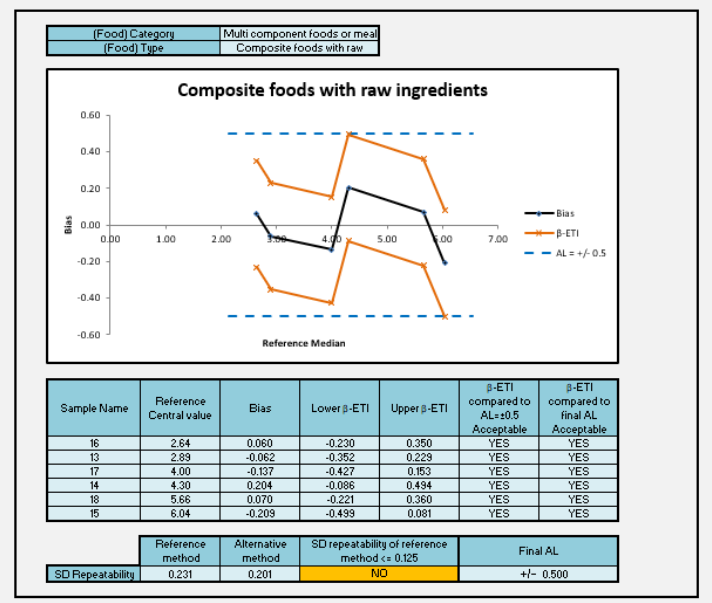
The high repeatability observed in the environmental category is likely due to differences in survivability of the organism on the surface. The surfaces were inoculated and then stored for 16-24 hours ambient before sampling.

The repeatability of the reference and alternative methods for the multicomponent foods was relatively high at 0.230 and 0.210 respectively.

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

46h incubation – selected categories

Figure 27 – Accuracy profile for the multicomponent food

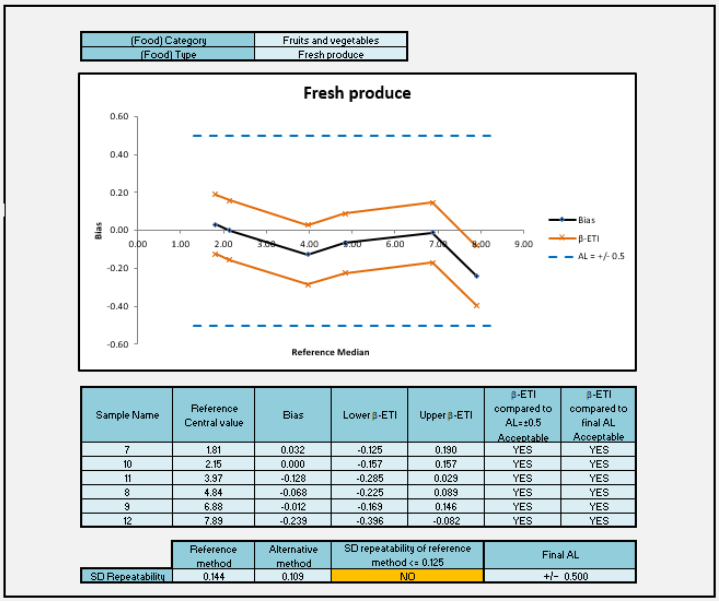


(Food) Category	Multi component foods or meal
(Food) Type	Composite foods with raw

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16	2.64	0.060	-0.230	0.350	YES	YES
13	2.89	-0.062	-0.352	0.229	YES	YES
17	4.00	-0.137	-0.427	0.153	YES	YES
14	4.30	0.204	-0.086	0.494	YES	YES
18	5.66	0.070	-0.221	0.360	YES	YES
15	6.04	-0.209	-0.499	0.081	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.231	0.201	NO	+/- 0.500

Figure 28 – Accuracy profile for the fresh produce category

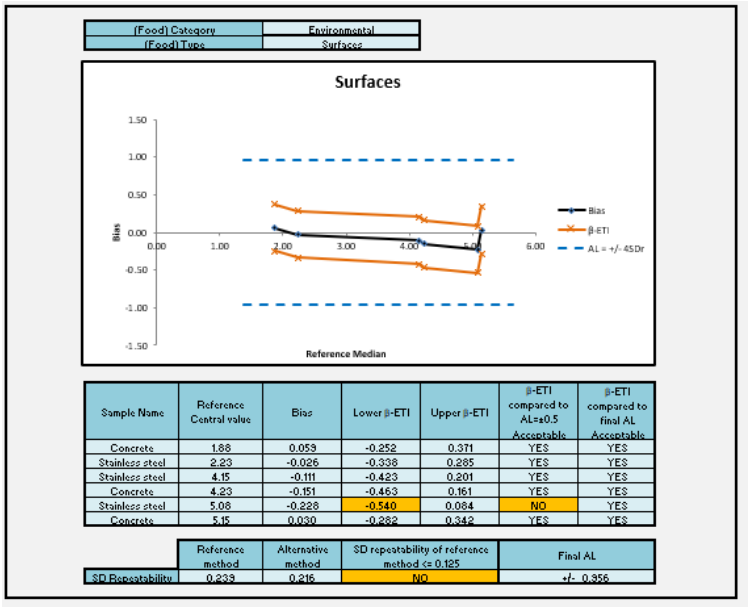


(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7	1.81	0.032	-0.125	0.190	YES	YES
10	2.15	0.000	-0.157	0.157	YES	YES
11	3.97	-0.128	-0.285	0.029	YES	YES
8	4.84	-0.068	-0.225	0.089	YES	YES
9	6.88	-0.012	-0.169	0.146	YES	YES
12	7.89	-0.239	-0.396	-0.082	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.109	NO	+/- 0.500

Figure 29 – Accuracy profile for environmental samples



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Concrete	1.88	0.059	-0.252	0.371	YES	YES
Stainless steel	2.23	-0.026	-0.338	0.285	YES	YES
Stainless steel	4.15	-0.111	-0.423	0.201	YES	YES
Concrete	4.23	-0.151	-0.463	0.161	YES	YES
Stainless steel	5.08	-0.228	-0.540	0.084	NO	YES
Concrete	5.15	0.030	-0.282	0.342	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.233	0.216	NO	+/- 0.956

Comments – 46h incubation for selected categories

In this study the multicomponent foods and fresh produce categories met the AL of 0.5log.

In this study, the environmental category required the AL to be recalculated. This category met the new AL value of 0.956.

The high repeatability observed in the environmental category is likely due to differences in survivability of the organism on the surface. The surfaces were inoculated and then stored for 16-24 hours ambient before sampling.

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

3.3 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.

3.3.1 Protocols

- Inclusivity

50 strains cultures were performed in Malt Extract Broth (MEB medium) at 25°C. Each strain was diluted to an appropriate level and tested once with the alternative method, the reference method and a non-selective agar.

- Exclusivity

30 strains cultures were performed in an appropriate non-selective broth at an appropriate incubation temperature. Each strain was tested once with the alternative method, the reference method and a non-selective agar.

3.3.2 Results

All raw data are given in Annex E.

46 hour incubation – selected categories

- Inclusivity

A total of 50 strains were tested for inclusivity. 49 of these strains showed a positive result at 46 hours. 1 strain, *Eurotium amstelomdami,* did not produce a count following 46h incubation. *Eurotium amstelomdami,* a xerophilic mould and therefore unlikely to be a source of contamination with the three categories (multicomponent foods fresh produce and environmental samples) that are selected for validation at 46 hours.

- Exclusivity

A total of 30 strains were tested for exclusivity. 30 of these strains showed a negative result on the alternative method.

3 strains showed a positive result on the reference methods, as shown in Table 11. The strains were *Pseudomonas fluorescens* (CRA7504), *Burkholderia cepacia* (CRA 16982) and *Asaia siamensis* (CRA 16653).

Further analysis was carried out on the 3 isolates giving unexpected results on the reference methods. MALDI analysis was performed on the isolates and the results are shown in Table 11 below.

Table 11. Positive results for exclusivity.

Strain	CRA Code	Growth on agar	MALDI Score
Pseudomonas fluorescens	7504	DRBCA, DG18	1.89
Burkholderia cepacia	16982	DRBCA	2.37
Asaia siamensis	16653	DRBCA, DG18	1.97

The exclusivity results indicate that the alternative method is more selective than the reference method.

72 hour incubation – all categories

- Inclusivity

A total of 50 strains were tested for inclusivity. 50 of these strains showed a positive result at 72 hours.

- Exclusivity

A total of 30 strains were tested for exclusivity. 30 of these strains showed a negative result on the alternative method.

3 strains showed a positive result on the reference methods, as shown in Table 12. The strains were *Pseudomonas fluorescens* (CRA7504), *Burkholderia cepacia* (CRA 16982) and *Asaia siamensis* (CRA 16653).

Further analysis was carried out on the 3 isolates giving unexpected results on the reference methods. MALDI analysis was performed on the isolates and the results are shown in Table 12 below.

Table 12. Positive results for exclusivity.

Strain	CRA Code	Growth on agar	MALDI Score
Pseudomonas fluorescens	7504	DRBCA, DG18	1.89
Burkholderia cepacia	16982	DRBCA	2.37
Asaia siamensis	16653	DRBCA, DG18	1.97

The exclusivity results indicate that the alternative method is more selective than the reference method.

3.3.3 Conclusion
The alternative method Easy Plate YM-R is selective and specific.

3.3.4 Conclusion (MCS)

Overall, the conclusions for the Method Comparison at 46 hours incubation are:

- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness for the multicomponent foods, fresh produce and environmental samples category
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile the multicomponent foods, fresh produce and environmental samples category
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific

Overall, the conclusions for the Method Comparison at 72 hours incubation for a broad range of foods and environmental samples are:

- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific.

4 Interlaboratory study

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

4.1 Study organisation

4.1.1 Collaborators
Samples were sent to 8 laboratories; 12 collaborators were involved in the study.

4.1.2 Matrix and strain used
Pasta salad samples were co-inoculated with *Candida sojae* CRA 16136 isolated from a soft drinks factory and *Penicillium chrysogenum* DSM 848 isolated from cheese.

4.1.3 Sample preparation
Samples were prepared and inoculated on Monday 6th November 2023 as described below:

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

The target levels and codes are shown in Table 13 below.

Table 13 : Contamination levels

Contamination level	Sample code
Uninoculated	4
Low (10^2 cfu/g)	1
Low (10^2 cfu/g)	5
Medium (10^4 cfu/g)	2
Medium (10^4 cfu/g)	6
High (10^6 cfu/g)	3
High (10^6 cfu/g)	7

4.1.4 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 had to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

4.1.5 Analysis of Samples

Collaborative study laboratories and the expert laboratory carried out the analyses on 13th November 2023 with the alternative and reference methods. The analyses by the reference method and the alternative method were performed on the same day.

4.2 Experimental parameters controls

4.2.1 Detection of yeasts and moulds in the matrix before inoculation

In order to detect the presence of yeasts and moulds the reference method was performed on five portions (25 g) before the inoculation. All the results were negative.

4.2.2 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 yeast and mould after 5 and 7 days of storage at 2-8°C and 7 days of storage at ≤-18°C (Table 14). Frozen samples were thawed under controlled conditions prior to analysis. The data shows good stability under the storage regime tested.

Table 14 – Yeast and mould stability in the matrix

Day	**Storage conditions**	**Reference method (log cfu/g)**							
		Level 1		**Level 2**		**Level 3**		**Level 4**	
		a	**b**	**a**	**b**	**a**	**b**	**a**	**b**
0	N/A	2.7	2.9	3.8	3.8	5.5	5.5	6.4	6.5
5	Storage at 2-8°C	2.5	2.7	3.9	3.5	5.9	5.6	7.1	7.2
7		2.7	2.7	3.4	3.4	5.8	5.7	7.1	7.1
7	Storage at ≤-18°C	2.8	2.9	3.6	3.6	5.5	5.4	6.4	6.5

It was selected to store samples at ≤-18°C due to the possibility of any delays in shipment.

4.2.3 Logistic conditions

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

Table 15 - Sample temperatures at receipt

Collaborator	Average temperature Measured by the probe (°C)	Temperature measured at receipt using water blank (°C)	Receipt date and time	Analysis date
1	-4.2	2.9	9/11/2023 11:30	13/11/2023
2	-3.9	3.4	9/11/2023 11:30	13/11/2023
3	-14.5	4.0	9/11/2023 9:00	13/11/2023
4	-14.2	4.2	9/11/2023 9:00	13/11/2023
5	-13.5	-1.0	8/11/2023 14:26	13/11/2023
6	Data not available	5.3	9/11/2023 11:00	13/11/2023
7	-2.0	0.0	8/11/2023 15:40	13/11/2023
8	Participant did not receive samples			
9	-15.3	3.8	9/11/2023	
10	-17.4	4.3		
11	-14.4	5.7	10/11/2023 11:00	13/11/2023
12	-13.3	5.1	10/11/2023 11:00	13/11/2023

No problem was encountered during the transport or at receipt for 10 collaborators. 1 participant did not receive samples,1 participant had damaged samples and so these results were excluded from the analysis. For the remaining 10 collaborators, temperatures during shipment and at receipt were all correct.

4.3 Calculation and summary of data

4.3.1 MicroVal Expert laboratory results

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

Table 16 – Results obtained by the expert lab – 46h and 72h incubation

Level	Log cfu/g		
	Reference method	**Alternative method – 46h incubation**	**Alternative method – 72h incubation**
Blank	<1.00	<1.00	<1.00
Low	2.15	2.00	2.20
Low	2.23	2.08	2.18
Medium	4.25	4.00	4.10
Medium	4.25	4.36	4.38
High	5.42	5.18	5.24
High	6.47	6.20	6.26

4.3.2 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 Figures 30 and 31 and the statistical analysis of the data shown in Tables 18 and 19.

Table 17. Summary of the results of the interlaboratory study per analyte level

Collaborator	**Level**	**Reference method (Log cfu/g)**		**Alternative method (Log cfu/g) – 46h incubation**		**Alternative method (Log cfu/g) – 72h incubation**	
		Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
1	low	2.7	2.6	2.6	2.7	2.6	2.7
2	low	2.9	2.6	3.5	2.7	3.5	2.7
3	low	2.5	2.5	2.3	2.2	2.7	2.5
4	low	2.3	2.2	2.3	2.1	2.3	2.3
6	low	2.4	2.4	3.0	2.4	3.0	2.5
7	low	3.1	2.7	3.2	2.4	3.4	2.4
9	low	2.2	2.1	2.3	2.2	2.3	2.2
10	low	2.5	2.2	2.5	2.1	2.5	1.8
11	low	1.6	2.0	1.7	2.0	1.7	2.2
12	low	1.8	1.6	1.6	1.6	1.8	2.0
1	medium	5.1	4.6	5.7	4.6	5.7	4.6
2	medium	3.7	4.5	3.6	4.6	3.6	4.6

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 46h incubation		Alternative method (Log cfu/g) – 72h incubation	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
3	medium	4.2	4.3	4.1	4.0	4.5	4.4
4	medium	3.9	4.1	3.9	3.9	4.0	4.0
6	Medium	4.2	4.4	4.1	4.4	4.2	4.4
7	Medium	4.2	4.3	4.1	4.3	4.1	4.4
9	Medium	3.7	3.3	3.8	3.7	4.0	3.9
10	Medium	4.3	4.0	3.9	4.2	4.1	3.9
11	Medium	4.0	4.0	3.9	4.0	4.0	4.1
12	Medium	3.8	4.6	3.7	4.5	3.8	4.5
1	high	6.7	6.2	6.7	6.8	6.7	6.8
2	high	6.6	6.8	6.4	6.7	6.4	6.7
3	high	5.9	6.0	5.8	5.9	5.9	6.2
4	high	5.7	5.8	5.7	5.7	5.8	5.8
6	high	6.0	6.1	6.0	6.0	6.1	6.1
7	high	6.1	6.2	6.4	6.2	6.3	6.2
9	high	5.4	5.7	5.4	5.6	5.5	5.6
10	high	6.0	6.1	5.7	5.8	5.8	5.9
11	high	5.5	5.0	5.6	5.3	5.7	5.4
12	high	5.4	5.3	5.1	5.4	5.3	5.4
1	blank	<1		<1		<1	
2	blank	<1		<1		<1	
3	blank	<1		<1		<1	
4	blank	<1		<1		<1	
6	blank	<1		<1		<1	
7	blank	<1		<1		<1	
9	blank	<1		<1		<1	
10	blank	<1		<1		<1	
11	blank	<1		<1		<1	
12	blank	<1		<1		<1	

Figure 30. Accuracy profile of Easy Plate YMR from the ILS – 46h incubation

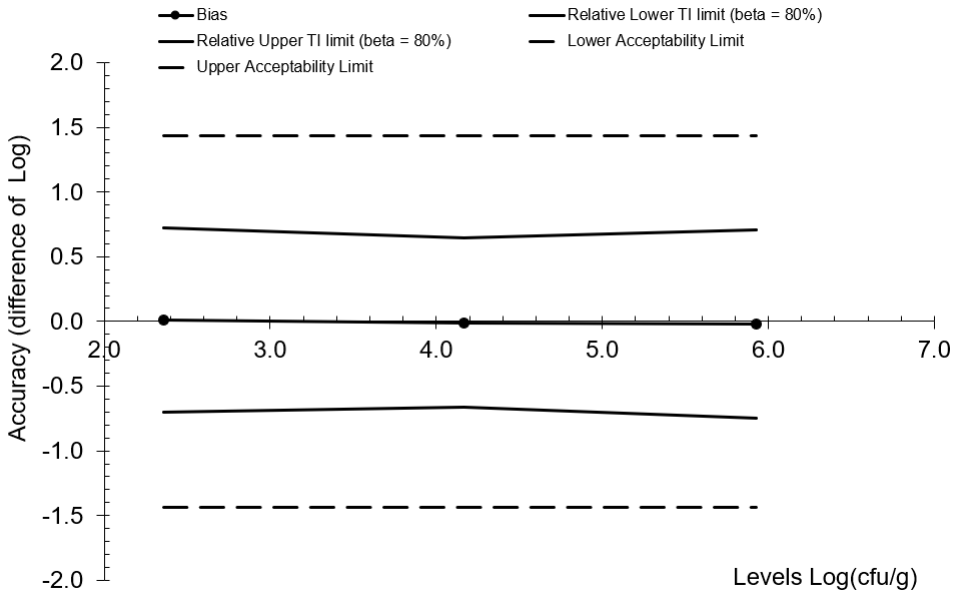


Figure 31. Accuracy profile of Easy Plate YMR from the ILS – 72h incubation

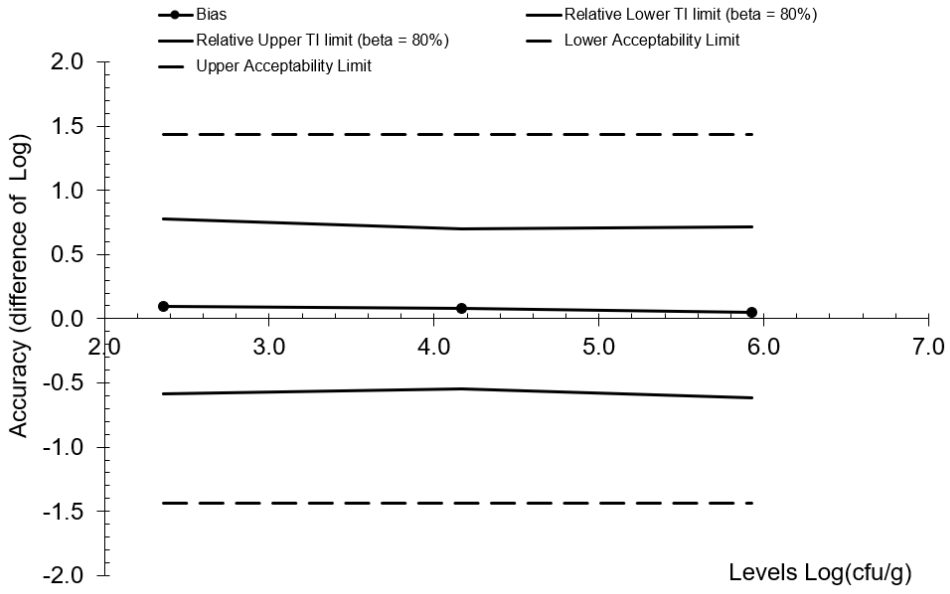


Table 18. Statistical analysis of the ILS data according to the ISO spreadsheet – 46h incubation

Levels	Alternative method Low	Medium	High	Reference method Low	Medium	High
Target value	2.356	4.166	5.929			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	2.366	4.156	5.910	2.356	4.166	5.929
Repeatability standard deviation (sr)	0.319	0.400	0.130	0.168	0.312	0.190
Between-labs standard deviation (sL)	0.395	0.258	0.485	0.375	0.257	0.445
Reproducibility standard deviation (sR)	0.508	0.476	0.502	0.411	0.404	0.484
Corrected number of dof	13.253	16.953	9.620	10.630	15.724	10.491
Coverage factor	1.402	1.376	1.441			
Interpolated Student t	1.349	1.334	1.376			
Tolerance interval standard deviation	0.5280	0.4914	0.5262			
Lower TI limit	1.654	3.501	5.185			
Upper TI limit	3.079	4.811	6.634			
Bias	**0.010**	**-0.010**	**-0.020**			
Relative Lower TI limit (beta = 80%)	**-0.702**	**-0.665**	**-0.744**	TRUE		
Relative Upper TI limit (beta = 80%)	**0.722**	**0.646**	**0.705**	TRUE		
Lower Acceptability Limit	**-1.43**	**-1.43**	**-1.43**			
Upper Acceptability Limit	**1.43**	**1.43**	**1.43**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.434					

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

Table 19. Statistical analysis of the ILS data according to the ISO spreadsheet – 72h incubation

Levels	Alternative method Low	Medium	High	Reference method Low	Medium	High
Target value	2.356	4.166	5.929			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	2.454	4.245	5.979	2.356	4.166	5.929
Repeatability standard deviation (sr)	0.377	0.383	0.123	0.168	0.312	0.190
Between-labs standard deviation (sL)	0.315	0.243	0.446	0.375	0.257	0.445
Reproducibility standard deviation (sR)	0.491	0.453	0.463	0.411	0.404	0.484
Corrected number of dof	15.633	17.030	9.660	10.630	15.724	10.491
Coverage factor	1.385	1.376	1.441			
Interpolated Student t	1.338	1.333	1.376			
Tolerance interval standard deviation	0.5084	0.4675	0.4845			
Lower TI limit	1.773	3.622	5.312			
Upper TI limit	3.134	4.868	6.645			
Bias	**0.097**	**0.079**	**0.050**			
Relative Lower TI limit (beta = 80%)	**-0.583**	**-0.544**	**-0.617**	TRUE		
Relative Upper TI limit (beta = 80%)	**0.778**	**0.703**	**0.716**	TRUE		
Lower Acceptability Limit	**-1.43**	**-1.43**	**-1.43**			
Upper Acceptability Limit	**1.43**	**1.43**	**1.43**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.434					

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

Minimal bias was observed at all levels at 46h incubation (0.010, -0.010, -0.020) and 72h incubation (0.097, 0.079, 0.050) respectively.

A review of the accuracy profile and statistical analysis revealed that there was a high acceptability limit of 1.43 and -1.43 observed in the ILS. To investigate possible reasons for the high AL seen in the ILS, a root cause analysis was carried out.

The same batches of media were used by all participants. The incubation time and temperature were correct.

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	0.168	0.312	0.190
46h alternative	0.319	0.400	0.130
72h alternative	0.377	0.383	0.123

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

Method	**Low**	**Medium**	**High**
Reference	0.375	0.257	0.445
46h alternative	0.395	0.258	0.485
72h alternative	0.315	0.243	0.446

The repeatability of the alternative method is relatively high for the low and medium levels. The medium level samples for both reference and alternative methods have high repeatability.

The standard deviation between labs is high with both reference and alternative methods. The trend for high standard deviation between labs is observed at all three levels of inoculation but is larger with the samples inoculated at the high level.

Samples analysed in the study were co-inoculated with a yeast and mould strain. Although the aim was to achieve comparable levels of contamination in both strains used, a higher level of yeast was observed in the samples. There are several potential reasons for this including instability of the mould in the matrix and different levels of yeast and mould in the inoculum. The samples were inoculated with mould spores, and so it is more likely that the difference in levels is likely due to the levels of yeast and mould inoculated into the samples.

Further investigation has shown that there were differences in the initial inoculation level between the yeast and mould strain. The growth of the mould ($5.2x10^7$ cfu/ml) was lower than that of the yeast ($3.6x10^8$ cfu/ml). In order for the high-level samples to produce the correct inoculation level (10^6 cfu/g) it was necessary to produce an inoculum with higher yeast counts as the maximum inoculation level that was possible for the mould to achieve was 10^5 cfu/g.

It was requested by the MVTC to report the yeast and mould results separately. The purpose of reporting separate yeast and mould counts is for the root cause analysis, the scope of the method validation is a combined yeast and mould count.

Yeast only analysis

The accuracy profile graphs for yeast only are shown in Figures 32 and 33.

Figure 32. Accuracy profile of Easy Plate YMR using yeast only counts from the ILS – 46h incubation

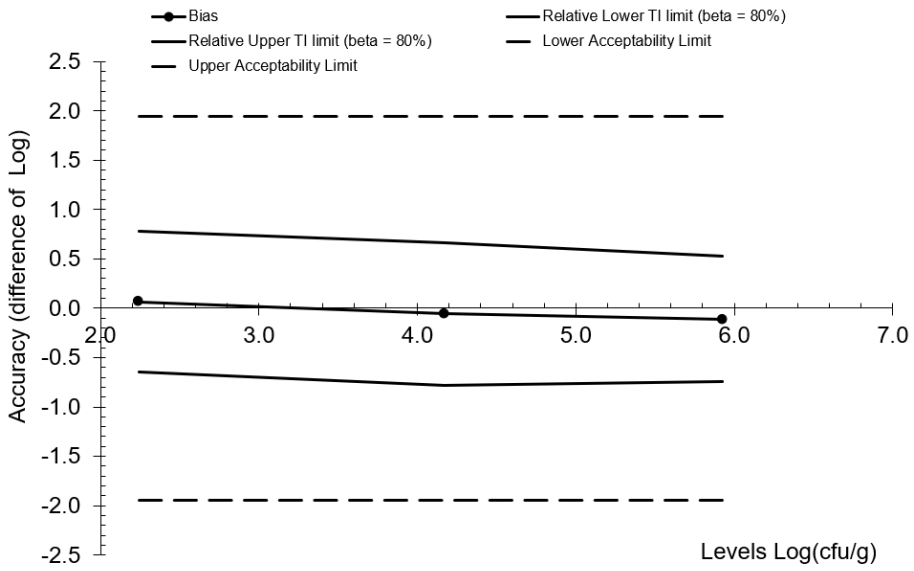
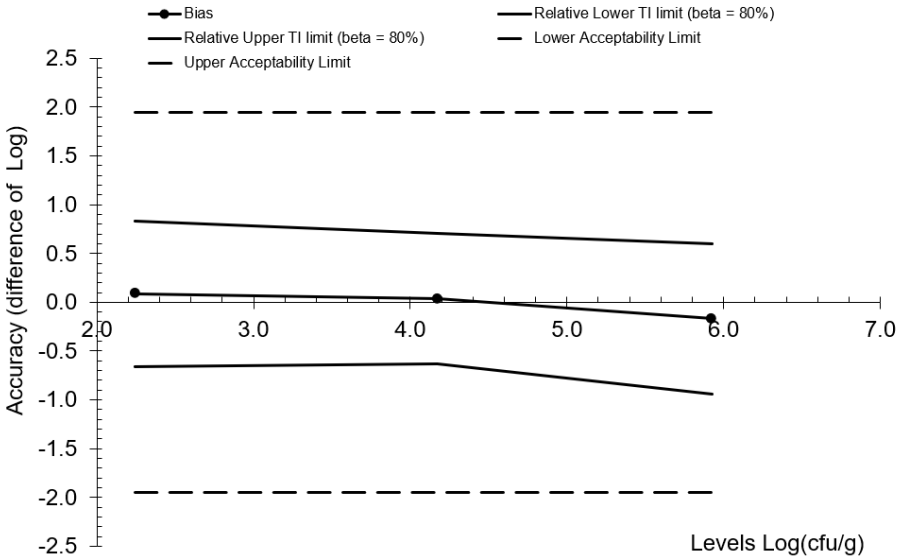


Figure 33. Accuracy profile of Easy Plate YMR using yeast only counts from the ILS – 72h incubation



The yeast-only interlaboratory study results meet the ISO 16140-2 criteria, as with the combined count, minimal bias was achieved by the Easy Plate YM-R method.

Mould only analysis

It was not possible to complete the calculations for the separate mould count. Due to the difference in yeast and mould levels inoculated into the samples, it was observed that there were too many yeasts to count the mould colonies present. Meaning that there is not adequate data to calculate cfu/g in the sample according to ISO 7218:2007.

The conclusion of the root cause analysis is that the large acceptability limit is due to high variability between samples and between labs.

Despite the large re-calculated acceptability limit observed, the interlaboratory study data for the Easy Plate YM-R meets the ISO 16140-2 criteria. There is good agreement between the reference and the alternative methods, this is consistent with the results of the method comparison study.

5 Overall conclusions of the validation study

Overall, the conclusions at 46 hours incubation are:

- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness for the multicomponent foods, fresh produce and environmental samples categories
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile for the multicomponent foods, fresh produce and environmental samples categories
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory performance in the ILS
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows comparable performance to the reference method ISO 21527:2008 Part 1 and BAM Chapter 18 for the multicomponent foods, fresh produce and environmental samples categories

Overall, the conclusions at 72 hours incubation are:

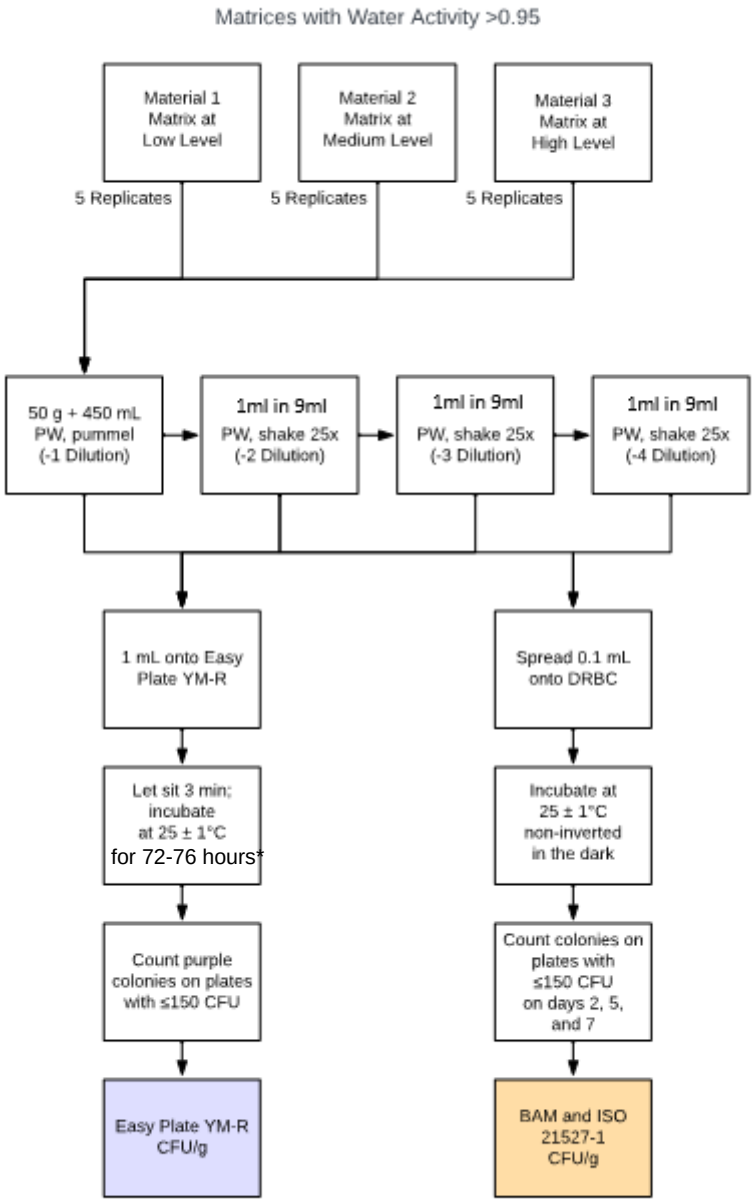
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for relative trueness;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory results for accuracy profile;
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds is selective and specific.
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows satisfactory performance in the ILS
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows comparable performance to the reference method ISO 21527:2008 Parts 1 for samples with Aw >0.95
- The alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows comparable performance to the reference method ISO 21527:2008 Part 2 for samples with Aw ≤0.95

21/10/2024

Alice Foxall

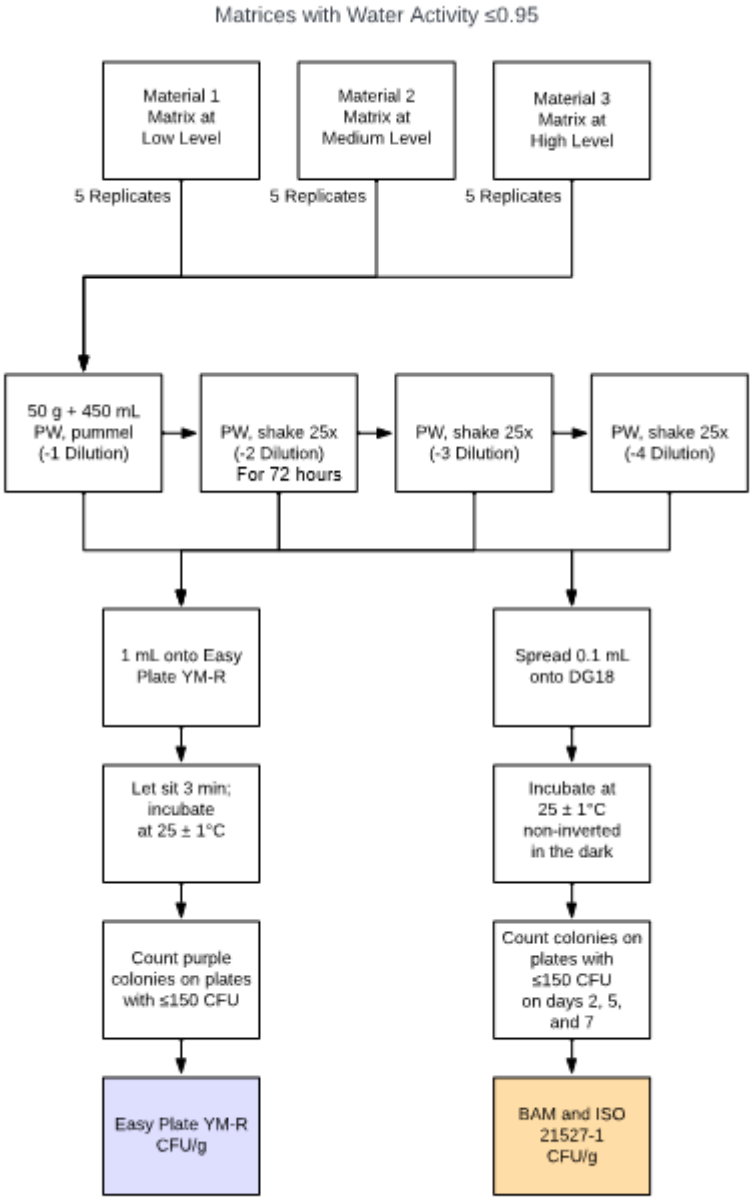
Project Manager – Molecular Microbiology and Methods

ANNEX A: flow diagram of the reference and alternative methods – Aw >0.95



*For multicomponent foods, fresh produce and environmental samples plates can be incubated for 46-76 hours

ANNEX B: Flow diagram of the reference and alternative methods – Aw ≤0.95



ANNEX C: Calculation and interpretation of relative trueness

72h incubation – all categories						
Category – Heat processed milk and dairy products						
Type	**Code**	**Sample**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Pasteurised dairy product	**R1**	Clotted cream	2.85	2.90	2.87	0.06
Pasteurised dairy product	**R2**	Vanilla milkshake	2.79	2.70	2.75	-0.09
Pasteurised dairy product	**R3**	British double cream	4.15	4.15	4.15	0.00
Pasteurised dairy product	**R4**	Soured cream	3.40	3.57	3.48	0.17
Pasteurised dairy product	**R5**	Ice cream	2.11	1.85	1.98	-0.27
Cheese	R6	Blue stilton cheese	5.58	5.58	5.58	0.00
Cheese	**R7**	Cottage cheese, fat free	3.28	2.93	3.11	-0.34
Cheese	**R8**	British Mature grated cheddar cheese	5.57	5.45	5.51	-0.12
Cheese	**R9**	Dairy soft cheese	2.11	2.18	2.15	0.06
Cheese	**R10**	Grated four cheese mix	2.15	2.11	2.13	-0.03
Fermented and acidified pasteurised dairy	**R11**	Peach fermented yogurt drink	3.45	3.04	3.24	-0.41
Fermented and acidified pasteurised dairy	**R12**	Fermented strawberry yogurt	1.90	1.85	1.87	-0.06
Fermented and acidified pasteurised dairy	**R13**	Fermented mango yogurt	3.28	3.62	3.45	0.34
Fermented and acidified pasteurised dairy	**R14**	Fermented apricot yogurt	2.15	2.24	2.19	0.09
Fermented and acidified pasteurised dairy	**R15**	Fermented kiwi yogurt	6.54	6.43	6.49	-0.11
Category – Fresh produce and fruits						
Fresh fruit salad and fruit puree	R16	Blueberries	3.83	3.91	3.87	0.09
Fresh fruit salad and fruit puree	R17	Strawberries	3.36	3.40	3.38	0.04
Fresh fruit salad and fruit puree	R18	Berry fruit salad	4.08	4.00	4.04	-0.08
Fresh fruit salad and fruit puree	R19	Apple banana strawberry and grape	8.40	8.40	8.40	0.00
Fresh fruit salad and fruit puree	R20	Melon kiwi and strawberry	5.63	6.34	5.99	0.71
Chilled fruit juices	**R21**	Vegetable juice	6.38	6.49	6.44	0.11
Chilled fruit juices	R22	Pure orange	3.96	3.57	3.76	-0.39
Chilled fruit juices	R23	Apple and pear juice	4.76	4.83	4.79	0.06
Chilled fruit juices	R24	Multivitamin boost fruit juice	5.77	5.52	5.64	-0.25
Chilled fruit juices	R25	Pure orange and mango fruit juice	5.93	5.90	5.92	-0.03
Fermented vegetables	**R26**	Sauerkraut with carrot	2.47	2.11	2.29	-0.36
Fermented vegetables	R27	Raw Kimchi	3.23	3.00	3.12	-0.23
Fermented vegetables	**R28**	Pitted Green Olives	6.59	6.58	6.59	-0.01
Fermented vegetables	R29	Pitted Black Olives	6.84	6.54	6.69	-0.29
Fermented vegetables	**R30**	Spicy kimchi	5.18	5.08	5.13	-0.10
Category - Multicomponent foods and meal components						
Composite foods with raw ingredients	**R31**	Ready to reheat pizza	3.15	3.43	3.29	0.29
Composite foods with raw ingredients	R32	Seafood cocktail sandwich	8.00	7.75	7.87	-0.25

Type	Code	Sample	log(Ref)	log(Alt)	Mean	Difference
Composite foods with raw ingredients	R33	Red leicester ploughman's sandwich	5.78	6.57	6.17	0.79
Composite foods with raw ingredients	R34	Tomato and sweet pepper salad	7.43	7.36	7.40	-0.07
Composite foods with raw ingredients	R35	Mediterranean salad	8.26	8.28	8.27	0.02
Mayonnaise based chilled salads	R36	Potato salad	3.15	3.43	3.29	0.29
Mayonnaise based chilled salads	**R37**	Potato salad 2	8.46	8.20	8.33	-0.26
Mayonnaise based chilled salads	R38	Potato and egg salad	7.83	7.68	7.76	-0.15
Mayonnaise based chilled salads	R39	Charlotte potato salad	6.08	6.00	6.04	-0.08
Mayonnaise based chilled salads	R40	Coleslaw	6.41	6.48	6.45	0.06
Ambient stable acidified foods	**R41**	Mayonnaise squeezy	2.04	2.11	2.08	0.07
Ambient stable acidified foods	**R42**	Sweet chilli sauce	3.08	3.11	3.10	0.03
Ambient stable acidified foods	**R43**	Tartare sauce	4.00	3.88	3.94	-0.12
Ambient stable acidified foods	**R44**	Bbq sauce	4.18	4.93	4.55	0.75
Ambient stable acidified foods	**R45**	Tomato ketchup	5.26	5.15	5.20	-0.11
Category – Confectionary, bakery and eggs						
Bakery products with custard	R46	Fresh Custard Slices	8.11	7.93	8.02	-0.18
Bakery products with custard	**R47**	Egg custard tarts	7.49	7.41	7.45	-0.08
Bakery products with custard	R48	Custard tart	4.46	4.36	4.41	-0.10
Bakery products with custard	R49	Custard slices	6.54	6.48	6.51	-0.07
Bakery products with custard	R50	Lemon and Elderflower tarts with custard	6.91	6.74	6.83	-0.17
Egg products without additives e.g. chilled quiches	**R51**	Crustless spinach, edamame and kale quiche	4.20	4.20	4.20	0.00
Egg products without additives e.g. chilled quiches	**R52**	Cheddar and onion quiche	4.08	4.15	4.11	0.07
Egg products without additives e.g. chilled quiches	R53	Crustless broccoli and tomato quiche	5.71	5.41	5.56	-0.29
Egg products without additives e.g. chilled quiches	**R54**	Crustless cheddar and bacon quiche	5.23	5.11	5.17	-0.12
Egg products without additives e.g. chilled quiches	R55	Quiche lorraine	2.30	2.20	2.25	-0.10
Par baked bread products Aw >0.95	R56	Part Baked Rustic White Rolls	7.45	7.48	7.46	0.03
Par baked bread products Aw >0.95	R57	Part Baked Rustic Seeded Rolls	7.18	7.26	7.22	0.08
Par baked bread products Aw >0.95	R58	Brown Baguettes Bake at Home	6.63	6.43	6.53	-0.20
Par baked bread products Aw >0.95	R59	Bake at Home White Petit Pains	7.70	7.66	7.68	-0.04
Par baked bread products Aw >0.95	R60	Bake at Home Stonebaked White baguettes	6.28	6.08	6.18	-0.20
Category – Ready to eat/ready to reheat meat and poultry products						
Cooked meat and poultry	**R61**	Cooked breaded chicken popcorn	4.76	4.46	4.61	-0.30
Cooked meat and poultry	**R62**	Deli turkey	3.53	3.51	3.52	-0.03
Cooked meat and poultry	**R63**	Cooked ham	6.23	6.15	6.19	-0.08
Cooked meat and poultry	**R64**	Cooked turkey fillet	3.64	3.95	3.80	0.31
Cooked meat and poultry	**R65**	Cooked chicken breast strips	3.99	3.80	3.89	-0.19
Fermented and dried products	**R66**	Beef Jerky	5.15	5.26	5.20	0.11

Type	Code	Sample	log(Ref)	log(Alt)	Mean	Difference
Fermented and dried products	**R67**	Beef biltong original	4.28	4.28	4.28	0.00
Fermented and dried products	**R68**	Pepparami tender jerky	5.38	5.20	5.29	-0.18
Fermented and dried products	**R69**	Truffle Saucisson Sec	6.08	5.92	6.00	-0.15
Fermented and dried products	**R70**	Petits Saucisson sec	6.15	5.94	6.04	-0.21
Raw cured products	**R71**	Chorizo	4.48	4.48	4.48	0.00
Raw cured products	**R72**	Spicy chorizo slices	3.54	3.18	3.36	-0.37
Raw cured products	**R73**	Pizza pepperoni slices	4.40	4.32	4.36	-0.08
Raw cured products	R74	Prosciutto crudo, salami plater	8.00	7.90	7.95	-0.10
Raw cured products	**R75**	Fuet	3.36	3.18	3.27	-0.19
Category – dried cereals, fruits, nuts, seeds and vegetables						
Dried cereals	**R76**	Rice snaps	4.38	4.62	4.50	0.24
Dried cereals	**R77**	Corn flakes	4.61	4.78	4.70	0.17
Dried cereals	**R78**	Low fat special flakes	4.46	4.63	4.55	0.17
Dried cereals	**R79**	Malt wheats	4.51	4.38	4.44	-0.12
Dried cereals	**R80**	Wheat biscuits	3.51	3.18	3.34	-0.33
Nuts and seeds	**R81**	Wholefoods 4 seed mix	3.97	4.18	4.07	0.20
Nuts and seeds	**R82**	Toasted 3 seed mix	4.53	4.62	4.58	0.09
Nuts and seeds	**R83**	Sunflower seeds	4.54	4.52	4.53	-0.03
Nuts and seeds	**R84**	Unsalted mixed nuts	3.58	3.60	3.59	0.02
Nuts and seeds	**R85**	Almond butter	3.79	3.46	3.62	-0.32
Low and IF fruits aw <0.85	**R86**	Sultanas	3.91	3.74	3.83	-0.17
Low and IF fruits aw <0.85	**R87**	Raisins	3.96	3.93	3.94	-0.03
Low and IF fruits aw <0.85	**R88**	Apricots	2.69	2.49	2.59	-0.20
Low and IF fruits aw <0.85	**R89**	Dried mixed fruit	2.90	2.50	2.70	-0.40
Low and IF fruits aw <0.85	**R90**	Italian mixed peel	4.76	4.79	4.77	0.04
Category – Chocolate and confectionary						
Dry and sugared low moisture aw<0.85	**R91**	Milk chocolate	2.51	2.43	2.47	-0.07
Dry and sugared low moisture aw<0.85	**R92**	Marzipan	2.40	2.23	2.31	-0.17
Dry and sugared low moisture aw<0.85	**R93**	Vanilla frosting	2.40	2.38	2.39	-0.02
Dry and sugared low moisture aw<0.85	**R94**	Chocolate frosting	2.60	2.51	2.55	-0.10
Dry and sugared low moisture aw<0.85	**R95**	Lemon icing	2.46	2.56	2.51	0.09
Dry and sugared low moisture aw<0.65	**R96**	Maple syrup	3.76	3.58	3.67	-0.18
Dry and sugared low moisture aw<0.65	**R97**	Golden syrup	3.74	3.08	3.41	-0.66
Dry and sugared low moisture aw<0.65	**R98**	Raspberry syrup	4.08	3.58	3.83	-0.50
Dry and sugared low moisture aw<0.65	**R99**	Black treacle	2.96	2.53	2.75	-0.43
Dry and sugared low moisture aw<0.65	**R100**	Honey	3.18	2.45	2.81	-0.73

Type	Code	Sample	log(Ref)	log(Alt)	Mean	Difference
Dry powders e.g. cake mixes	**R101**	Choc chip cookie mix	2.11	2.15	2.13	0.03
Dry powders e.g. cake mixes	**R102**	Choc cake mix	1.90	1.74	1.82	-0.16
Dry powders e.g. cake mixes	**R103**	Vanilla cupcake mix	2.26	1.88	2.07	-0.38
Dry powders e.g. cake mixes	**R104**	Lemon drizzle mix	2.18	2.20	2.19	0.03
Dry powders e.g. cake mixes	**R105**	Victoria sponge mix	1.60	1.60	1.60	0.00
Category – Pet food and animal feed						
Dry food	**R106**	Complete dry kitten food turkey and rice	4.53	4.23	4.38	-0.30
Dry food	**R107**	Complete adult dry cat food turkey and rice	2.52	2.51	2.51	-0.01
Dry food	**R108**	Health dry kitten food	2.11	2.36	2.24	0.25
Dry food	**R109**	Dry adult dog food lamb and rice	4.28	4.28	4.28	0.00
Dry food	**R110**	Mature dog biscuit bones	2.58	2.48	2.53	-0.10
Wet food (raw and canned)	R111	Chicken with vegetables	6.75	6.51	6.63	-0.24
Wet food (raw and canned)	R112	Tuna fillet natural cat food	6.51	6.53	6.52	0.03
Wet food (raw and canned)	R113	Wet adult dog food lamb with brown rice	6.74	6.61	6.68	-0.13
Wet food (raw and canned)	R114	Luxury mousse wet adult cat food salmon	6.86	6.89	6.87	0.02
Wet food (raw and canned)	**R115**	Mouse with salmon and gravy	4.81	4.74	4.78	-0.07
Animal feeds (poultry and fish)	**R116**	Organic mixed corn for chickens	4.30	4.38	4.34	0.08
Animal feeds (poultry and fish)	**R117**	Chicken oyster shell supplement	4.45	4.40	4.42	-0.05
Animal feeds (poultry and fish)	**R118**	Goldfish flakes with mealworms	4.49	4.23	4.36	-0.26
Animal feeds (poultry and fish)	**R119**	Tropical fish mini pellets	3.15	2.92	3.04	-0.22
Animal feeds (poultry and fish)	**R120**	Chicken feed - corn	4.15	3.73	3.94	-0.41
Category - Environmental						
Surfaces (sponges, swabs)	**T200**	Metal tabletop outside kitchen 1	5.23	5.18	5.20	-0.05
Surfaces (sponges, swabs)	**T201**	Refrigeration metal machine surface	5.28	5.36	5.32	0.08
Surfaces (sponges, swabs)	**T202**	Main factory floor left	4.30	4.26	4.28	-0.05
Surfaces (sponges, swabs)	**T203**	Fume hood inside	3.40	3.40	3.40	0.00
Surfaces (sponges, swabs)	**T204**	Floor right side tile	2.11	2.30	2.21	0.19
Wash water - cooling water	**T205**	Canning process water	5.00	4.95	4.98	-0.05
Wash water - cooling water	**T206**	Wash water	4.72	4.81	4.77	0.09
Wash water - cooling water	**T207**	kitchen 1 sink water	3.43	3.51	3.47	0.07

Type	Code	Sample	log(Ref)	log(Alt)	Mean	Difference
Wash water - cooling water	**T208**	Process hall water	2.34	2.43	2.39	0.09
Wash water - cooling water	**T209**	Canning process water 2	6.20	6.08	6.14	-0.12
Dusts	**T210**	Plastic sheet conveyor machine	5.74	5.54	5.64	-0.20
Dusts	**T211**	Tiled wall 1	5.92	5.75	5.83	-0.17
Dusts	**T212**	Kitchen 1 plastic window sill	5.04	5.08	5.06	0.04
Dusts	**T213**	Conyeor belt 1	4.43	4.20	4.32	-0.23
Dusts	**T214**	Metal bench 1	3.59	3.56	3.57	-0.03
46 hour incubation - multicomponent foods, fresh produce and environmental						
Category – Fresh produce and fruits						
Fresh fruit salad and fruit puree	R16	Blueberries	3.83	3.88	3.85	0.05
Fresh fruit salad and fruit puree	R17	Strawberries	3.36	3.34	3.35	-0.02
Fresh fruit salad and fruit puree	R18	Berry fruit salad	4.08	3.70	3.89	-0.38
Fresh fruit salad and fruit puree	R19	Apple banana strawberry and grape	8.40	8.36	8.38	-0.04
Fresh fruit salad and fruit puree	R20	Melon kiwi and strawberry	5.63	6.34	5.99	0.71
Chilled fruit juices	**R21**	Vegetable juice	6.38	6.49	6.44	0.11
Chilled fruit juices	R22	Pure orange	3.96	3.56	3.76	-0.40
Chilled fruit juices	R23	Apple and pear juice	4.76	4.81	4.79	0.05
Chilled fruit juices	R24	Multivitamin boost fruit juice	5.77	5.52	5.64	-0.25
Chilled fruit juices	R25	Pure orange and mango fruit juice	5.93	5.90	5.92	-0.03
Fermented vegetables	**R26**	Sauerkraut with carrot	2.47	2.04	2.26	-0.43
Fermented vegetables	R27	Raw Kimchi	3.23	2.43	2.83	-0.80
Fermented vegetables	**R28**	Pitted Green Olives	6.59	6.58	6.59	-0.01
Fermented vegetables	R29	Pitted Black Olives	6.84	6.52	6.68	-0.32
Fermented vegetables	**R30**	Kimchi Spicy	5.18	5.23	5.20	0.05
Category – multicomponent foods and meal components						
Composite foods with raw ingredients	**R31**	Ready to reheat pizza	3.15	3.20	3.18	0.06
Composite foods with raw ingredients	R32	Seafood cocktail sandwich	8.00	7.75	7.87	-0.25
Composite foods with raw ingredients	R33	Red Leicester ploughman's sandwich	5.78	6.52	6.15	0.74
Composite foods with raw ingredients	R34	Pomodoro tomato and sweet pepper salad	7.43	6.36	6.90	-1.07
Composite foods with raw ingredients	R35	Mediterranean salad	8.26	7.23	7.74	-1.02
Mayonnaise based chilled salads	R36	Potato salad	3.15	3.20	3.18	0.06
Mayonnaise based chilled salads	**R37**	Potato salad 2	7.46	7.40	7.43	-0.06
Mayonnaise based chilled salads	R38	Potato and egg salad	7.83	7.64	7.74	-0.19
Mayonnaise based chilled salads	R39	Charlotte potato salad	6.08	6.00	6.04	-0.08
Mayonnaise based chilled salads	R40	Coleslaw	6.41	6.45	6.43	0.03

Type	Code	Sample	log(Ref)	log(Alt)	Mean	Difference
Ambient stable acidified foods	**R41**	Mayonnaise squeezy	2.04	2.00	2.02	-0.04
Ambient stable acidified foods	**R42**	Sweet chilli sauce	5.71	5.88	5.79	0.17
Ambient stable acidified foods	**R43**	Tartare sauce	4.00	3.62	3.81	-0.38
Ambient stable acidified foods	**R44**	Bbq sauce	4.18	4.57	4.37	0.39
Ambient stable acidified foods	**R45**	Tomato ketchup	5.26	4.59	4.92	-0.66
Category - environmental						
Dusts	T210	Metal tabletop outside kitchen 1	5.23	5.15	5.19	-0.08
Dusts	T211	Refrigeration metal machine surface	5.28	5.36	5.32	0.08
Dusts	T212	Main factory floor left	4.30	4.20	4.25	-0.10
Dusts	T213	Fume hood inside	3.40	3.38	3.39	-0.02
Dusts	T214	Floor right side tile	2.11	2.30	2.21	0.19
Surfaces (sponges, swabs)	T200	Canning process water	5.00	4.94	4.97	-0.06
Surfaces (sponges, swabs)	T201	Wash water	4.72	4.76	4.74	0.04
Surfaces (sponges, swabs)	T202	kitchen 1 sink water	3.43	3.51	3.47	0.07
Surfaces (sponges, swabs)	T203	Process hall water	2.34	2.41	2.38	0.07
Surfaces (sponges, swabs)	T204	Canning process water 2	6.20	5.11	5.66	-1.09
Wash water - cooling water	T205	Plastic sheet conveyor machine	5.74	5.54	5.64	-0.20
Wash water - cooling water	T206	Tiled wall 1	5.92	5.72	5.82	-0.20
Wash water - cooling water	T207	Kitchen 1 plastic windowsill	5.04	5.04	5.04	0.00
Wash water - cooling water	T208	Conveyor belt 1	4.43	4.20	4.32	-0.23
Wash water - cooling water	T209	Metal bench 1	3.59	3.54	3.57	-0.05

ANNEX D: Summary tables accuracy profile study

Summary Tables 72h incubation

(Food) Category 1	Heat processed
(Food) Type 1	Pasteurised dairy

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
1	Fermented yogurt drink	low	385	467	480	465	425	391	359	441	391	382
4	Cream cheese	low	500	1400	1300	344	227	275	920	1500	145	500
5	Cream cheese	interme diate	7500	18000	5300	13000	16000	17000	9400	9500	14000	5100
2	Fermented yogurt drink	interme diate	44000	32000	39000	20000	38000	43000	36000	39000	26000	26000
3	Fermented yogurt drink	high	310000	390000	210000	250000	490000	350000	290000	300000	350000	350000
6	Cream cheese	high	610000	430000	260000	750000	550000	530000	470000	320000	460000	650000

(Food) Category 2	Fruits and
(Food) Type 2	Fresh produce

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7	Vegetable juice	low	65	55	75	50	180	75	65	70	40	75
10	Beetroot salad	low	130	140	120	160	180	150	110	130	140	160
11	Beetroot salad	interme diate	7300	9400	5600	9800	13000	6600	7000	9500	6100	9800
8	Vegetable juice	interme diate	65000	75000	47000	69000	74000	57000	91000	50000	81000	59000
9	Vegetable juice	high	7500000	6800000	7700000	4000000	8500000	8000000	5500000	6500000	7300000	8100000
12	Beetroot salad	high	83000000	78000000	51000000	44000000	110000000	62000000	50000000	50000000	21000000	52000000

(Food) Category 3	Multi component
(Food) Type 3	Composite foods

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
16	Frozen ready to reheat pizza	low	415	665	503	400	440	536	705	532	364	377
13	Raw vegetable salad with dressing	low	540	480	660	780	810	610	550	690	732	782
17	Frozen ready to reheat pizza	interme diate	1000	7000	22000	10000	17000	2000	7000	23000	7500	23000
14	Raw vegetable salad with dressing	interme diate	14000	22000	22000	18000	20000	21000	42000	32000	17000	38000
18	Frozen ready to reheat pizza	high	420000	460000	570000	430000	480000	520000	630000	540000	420000	490000
15	Raw vegetable salad with dressing	high	1400000	900000	1000000	1400000	1100000	1000000	540000	590000	1100000	840000

(Food) Category 4	Confectionary
(Food) Type 4	Chilled RTE foods

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
19	Quiche	low	450	797	1300	630	520	323	550	650	427	500
22	Custard tart	low	1800	1900	1200	1100	1300	632	660	382	510	1200
21	Quiche	interme diate	12000	15000	31000	24000	17000	15000	15000	21000	29000	9000
23	Custard tart	interme diate	22000	27000	27000	24000	16000	19000	24000	19000	29000	19000
24	Custard tart	high	190000	220000	200000	220000	200000	170000	200000	150000	220000	290000
20	Quiche	high	320000	360000	470000	180000	380000	180000	160000	380000	240000	280000

(Food) Category 5	RTE and RTRH foods
(Food) Type 5	Ready to eat meat and poultry

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
25	Cooked breaded chicken patties	low	70	110	115	95	135	110	80	90	80	55
28	Deli turkey	low	70	80	100	80	75	30	55	50	65	105
26	Cooked breaded chicken patties	intermediate	16000	18000	14000	19000	12000	8800	6900	11000	8600	8300
29	Deli turkey	intermediate	66000	50000	75000	65000	64000	29000	46000	38000	31000	37000
27	Cooked breaded chicken patties	high	210000	260000	120000	190000	140000	360000	270000	110000	190000	100000
30	Deli turkey	high	120000	81000	120000	87000	110000	82000	53000	64000	51000	130000

(Food) Category 2	Chocolate, bakery and confectionary
(Food) Type 2	Dry and sugared low moisture aw <0.85

			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
Milk chocolate	37	low	1300	1000	520	830	1800	570	850	860	800	870
Cream puff	40	low	1100	1100	1200	1300	1100	1100	1100	1400	1000	1600
Milk chocolate	38	intermediate	5100	3100	5200	3800	5100	3600	3800	3500	3600	3900
Cream puff	41	intermediate	87000	46000	79000	86000	68000	65000	52000	67000	76000	66000
Milk chocolate	39	high	390000	350000	330000	190000	330000	250000	270000	210000	220000	200000
Cream puff	42	high	5000000	2200000	3300000	3500000	7700000	8500000	3800000	2400000	3300000	7400000

(Food) Category 1	Dried cereals fruits nuts seeds vegetables
(Food) Type 1	Nuts and seeds

			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
Dried apricots	33	low	150	130	125	150	100	260	230	250	260	200
Almond butter	34	low	2500	6500	2300	1900	1800	2300	1800	2000	2900	2400
Dried apricots	31	intermediate	7200	8900	6300	7500	14000	8700	6500	7500	6400	11000
Almond butter	35	intermediate	20000	27000	29000	25000	15000	19000	14000	19000	18000	35000
Almond butter	36	high	340000	290000	420000	290000	470000	270000	120000	170000	610000	160000
Dried apricots	32	high	330000	300000	420000	300000	480000	360000	410000	350000	360000	430000

(Food) Category 3	Pet food and animal feed
(Food) Type 3	Dried pet food and feed

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Meat and bone meal	46	low	70	60	60	70	90	30	130	50	70	70
Kibbles	43	low	90	70	90	70	85	160	50	70	60	90
Kibbles	44	intermediate	9200	7500	8500	8000	5500	9300	9500	11000	6400	9800
Meat and bone meal	47	intermediate	12000	26000	17000	23000	13000	19000	26000	19000	18000	19000
Kibbles	45	high	130000	130000	140000	200000	130000	150000	150000	130000	120000	130000
Meat and bone meal	48	high	260000	260000	290000	320000	200000	210000	210000	220000	250000	250000

(Food) Category 6	Environmental
(Food) Type 6	Surfaces

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Concrete	52	low	70	110	330	70	75	110	86	230	50	29
Stainless steel	49	low	180	160	135	250	170	120	160	164	245	160
Stainless steel	50	intermediate	10000	21000	34000	13000	14200	6100	11000	23000	6200	12000
Concrete	53	intermediate	6200	17000	27000	15000	62000	7500	12000	25000	8100	13000
Stainless steel	51	high	70000	120000	150000	100000	200000	71000	56000	79000	65000	150000
Concrete	54	high	330000	300000	140000	130000	130000	250000	240000	130000	120000	150000

Accuracy Profile - Summary Tables - 46h incubation

(Food) Category 2	Fruits and
(Food) Type 2	Fresh produce

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7	Vegetable juice	low	65	55	75	50	58	75	65	70	40	75
10	Beetroot salad	low	130	140	120	160	180	150	110	130	140	160
11	Beetroot salad	interme diate	7900	9400	5600	9800	13000	6600	7000	9500	6100	9800
8	Vegetable juice	interme diate	65000	75000	47000	69000	74000	57000	91000	50000	81000	59000
9	Vegetable juice	high	7500000	6800000	7700000	4000000	8500000	8000000	5500000	6500000	7300000	8100000
12	Beetroot salad	high	83000000	78000000	51000000	44000000	110000000	57000000	45000000	48000000	21000000	45000000

(Food) Category 5	Environmental
(Food) Type 5	Surfaces

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Concrete	52	low	70	110	330	70	75	56	72	210	45	27
Stainless steel	49	low	180	160	135	250	170	99	125	110	140	126
Stainless steel	50	interme diate	10000	21000	34000	13000	14200	6100	11000	23000	6200	12000
Concrete	53	interme diate	6200	17000	27000	15000	62000	3400	13000	16000	18000	56000
Stainless steel	51	high	70000	120000	150000	100000	200000	71000	56000	79000	65000	150000
Concrete	54	high	330000	300000	140000	130000	130000	230000	220000	120000	110000	140000

(Food) Category 3	Multi component
(Food) Type 3	Composite foods

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
16	Frozen ready to reheat pizza	low	415	665	503	400	440	505	650	505	359	350
13	Raw vegetable salad with dressing	low	540	480	660	780	810	610	532	677	727	768
17	Frozen ready to reheat pizza	interme diate	1000	7000	22000	10000	17000	2000	6600	20000	7300	22000
14	Raw vegetable salad with dressing	interme diate	14000	22000	22000	18000	20000	19000	36000	32000	17000	35000
18	Frozen ready to reheat pizza	high	420000	460000	570000	430000	480000	560000	620000	540000	420000	480000
15	Raw vegetable salad with dressing	high	1400000	900000	1000000	1400000	1100000	890000	450000	520000	860000	680000

ANNEX E: Raw data inclusivity and exclusivity study

Inclusivity

Number	Inclusivity Organism	CRA code	Source	Alternate method - YMR 46H								Alternate method - YMR 72H								DG18 - Reference method 5 days								DRBCA - Reference method 5 days								Non selective - MEA							
				-3	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml	-3	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml	-2	-3	-4	-5	-6	-7	calculated count cfu/ml	log cfu/ml	-2	-3	-4	-5	-6	-7	calculated count cfu/ml	log cfu/ml	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml
1.	*Aureobasidium pullulans*	16148	Soft drinks factory	nt	T	T	36	2	0	3.50E+07	7.5	nt	T	T	36	2	0	3.50E+07	7.5	nt	T	T	31	4	1	3.20E+07	7.5	nt	T	T	32	3	0	3.20E+07	7.5	T	182	22	1	0	1.90E+07	7.3
2.	*Aspergillus niger*	16667	Grapes	23	2	0	0	0	0	2.30E+04	4.4	23	2	0	0	0	0	2.30E+04	4.4	24	2	0	0	0	0	2.40E+04	4.4	43	5	0	0	0	0	4.40E+04	4.6	5	0	0	0	0	5.00E+04	4.7
3.	*Aspergillus brasiliensis*	17002	Plants	T	52	7	3	0	0	5.40E+05	5.7	T	52	7	3	0	0	5.40E+05	5.7	T	65	2	0	0	0	6.10E+05	5.8	T	73	6	0	0	0	7.20E+05	5.9	84	9	0	0	0	8.50E+05	5.9
4.	*Aspergillus restrictus*	ATCC42693	Dried chillies	39	11	0	0	0	0	3.90E+04	4.6	T	T	T	36	9	0	4.10E+06	6.6	nt	T	70	5	3	0	6.80E+06	5.3	nt	T	103	11	1	0	1.00E+07	7.0	T	64	4	0	0	6.20E+06	5.3
5.	*Botrytis cinerea*	6728	Dried chillies	nt	T	27	1	0		2.50E+06	6.4	nt	T	35	1	0		3.30E+06	6.5	nt	nt	nt	nt	nt	nt	nt	n/a	nt	T	46	2	0	0	4.40E+06	6.6	T	26	3	0		2.60E+06	6.4
6.	*Byssochlamys spectabilis*	17025	Factory isolate	nt	58	0	0	0	0	5.80E+05	5.8	nt	T	40	4	0	0	4.00E+06	6.6	nt	T	37	1	0	0	3.50E+06	6.5	nt	T	35	3	0	1	3.50E+06	6.5	T	36	5	0	0	3.70E+06	6.6
7.	*Byssochlamys fulva*	16668	Pasteurised fruit juice	nt	T	20	0	0	0	2.00E+06	6.3	nt	T	96	10	0	0	9.60E+06	7.0	nt	T	SP	5	1	0	5.00E+06	6.7	nt	T	51	9	3	0	5.50E+06	6.7	T	T	16	2	0	1.60E+07	7.2
8.	*Candida magnoliae*	861	Strawberry ingredient	nt	T	T	T	17	2	1.70E+08	8.2	nt	T	T	T	18	3	1.90E+08	8.3	nt	T	T	149	18	0	1.50E+08	8.2	nt	T	T	T	14	3	1.50E+08	8.2	T	T	135	12	1	1.30E+08	8.1
9.	*Candida krusei*	629	Yogurt base	nt	T	T	64	7	1	6.50E+07	7.8	nt	T	T	64	7	1	6.50E+07	7.8	nt	T	T	44	5	1	4.50E+07	7.7	nt	T	T	46	3	0	4.50E+07	7.7	T	T	39	5	0	4.00E+07	7.6
10.	*Candida sojae*	16138	Soft drinks factory	nt	T	T	T	33	3	3.30E+08	8.5	nt	T	T	T	34	3	3.40E+08	8.5	nt	T	T	T	33	2	3.20E+08	8.5	nt	T	T	T	44	2	4.20E+08	8.6	T	T	T	31	2	3.00E+08	8.5
11.	*Candida wyomingensis*	16144	Soft drinks factory	nt	T	T	39	0	0	3.90E+07	7.6	nt	T	T	47	4	0	4.60E+07	7.7	nt	T	T	61	2	0	5.70E+07	7.8	nt	T	T	63	4	0	6.10E+07	7.8	T	T	61	7	0	6.20E+07	7.8
12.	*Clavispora lusitaniae*	15967	Strawberry yogurt	nt	T	T	60	7	1	6.10E+07	7.8	nt	T	T	61	7	1	6.20E+07	7.8	nt	T	T	56	5	0	5.50E+07	7.7	nt	T	T	55	8	0	5.70E+07	7.8	T	T	62	6	1	6.20E+07	7.8
13.	*Cryptococcus laurenti*	16139	Soft drinks factory	nt	T	67	4	0	0	6.50E+06	6.8	nt	T	137	9	0	0	1.30E+07	7.1	nt	T	123	13	0	0	1.20E+07	7.1	nt	T	142	10	0	0	1.40E+07	7.1	T	60	2	1	0	5.60E+06	6.7
14.	*Cryptococcus uzbekistanensis*	15967	South African factory	nt	T	31	4	0	0	3.00E+06	6.5	nt	T	31	3	0	0	3.10E+06	6.5	nt	T	27	3	0	0	2.70E+06	6.4	nt	T	28	3	0	0	2.80E+06	6.4	T	23	4	0	0	2.50E+06	6.4
15.	*Debaryomyces hansenii*	16833	Factory isolate	nt	T	T	18	0	0	1.80E+07	7.3	nt	T	T	19	0	0	1.90E+07	7.3	nt	T	T	16	1	0	1.50E+07	7.2	nt	T	T	12	2	0	1.30E+07	7.1	T	158	20	0	0	1.60E+07	7.2
16.	*Dekkera naardensis*	3237	cola	T	T	T	T	23	3	2.40E+08	8.4	T	T	T	T	23	3	2.40E+08	8.4	T	T	T	T	45	5	4.50E+08	8.7	T	T	T	T	53	5	5.30E+08	8.7		T	T	65	6	6.50E+08	8.8
17.	*Eurotium amstelodami*	DSM62629	Mouldy kernel	nt	0	0	0	0	0	<1E+04	n/a	16	5	0	0	0	0	1.90E+04	4.3	22	2	0	0	0	0	2.20E+04	4.3	17	3	0	0	0	0	1.80E+04	4.3	30	3	0	0	0	3.00E+04	4.5
18.	*Eurotium chevaleri*	16902	Not known	12	1	0	0	0	0	1.20E+04	4.1	T	33	4	0	0	0	3.40E+05	5.5	20	3	0	0	0	0	2.20E+04	5.0	nt	T	20	1	0	0	1.90E+06	6.3	T	17	5	0	0	2.00E+06	5.3
19.	*Fusarium proliferatum*	CBS134066	grass	T	T	88	13	0	0	9.20E+06	7.0	T	T	121	13	1	0	1.20E+07	7.1	T	T	23	2	0	0	2.30E+07		T	T	22	1	0	0	2.20E+07	7.3	T	T	33	4	0	3.00E+07	5.3
20.	*Fusarium solani*	16976	Factory isolate	nt	T	39	2	0	0	3.70E+06	6.6	nt	T	94	5	0	0	9.00E+06	7.0	nt	T	80	9	1	0	8.10E+06	6.9	nt	T	93	8	1	0	9.20E+06	7.0	T	60	7	2	0	6.10E+06	6.8
21.	*Geotrichum candidum*	14398	Factory isolate	nt	T	T	T	19	2	1.90E+08	8.3	nt	T	T	T	19	2	1.90E+08	8.3	nt	T	T	T	21	1	2.00E+08	8.3	nt	nt	nt	nt	nt	nt	nt	n/a	T	T	183	13	0	1.80E+08	8.3
22.	*Hanseniaspora uvarum*	15958	Factory isolate	nt	T	57	3	1	0	5.40E+06	6.7	nt	T	69	4	1	0	6.60E+06	6.8	nt	T	89	8	1	0	8.80E+06	6.9	nt	T	50	5	0	0	5.50E+06	6.7	T	10	0	0	0	1.00E+06	6.0
23.	*Hansenula anomala*	626	Sugar solution	nt	T	T	158	13	4	1.50E+08	8.2	nt	T	T	T	14	4	1.60E+08	8.2	nt	T	T	T	12	2	1.30E+08	8.1	nt	T	T	T	18	2	1.80E+08	8.3	T	T	T	17	3	1.80E+08	8.3
24.	*Kloeckera apiculata*	6412	Bakery isolate	nt	T	T	44	3	0	4.30E+07	7.6	nt	T	T	44	3	0	4.30E+07	7.6	nt	T	T	43	0	2	4.30E+07	7.6	nt	T	T	47	2	0	4.50E+07	7.7	T	T	39	5	1	4.00E+07	7.6
25.	*Kluyveromyces lactis var. lactis*	16455	Gassy Cheese	nt	T	T	55	10	0	5.90E+07	7.8	nt	T	T	55	10	0	5.90E+07	7.8	nt	T	T	63	5	0	6.20E+07	7.8	nt	nt	nt	nt	nt	nt	nt	n/a	T	T	44	5	1	4.50E+07	7.7
26.	*Kluyveromyces marxianus*	6749	Dairy isolate	nt	T	T	19	0	0	1.90E+07	7.3	nt	T	T	21	6	0	2.50E+07	7.4	nt	nt	nt	nt	nt	nt	nt	n/a	nt	T	T	66	10	0	6.90E+07	7.8	T	T	52	3	0	5.00E+07	7.7
27.	*Metschenikowia pulcherrima*	16167	Food spoilage	nt	T	39	5	0	1	4.00E+06	6.6	nt	T	43	5	0	1	4.40E+06	6.6	nt	T	56	11	0	0	6.10E+06	6.8	nt	T	66	11	0	1	7.00E+06	6.8	T	63	10	0	1	6.60E+06	6.8
28.	*Monascus ruber*	16725	Acid product	32	3	0	0	0	0	3.20E+04	4.5	43	3	0	0	0	0	4.20E+04	4.6	45	4	0	0	0	0	4.50E+04	4.7	52	6	0	0	0	0	5.30E+04	4.7	6	0	0	0	0	6.00E+04	4.8
29.	*Neosartorya fischeri*	16669	Grape juice	nt	2	1	0	0	0	2.00E+04	4.3	nt	122	12	1	0	0	1.20E+06	6.1	nt	T	20	1	0	0	1.90E+06	6.3	nt	77	13	2	0	0	8.20E+05	5.9	T	13	3	0	0	1.50E+06	6.2

2022LR109 Easy Plate YM-R MCS ILS Summary report

MICROVAL® NEN

Number	Inclusivity Organism	CRA code	Source	Alternate method - YMR 46H								Alternate method - YMR 72H								DG18 - Reference method 5 days								DRBCA - Reference method 5 days								Non selective - MEA							
				-3	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml	-3	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml	-2	-3	-4	-5	-6	-7	calculated count cfu/ml	log cfu/ml	-2	-3	-4	-5	-6	-7	calculated count cfu/ml	log cfu/ml	-4	-5	-6	-7	-8	calculated count cfu/ml	log cfu/ml
30.	*Paecilomyces variotii*	16670	Fruit concentrate	4	1	0	0	0	0	4.00E+03	3.6	82	17	0	0	0	0	9.00E+04	5.0	nt	T	40	0	0	0	4.00E+05	4.7	nt	41	4	0	0	0	4.10E+05	5.6	20	6	0	0	0	2.40E+05	5.4
31.	*Penicillium aurantiogriseum*	16192	Acid product	nt	12	0	0	0	0	1.20E+05	5.1	nt	20	1	0	0	0	1.90E+05	5.3	nt	34	5	0	0	0	3.50E+05	5.5	nt	28	3	0	9	0	2.80E+05	5.4	34	1	0	0	0	3.20E+05	5.5
32.	*Penicillium crustosum*	16671	Lemon	nt	25	3	1	0	0	2.50E+05	5.4	nt	28	3	1	0	0	2.80E+05	5.4	nt	25	4	1	1	0	2.60E+05	5.4	nt	32	4	1	0	0	3.30E+05	5.5	20	2	0	0	1	2.00E+05	5.3
33.	*Penicillium expansum*	16672	Mouldy cherry juice	nt	28	3	0	0	0	2.80E+05	5.4	nt	32	4	1	0	0	3.30E+05	5.5	nt	43	6	0	2	2	4.50E+05	5.7	nt	37	10	0	0	0	4.30E+05	5.6	24	5	0	0	0	2.60E+05	5.4
34.	*Penicillium roqueforti*	16726	Acidic product	nt	15	2	0	0	0	1.50E+05	5.2	nt	125	25	2	0	0	2.50E+06	6.4	nt	T	18	2	1	0	1.80E+06	6.3	nt	T	20	1	0	0	1.90E+06	6.3	T	15	1	1	0	1.50E+06	6.2
35.	*Pichia anomala*	4901	Carbonated drink	nt	T	T	49	4	1	4.80E+07	7.7	nt	T	T	50	4	1	4.90E+07	7.7	nt	T	T	46	9	1	5.00E+07	7.7	nt	T	T	41	7	0	4.40E+07	7.6	T	T	45	7	1	4.70E+07	7.7
36.	*Pichia galeiformis*	16015	Spoiled tomato juice	nt	T	141	11	1	0	1.40E+07	7.1	nt	T	134	14	1	0	1.30E+07	7.1	nt	T	T	27	3	0	2.70E+07	7.4	nt	T	T	18	0	0	1.80E+07	7.3	T	97	13	0	2	1.00E+07	7.0
37.	*Issatchenkia orientalis*	16164	Spoiled yogurt	nt	T	T	19	1	0	1.80E+07	7.3	nt	T	T	19	1	0	1.80E+07	7.3	nt	T	T	24	0	2	2.40E+07	7.4	nt	T	T	27	1	1	2.50E+07	7.4	T	T	23	1	0	2.20E+07	7.3
38	*Rhodotorula graminis*	16003	unknown	T	T	34	0	0	0	3.40E+06	6.5	T	T	34	3	0	0	3.40E+06	6.5	T	T	33	3	0	0	3.30E+06	6.5	T	T	45	5	0	0	4.50E+06	6.7	T	55	6	0	0	5.50E+06	6.7
39.	*Saccharomyces bayanus*	16434	Lager production	nt	T	T	24	1	0	2.30E+07	7.4	nt	T	T	24	1	0	2.30E+07	7.4	nt	T	83	7	0	0	8.20E+06	6.9	nt	T	124	14	5	0	1.30E+07	7.1	T	74	8	0	0.0	7.50E+06	6.9
40.	*Saccharomyces exiguus*	16017	Spoiled mayonnaise	nt	T	T	16	2	0	1.60E+07	7.2	nt	T	T	16	2	0	1.60E+07	7.2	nt	T	T	21	2	0	2.10E+07	7.3	nt	T	T	21	2	0	2.10E+07	7.3	T	T	101	6	1	9.70E+07	8.0
41.	*Saccharomyces cerevisiae*	720	Frozen fruit juice	nt	T	T	17	0	1	1.70E+07	7.2	nt	T	T	17	0	1	1.70E+07	7.2	nt	T	T	14	3	2	1.50E+07	7.2	nt	T	T	25	2	1	2.50E+07	7.4	T	T	20	2	0	2.00E+07	7.3
42.	*Torulaspora delbrukii*	16154	Spoiled yogurt	nt	T	163	12	0	0	1.60E+07	7.2	nt	T	163	12	0	0	1.60E+07	7.2	nt	T	T	17	2	0	1.70E+07	7.2	nt	T	151	10	2	1	1.50E+07	7.2	T	130	15	1	0	1.30E+07	7.1
43.	*Trichosporon jirovecii*	15964	Powder	nt	T	144	9	2	0	1.40E+07	7.1	nt	T	150	9	2	0	1.40E+07	7.1	nt	T	T	12	0	0	1.20E+07	7.1	nt	T	T	13	2	0	1.40E+07	7.1	T	116	12	0	0	1.20E+07	7.1
44.	*Trichosporon coremiiforme*	15962	Powder	nt	T	106	9	1	0	1.00E+07	7.0	nt	T	108	9	1	0	1.10E+07	7.0	nt	T	84	3	0	0	7.90E+06	6.9	nt	T	100	6	0	0	9.60E+06	7.0	T	91	6	0	0	8.80E+06	6.9
45.	*Yarrowia lipolytica*	16146	Soft drinks factory	nt	T	T	23	5	0	2.50E+07	7.4	nt	T	T	41	9	0	4.50E+07	7.7	nt	T	T	23	2	1	2.30E+07	7.4	nt	T	T	23	2	0	2.30E+07	7.4	T	T	25	1	0	2.40E+07	7.4
46.	*Wallemia sebi*	17533	Blackcurrant jelly	nt	61	6	1	0	0	6.10E+05	5.8	nt	61	6	1	0	0	6.10E+05	5.8	nt	102	8	2	0	0	1.00E+06	6.0	nt	nt	nt	nt	nt	nt	nt	n/a	70	10	0	0	0	7.30E+05	5.9
47.	*Willopsis saturnus*	6423	Bakery isolate	nt	T	T	33	3	0	3.30E+07	7.5	nt	T	T	33	3	0	3.30E+07	7.5	nt	T	T	21	3	0	2.20E+07	7.3	nt	T	T	31	1	0	2.90E+07	7.5	T	T	19	3	1	2.00E+07	7.3
48.	*Xeromyces bisporus*	17179	Fruit cake	8	0	0	0	0	0	8.00E+03	3.9	10	1	0	0	0	0	1.00E+04	4.0	33	3	0	0	0	0	3.30E+04	7.3	nt	nt	nt	nt	nt	nt	nt	7.5	6	0	0	0		6.00E+04	7.3
49.	*Zygosaccharomyces bailii*	16125	Nougat	nt	T	19	1	0	0	1.80E+06	6.3	nt	T	29	5	0	0	3.10E+06	6.5	nt	T	48	2	0	0	4.50E+06	6.7	nt	T	42	5	3	0	4.30E+06	6.6	T	45	13	2	0	1.40E+07	7.1
50.	Zygosaccharomyces rouxii	16127	Spoiled fruit	T	80	17	0	0	0	8.00E+05	5.9	nt	T	50	5	0	0	5.00E+06	6.7	T	T	80	6	0	0	7.80E+06	6.9	nt	T	81	13	1	0	8.50E+06	6.9	T	81	6	0	0	7.90E+06	6.9

Exclusivity

Code	Exclusivity Organism	CRA Code	Source	Alternate method - YMR				Reference method - DG18				Reference method - DRBCA				Non-selective (PCA or MRSA)				
				N	-1	-2	-3	N(0.3)	N(0.3)	N(0.3)	N (0.1)	N(0.3)	N(0.3)	N(0.3)	N (0.1)	-6	-7	-8	count cfu/ml	log cfu/ml
1	*Asaia siamensis*	16653	Soft drinks factory	nt	3	1	0	spread	spread	spread	spread	spread	spread	spread	spread	41	10	0	4.10E+07	7.5
2	Acinetobacter calcoaceticus	7421	Sesame seeds	nt	nt	0	0	0	0	0	0	spread	spread	spread	spread	170	27	0	1.80E+08	8.3
3	Allicyclobacillus acidoterrestris	5331	Apple juice	0	0	0	0	0	0	0	0	0	0	0	0	22	2	0	2.20E+07	8.3
4	*Bacillus coagulans*	16020	Evaporated milk	nt	nt	0	0	0	0	0	0	0	0	0	0	60	6	3	6.00E+07	7.8
5	*Bacillus amyloliquefaciens*	6317	Crumpet	nt	nt	0	0	0	0	0	0	0	0	0	0	31	5	0	3.30E+07	7.5
6	*Bacillus subtilis*	16597	UHT custard	nt	nt	0	0	0	0	0	0	0	0	0	0	18	3	0	1.90E+07	7.3
7	*Brochothrix thermosphacta*	16019	Fresh pork sausage	nt	nt	0	0	0	0	0	0	0	0	0	0	7	0	0	7.00E+06	6.8
8	*Burkholderia cepacia*	16982	Soil	nt	nt	0	0	0	0	0	0	spread	spread	spread	spread	196	33	6	3.50E+08	8.5
9	*Carnobacterium divergens*	3190	Brie	nt	nt	0	0	0	0	0	0	0	0	0	0	84	6	1	8.20E+07	7.9
10	*Citrobacter freundii*	3659	Salami	nt	nt	0	0	0	0	0	0	0	0	0	0	T	54	6	5.50E+08	8.7
11	*Enterococcus faecium*	16465	Cheese	nt	nt	0	0	0	0	0	0	0	0	0	0	119	16	1	1.20E+08	8.1
12	*Enterobacter agglomerans*	1488	Raw mince	0	0			0	0	0	0	0	0	0	0	18	2	2	1.80E+07	7.3
13	*Flavobacterium resinovorum*	9000	Soil	nt	nt	0	0	0	0	0	0	0	0	0	0	65	6	1	6.50E+07	7.8
14	*Lactobacillus buchneri*	16628	Brie	nt	nt	0	0	0	0	0	0	0	0	0	0	T	49	2	4.60E+08	8.7
15	*Lactobacillus plantarum*	561	Bamboo shoots	nt	nt	0	0	0	0	0	0	0	0	0	0	270	20	5	2.60E+08	8.4
16	*Leclercia adecarboxylata*	5121	Oregano	0	0			0	0	0	0	0	0	0	0	174	15	5	1.70E+08	8.2
17	*Leuconostoc mesenteroides*	16023	Green ham	nt	nt	0	0	0	0	0	0	0	0	0	0	44	3	0	4.30E+07	8.2
18	*Listeria ivanovii*	1123	Soft cheese	nt	nt	0	0	0	0	0	0	0	0	0	0	85	10	0	8.60E+07	7.9
19	*Morganella morganii*	7533	Beansprouts	nt	0	0	0	0	0	0	0	0	0	0	0	T	61	8	6.30E+08	8.8
20	*Micrococcus luteus*	3503	Tea factory	nt	nt	0	0	0	0	0	0	0	0	0	0	14	4	0	1.60E+07	7.2
21	*Pediococcus pentosaceus*	16030	Brine	nt	nt	0	0	0	0	0	0	0	0	0	0	T	29	3	2.90E+08	7.2
22	*Proteus mirabilis*	1588	Poultry	nt	nt	0	0	0	0	0	0	0	0	0	0	T	36	8	4.00E+08	8.6
23	*Proteus vulgaris*	1581	Poultry	nt	0			0	0	0	0	0	0	0	0	T	31	4	3.20E+08	8.5
24	*Providencia rettgeri*	8386	Faeces	nt	0			0	0	0	0	0	0	0	0	T	51	6	5.20E+08	8.7
25	*Pseudomonas fluorescens*	7504	Mixed salad	nt	nt	0	0	spread	spread	spread	spread	spread	spread	spread	spread	112	13	3	1.10E+08	8.0
26	*Pseudomonas syringae*	8252	Sugar beet	nt	nt	0	0	0	0	0	0	0	0	0	0	118	13	2	1.20E+08	8.1
27	*Serratia marcescens*	1521	Raw mince	nt	nt	0	0	0	0	0	0	0	0	0	0	T	92	12	9.50E+08	9.0
28	*Shewanella putrefaciens*	17042	Chilled chicken	nt	nt	0	0	0	0	0	0	0	0	0	0	172	17	2	1.70E+08	8.2
29	*Sphingomonas aquatilis*	16632	Natural mineral water	nt	nt	0	0	0	0	0	0	0	0	0	0	44	6	0	4.50E+07	7.7
30	*Streptococcus lactis*	179	Fresh beef mince	nt	nt	0	0	0	0	0	0	0	0	0	0	T	62	8	6.40E+08	7.7