ISO 16140-2:2016 validation of Neogen One Plate Yeast and Moulds, for the enumeration of yeast and moulds in broad range of foods

MicroVal study number: 2021LR99

Method/Kit name: Neogen One plate Yeast and Moulds

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MicroVal Expert Laboratory Campden BRI

Suzanne Jordan and Alice Foxall
Station Road,
Chipping Campden,
Gloucs,
GL55 6LD, UK
Tel: 0044 1386 842000
Email: suzanne.jordan@campdenbri.co.uk
www.campdenbri.co.uk

Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v.1.0

Company: Neogen Corporation
The Dairy School,
Auchincruive,
Ayr,
KA6 5HU, Scotland, UK

Expert Laboratory: Campden BRI
Station Road
Chipping Campden
Gloucs,
GL55 6LD, UK

Method/Kit name: Neogen One plate Yeast and Moulds

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 parts 1 and 2

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: borad range of foods Aw >0.95:

- Heat processed dairy products
- Confectionary, bakery and eggs (combined category)
- Fresh produce and fruit
- Ready to eat foods (combined category: meat, poultry and fishery products)
- Multi component foods or meal components

2 categories Aw <0.95

- Dried cereals, fruits, nuts, seeds and vegetables
- Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

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
- PSD Peptone Salt Diluent

Contents

1 Introduction

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

The alternative method used was:

The alternative method principle is based on a chromogenic medium for the enumeration of yeasts and moulds. Yeasts grow on the media as lilac to purple colonies. Moulds appear with typical morphologies on the spread and pour plates. The agar can be used as a surface plating technique with 0.1ml volumes or a pour plating technique with 1ml volumes plated. The alternative method can be used to count yeasts and moulds separately or as a combined count if needed. For the purpose of the study the yeasts and moulds were counted and reported as perfomed in the reference method.

Reference method is: ISO 21527:2008 parts 1 and 2

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 the validation study is a broad range of foods for Aw >0.95 and 2 further categories with Aw <0.95

Scope of validation: borad range of foods Aw >0.95:

- Heat processed dairy products
- Confectionary, bakery and eggs (combined category)
- Fresh produce and fruit
- Ready to eat foods (combined category: meat, poultry and fishery products)
- Multi component foods or meal components

2 categories Aw <0.95

- Dried cereals, fruits, nuts, seeds and vegetables
- Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Inclusivity and exclusivity.

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

- The alternative method One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for relative trueness;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for accuracy profile;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould is selective and specific.
- The combined data meets the requirements of ISO 16140-2 and was approved for analysis by the MVTC. All interlaboratory study results show good agreement between the reference and alternative methods for both incubation times analysed in the study.
- The alternative method, OPYM, shows comparable performance to the reference method, ISO 21527:2008 parts 1 and 2 for the enumeration of yeasts and moulds in a broad range of foods for both plating formats (1ml pour plate and 0.1ml spread plate) at the 2 time points tested (54 h and 72 h).

See conclusions outlined in *Section 3.4.1* for more details.

2 Method protocols

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

2.1 Reference method

A flow diagram of the reference method is shown in Annex A for reference.

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

2.2 Alternative method

See the flow diagram in Annex A.

The alternative method principle is based on a chromogenic medium for the enumeration of yeasts and moulds. Yeasts grow on the media as lilac to purple colonies. Moulds appear with typical morphologies on the spread and pour plates. The agar can be used as a surface plating technique with 0.1ml volumes or a pour plating technique with 1ml volumes plated. One plate yeast and moulds is incubated at 25 ±1°C for 54-72h.

2.3 Study design

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

3 Method comparison study

3.1 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 and artificially contaminated samples. Different categories, types and items were tested for this.

A total of 7 categories were included in this validation study. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category.

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

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

Table 1 – Categories, types and number of samples analyzed

Category	Types/Items	Preparation	Number of samples analyzed	Number of samples with interpretable results
Heat processed milk and dairy products	Pasteurised dairy products e.g. milk-based dessert, drinks, creams	PSD @ room temperature	5	5
	Cheese e.g. grated cheese, soft cheese, blue cheese	ISO 6887-5	5	5
	Fermented and acidified pasteurised milk and yogurt e.g. Yogurts with fruit, fermented milk drinks		5	5
Fresh produce and fruits	Fresh fruit salad and fruit purees	PSD @ room temperature.	5	5
	Chilled fruit juices		5	5
	Fermented vegetables e.g. sauerkraut, olives	ISO 6887-4	5	5
Multi component foods or meal components	Composite foods with raw ingredients e.g. sandwiches, pasta salads.	PSD @ room temperature.	5	5
	Mayonnaise based chilled salads	ISO 6887-1	5	5
	Ambient stable acidified foods e.g. ketchup	ISO 6887-4	5	5

Category	Types/Items	Preparation	Number of samples analyzed	Number of samples with interpretable results
Confectionery, bakery and eggs Combined category: Chocolate, bakery products and confectionary (high moisture aw > 0.95) Eggs and egg products (derivates)	Bakery products with custard	PSD @ room temperature. ISO 6887-4	5	5
	Egg products without additives e.g. chilled quiches		5	5
	Par baked bread products Aw >0.95		5	5
RTE/RTRH foods (combined category: RTE/RTRH meat, RTE/RTRH poultry, RTE/RTRH fishery products)	Ready to eat meat and poultry e.g. turkey fillet, pate	PSD @ room temperature. ISO 6887-2 ISO 6887-3	5	5
	Cooked and cured fish products e.g. roll herring, seafood terrine		5	5
	Raw cured meat and poultry e.g. salami, ham		5	5
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 ISO 6887-4	5	5
	Nuts and seeds		5	5
	Low and IF fruits aw <0.85		5	5
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	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	5	5
	Dry and sugared low moisture aw<0.65 e.g. syrups		5	5
	Dry powders e.g. cake mixes		5	5

Category	Types/Items	Preparation	Number of samples analyzed	Number of samples with interpretable results
		for up to 1 hr before testing.		

105 samples were analyzed, leading to 105 exploitable results.

3.1.2 Test sample preparation

Naturally contaminated samples were preferentially analyzed. 63 samples were screened for the presence of the target organism. From these samples 60.3% 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 2. The remaining 39.7% were negative for the target organism. It was therefore necessary to use artificial contamination procedures.

46 % of the samples were naturally contaminated.

Table 2 – Screening of samples for target organism

Category	Samples screened	Natural contamination – positive for target	% of category naturally contaminated
Heat processed milk and dairy products	3/15	3/15	20
Confectionery, bakery and eggs (combined category)	15/15	13/15	87
Fresh produce and fruits	15/15	10/15	67
Ready to eat/ready to reheat foods (combined category: meat, poultry fish)	15/15	12/15	80
Multi component foods meal components	15/15	10/15	67
Dried cereals, fruits, nuts, seeds and vegetables	0/15	0/15	0

Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	0/15	0/15	0

Artificial contaminations were obtained using a seeding protocol. Samples with Aw >0.95 were inoculated with yeast isolates and mould spores before storage of the inoculated samples, e.g. frozen foods were stored for at least 2 weeks at -20 °C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature. 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 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. 12 strains were used to inoculate samples.

3.1.3 Protocols applied during the validation study

Incubation time

An incubation time of 54 hours and 72 hours was used for the study and both are detailed in this report.

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).

Figures 1a-i show the plots for 0.1ml spread plates with 54 hour incubation. Figures 1j-r show the plots for 0.1ml spread plates with 72 hour incubation. Figures 2a-i show the plots for 1ml pour plates with 54 hour incubation. Figures 2j-r show the scatter plots with 1ml pour plates with 72 hour incubation.

0.1ml spread plate – 54h incubation

Figure 1a – scatter plot for Heat processed milk and dairy products

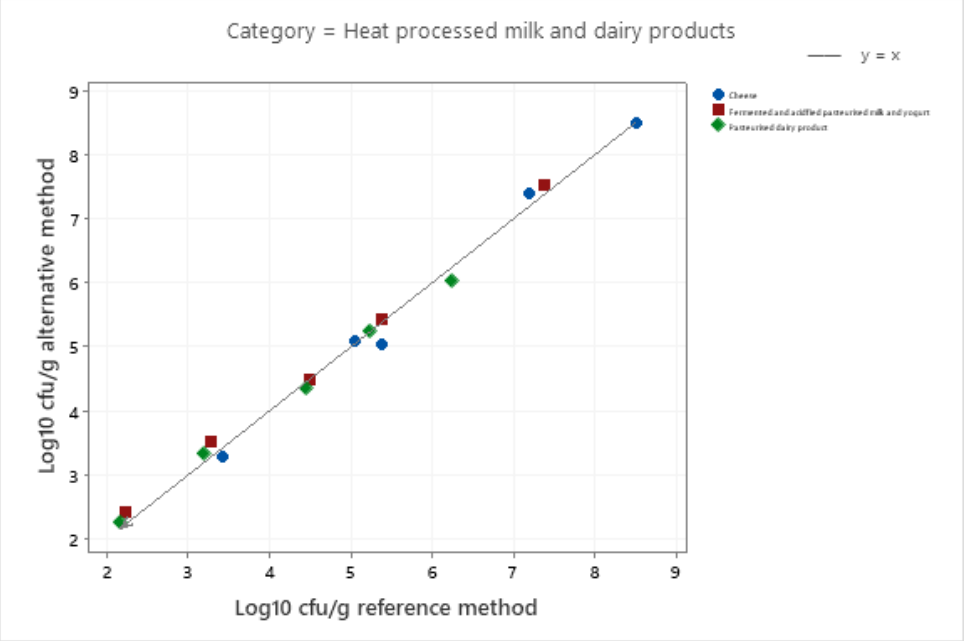


Figure 1b – scatter plot for Fresh produce and fruits

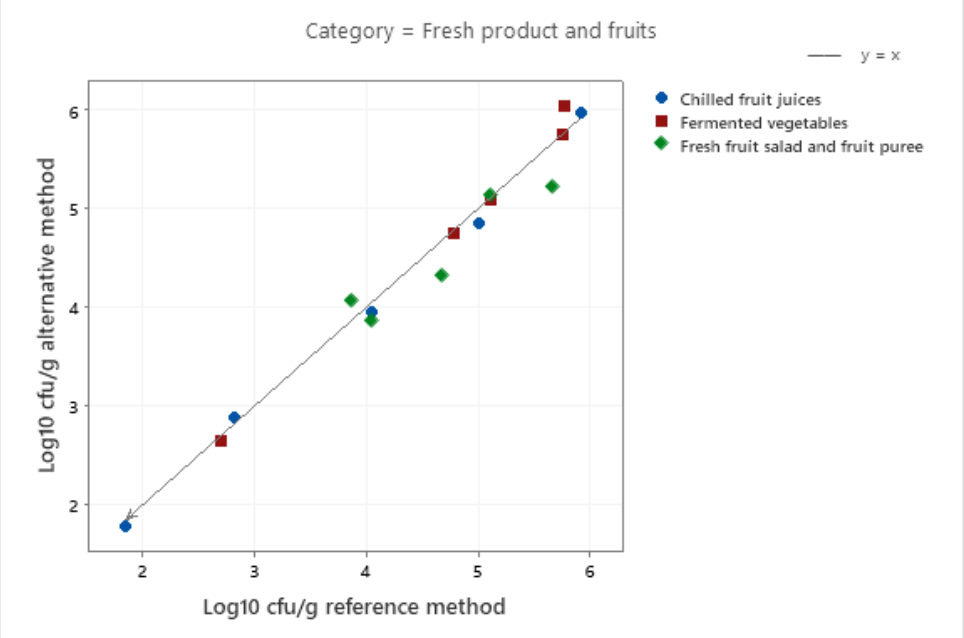


Figure 1c – scatter plot for Multi component foods or meal components

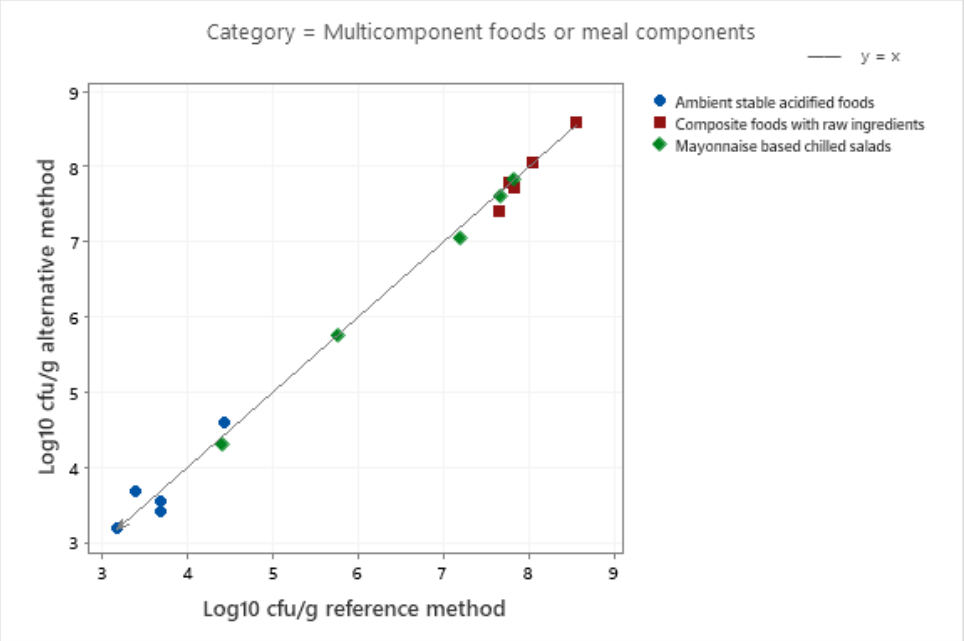


Figure 1d – scatter plot for Confectionery bakery and eggs (combined category)

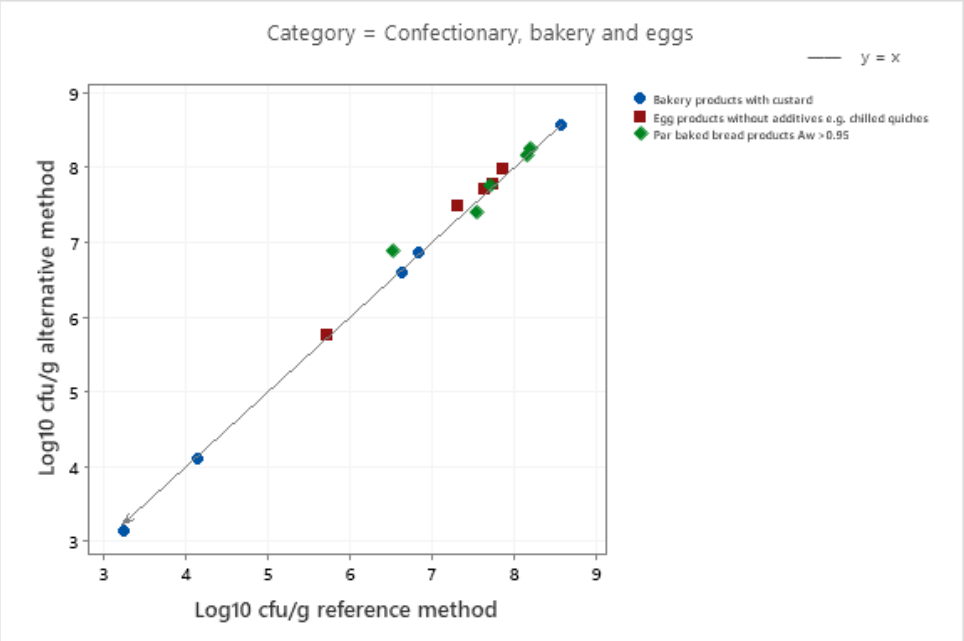


Figure 1e – scatter plot for RTE/RTRH foods (combined category: meat, poultry and fish)

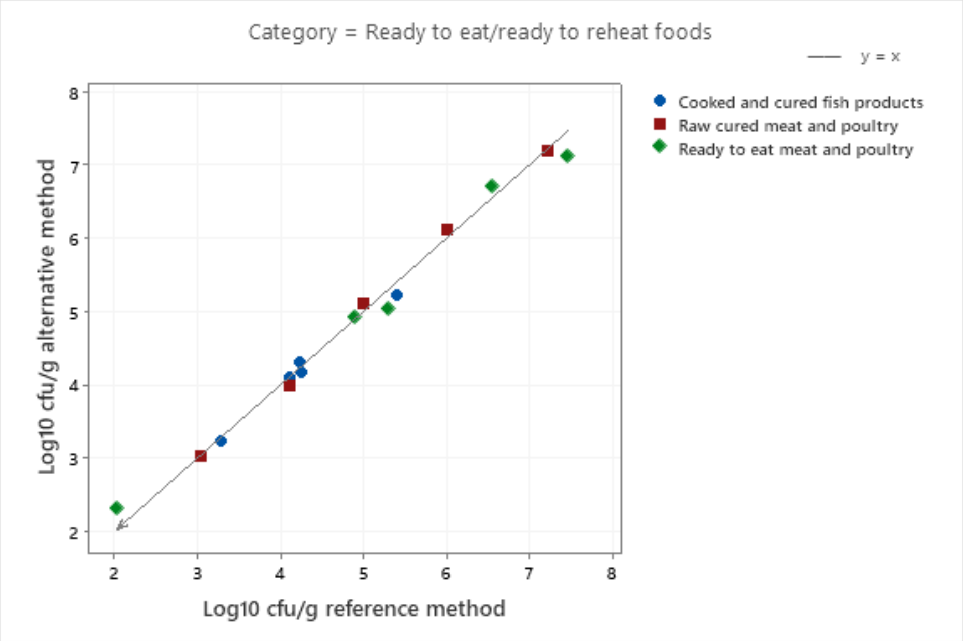


Figure 1f – scatter plot for Dried cereals fruits nut seeds and vegetables

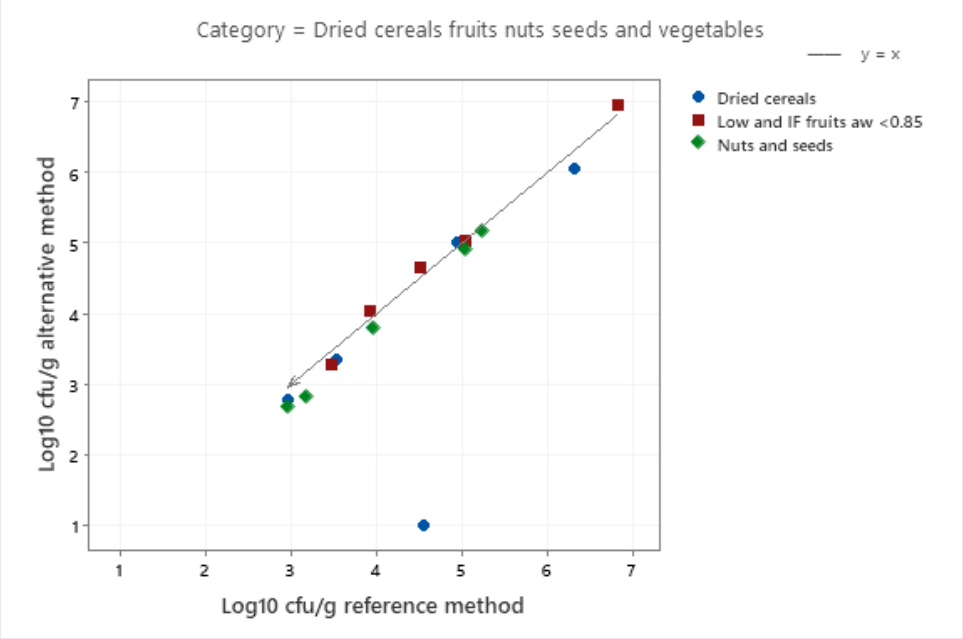


Figure 1g – scatter plot for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

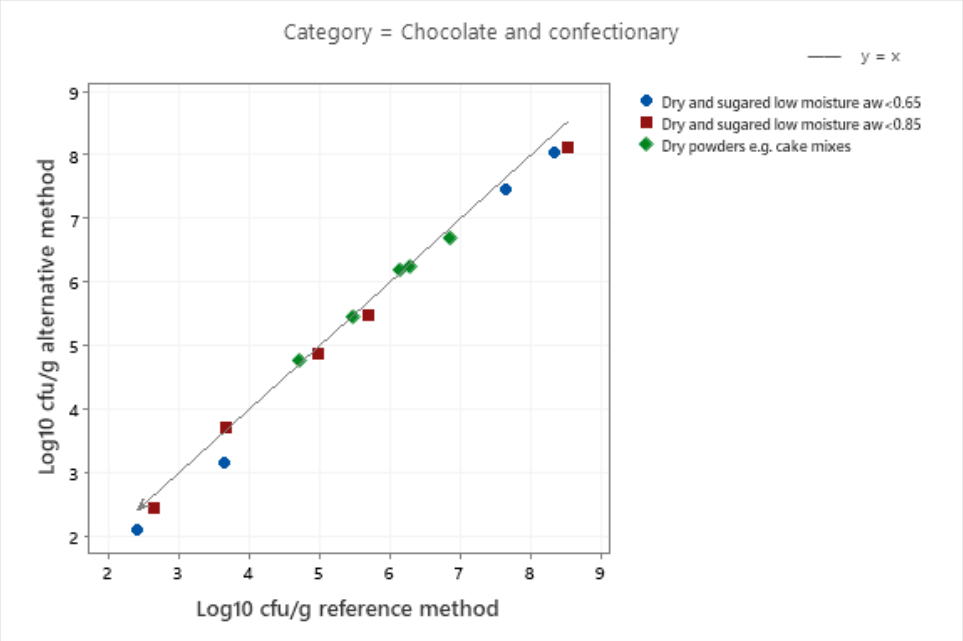
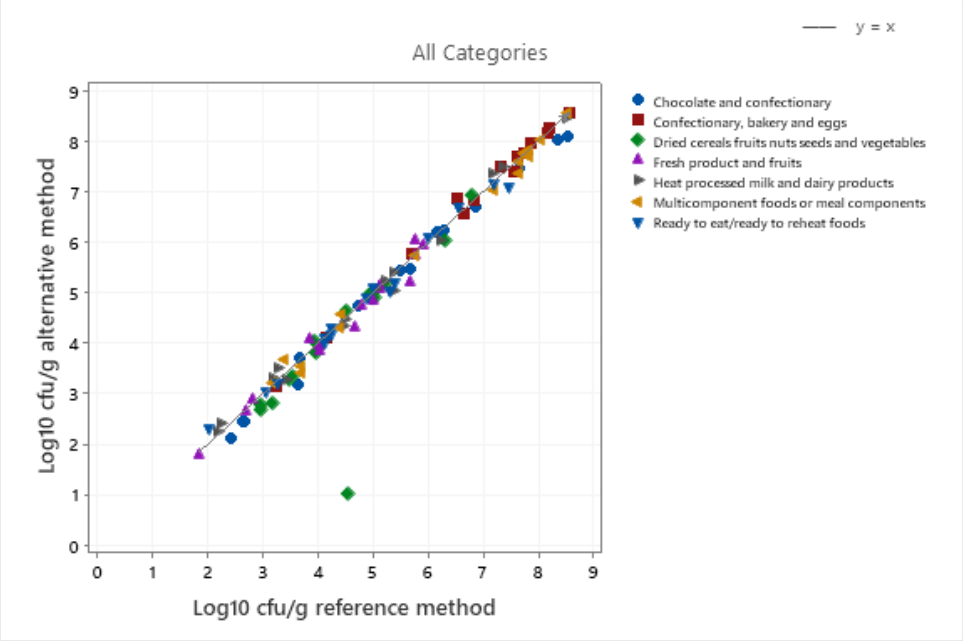


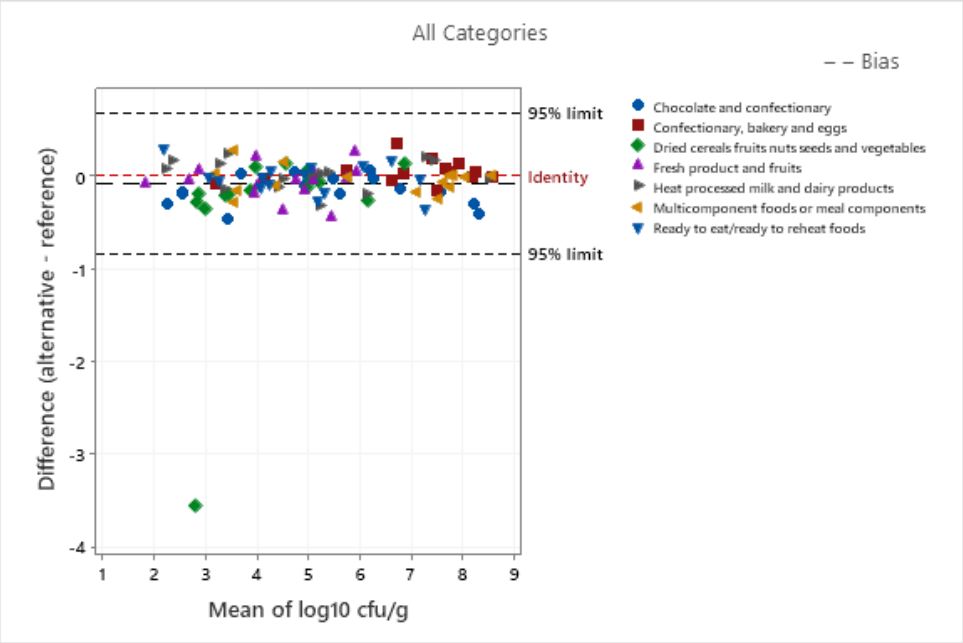
Figure 1h – scatter plot for all categories



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 obvious disagreement.

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

Figure 1i – Bland-Altman difference plot for all the samples



There is one outlier, the details of which are shown in Table 3. The alternative method outlying result is below the quantification limit, the datapoint is included in the Bland-Altman analysis and was excluded in the calculated values in Table 3. The result is reported in Annex E. To meet the ISO 16140-2 statistical analysis, the sample was repeated following the same artifical contamination protocol using a different strain for inoculation (*Paecilomyces variotti* CRA 16670). On the repeat, the sample produced counts and the log difference was -0.78. The count improved with a total incubation of 72 hours.

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

Table 3 - Data which are outside of the accepted limits

Category	**Item /Type**	**N° Sample**	**Reference method** **Log cfu/g**	**Alternative method** **Log cfu/g**	**Mean** **Log cfu/g**	**Difference** **Alternative – reference)**	**Inoculation**
Dried cereals fruits nuts seeds and vegetables	Wheat biscuits / Dried cereals	T80	4.56	1.00	2.78	-3.56	*Eurotium amstelodami* DSM62629 Inoculated using lyophilised culture and stored for 2 weeks ambient

Table 4 - Summary of the calculated values per category

Category.	**n**	$\bar{D}$	**SD**	**95% Lower limit**	**95% Upper limit**
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	15	-0.04	0.06	-0.17	0.09
Confectionary, bakery and eggs (combined category)	15	0.00	0.13	-0.29	0.28
Dried cereals fruits nuts seeds and vegetables	15	-0.17	0.22	-0.66	0.32
Fresh produce and fruits	15	-0.03	0.06	-0.16	0.09
Heat processed milk and dairy products	15	0.00	0.04	-0.08	0.08
Multicomponent foods or meal components	15	-0.07	0.14	-0.37	0.23
Ready to eat/ready to reheat foods (combined category: meat, poultry fish)	15	-0.04	0.09	-0.25	0.17
All Categories	**105**	**-0.05**	**0.13**	**-0.31**	**0.21**

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

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 1 data points from a total of 105 data points which were outside of the accepted limits. The scatter plots show good agreement between the reference and alternative methods.

0.1ml spread plates – 72h incubation

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

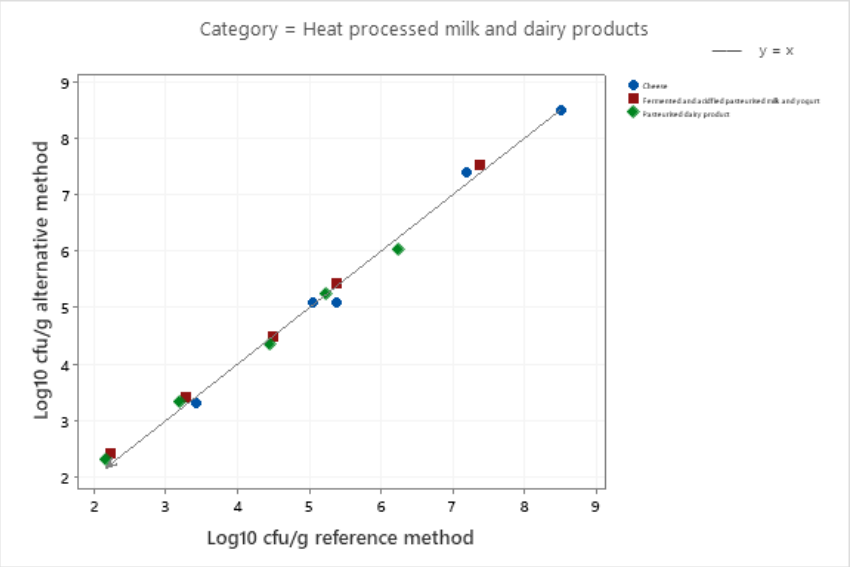


Figure 1g – Scatter plot of the reference method versus alternative method results for Fresh produce and fruit

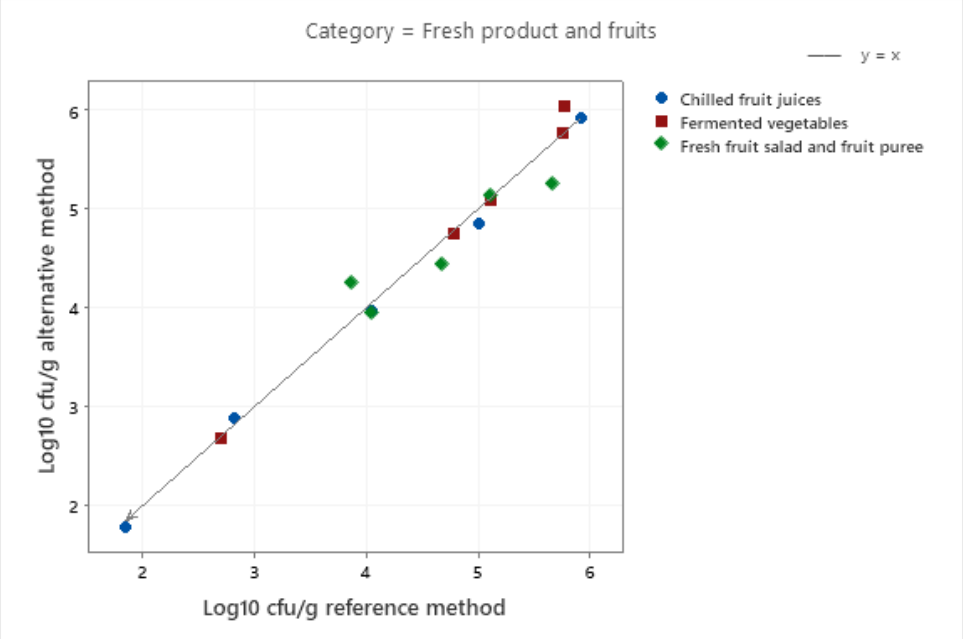


Figure 1l – Scatter plot of the reference method versus alternative method results for Multi component foods or meal components

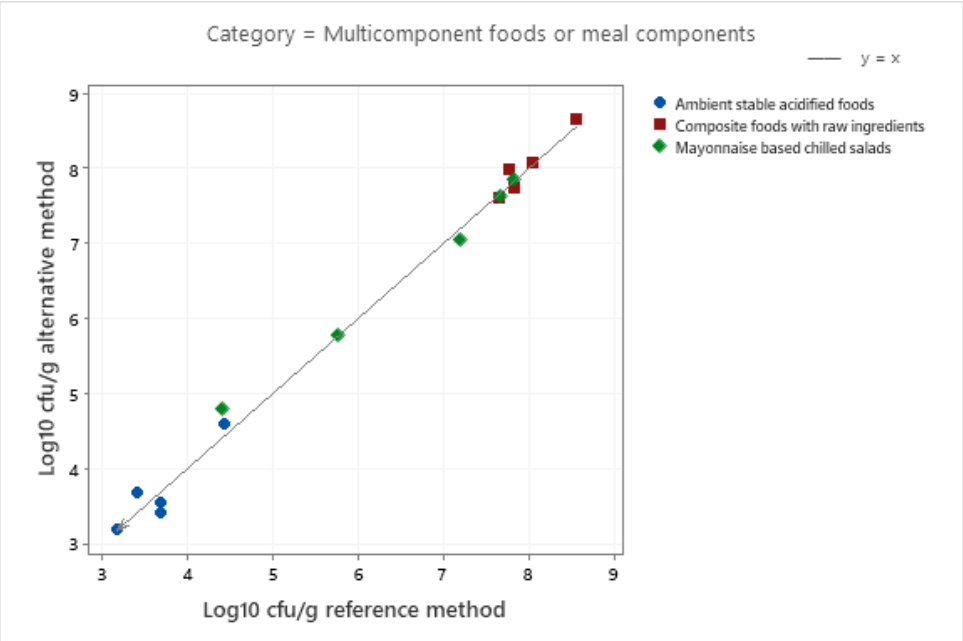


Figure 1m – Scatter plot of the reference method versus alternative method results for Confectionery bakery and eggs (combined category)

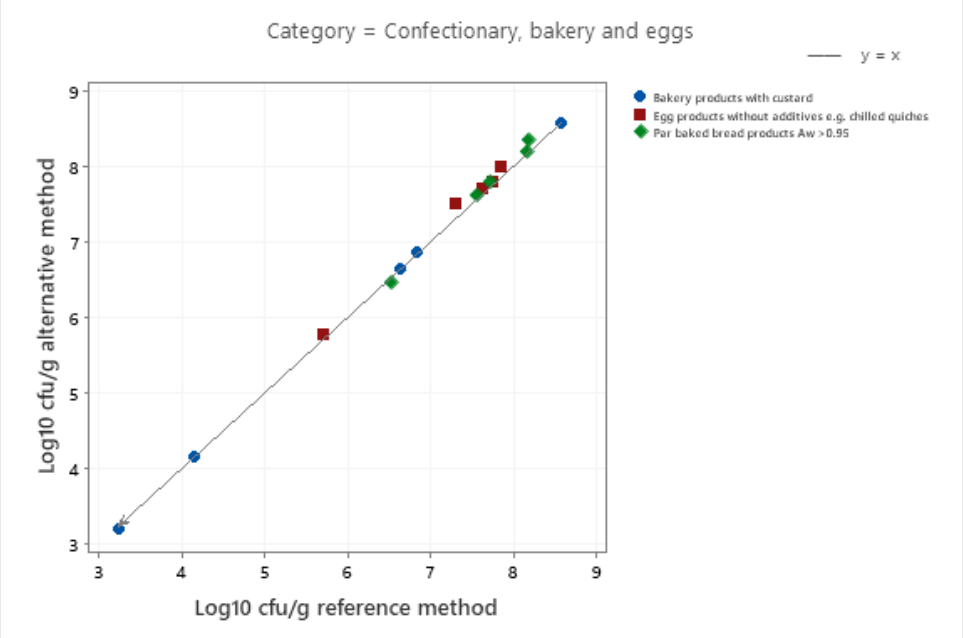


Figure 1n – Scatter plot of the reference method versus alternative method results for RTE/RTRH foods (combined category: meat, poultry and fishery products)

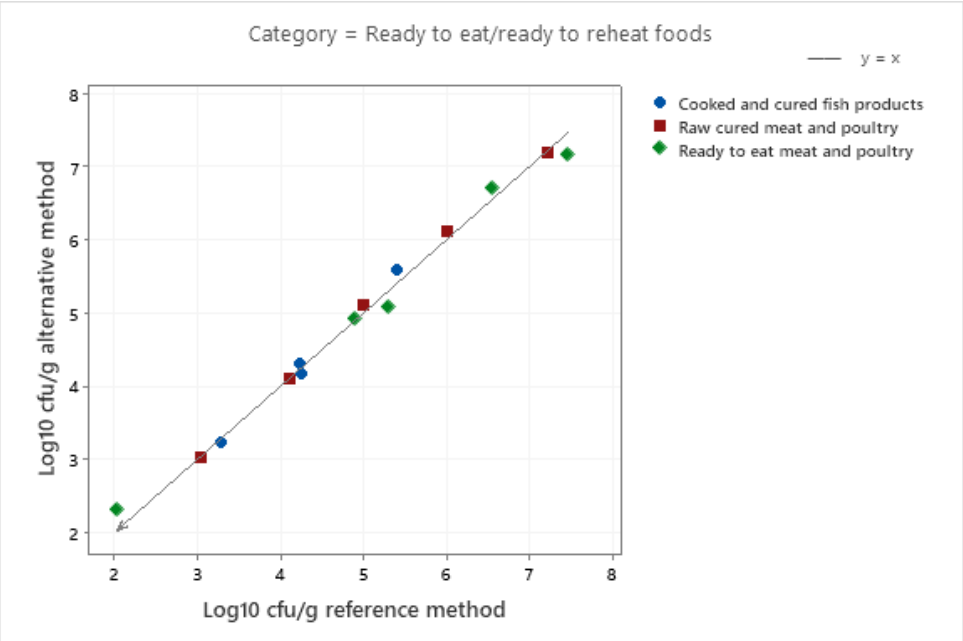


Figure 1o – Scatter plot of the reference method versus alternative method results for Dried cereals, fruits, nuts, seeds and vegetables

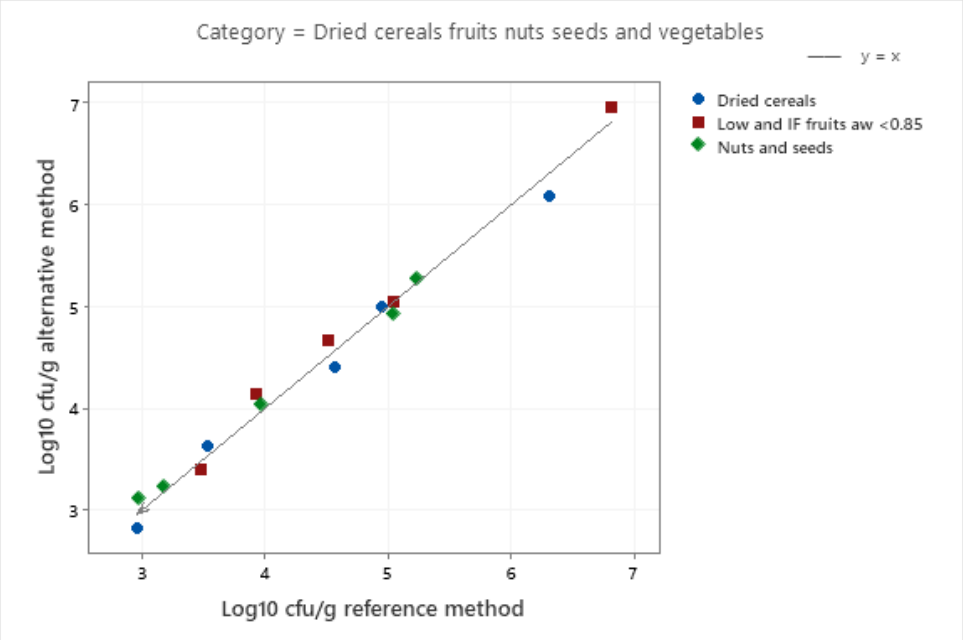


Figure 1p – Scatter plot of the reference method versus alternative method results for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

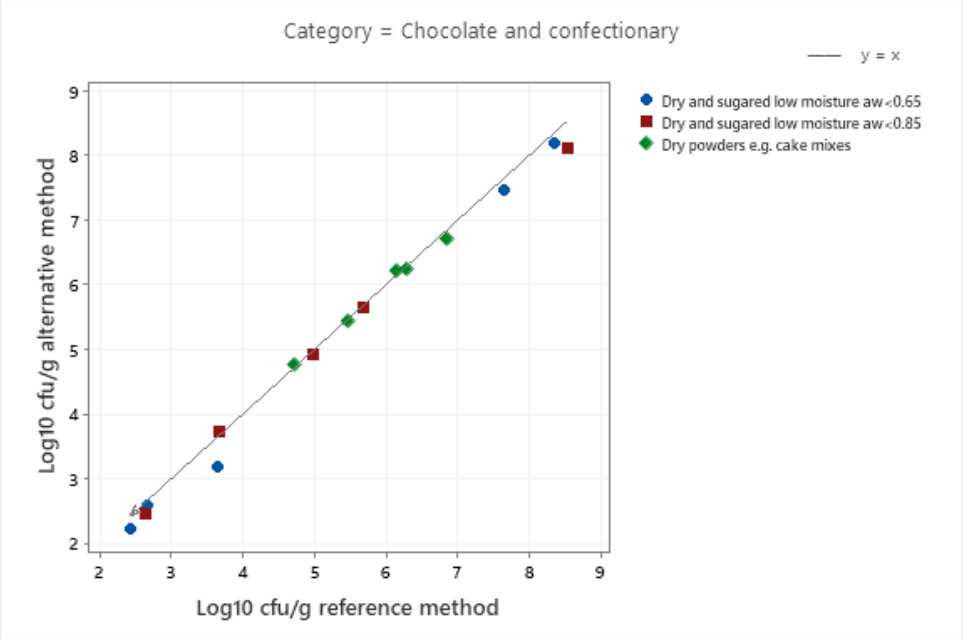
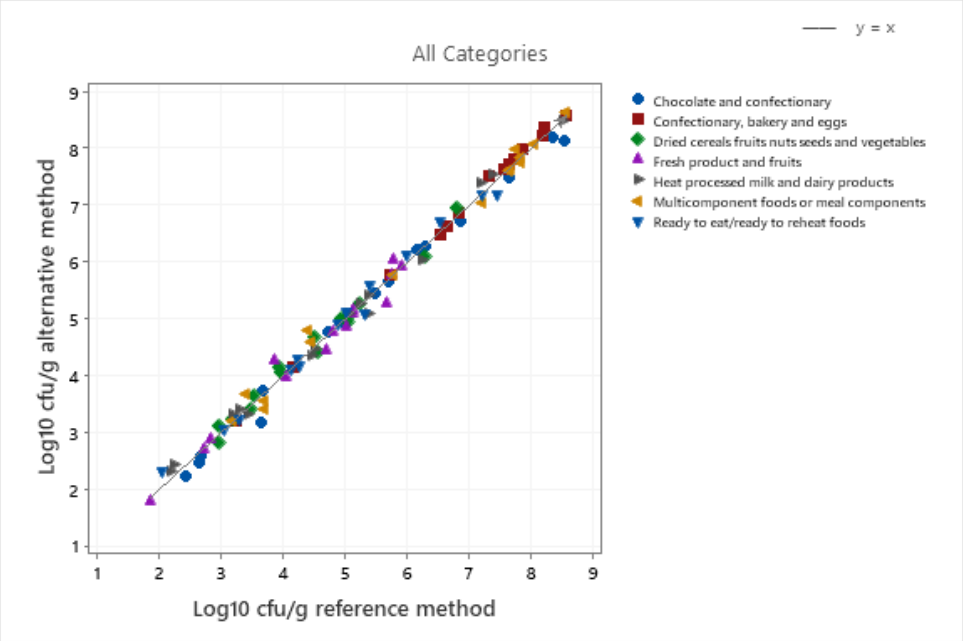


Figure 1q - Scatter plot of the reference method versus alternative method results for all the categories



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 obvious disagreement.

A summary of the calculated values per category is provided in Table 5.

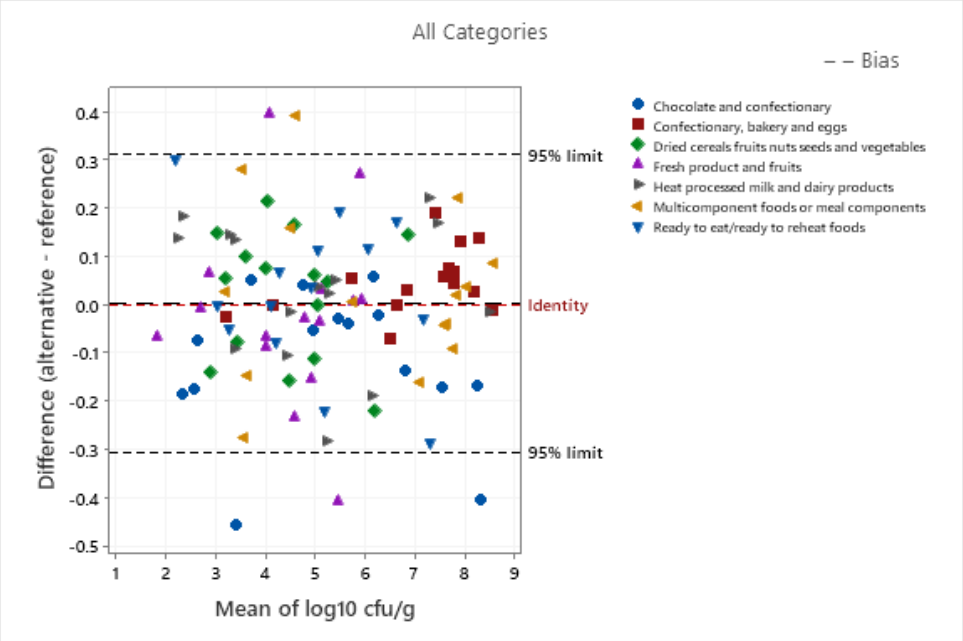
Table 5 - Summary of the calculated values per category

Category	**n**	$\bar{D}$	**SD**	**95% Lower limit**	**95% Upper limit**
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	15	-0.12	0.15	-0.45	0.22
Confectionary, bakery and eggs (combined category)	15	0.05	0.07	-0.10	0.20
Dried cereals fruits nuts seeds and vegetables	15	0.02	0.13	-0.28	0.32
Fresh produce and fruits	15	-0.02	0.19	-0.44	0.40
Heat processed milk and dairy products	15	0.03	0.15	-0.30	0.35
Multicomponent foods or meal components	15	0.03	0.18	-0.36	0.43
Ready to eat/ready to reheat foods (combined category: meat, poultry fish)	15	0.02	0.15	-0.31	0.36
All Categories	**105**	**0.00**	**0.15**	**-0.31**	**0.31**

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

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

Figure 1r – 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 6.

Table 6 - Data which are outside of the accepted limits

Category	**Sample and Type**	**N° Sample**	**Reference method** **Log cfu/g**	**Alternative method** **Log cfu/g**	**Mean** **Log cfu/g**	**Difference** **Alternative – reference)**	**Contamination**
Fresh produce and fruit	Apple, banana, strawberry and grape fruit salad Fresh fruit salad and fruit puree	T19	3.9	4.3	4.1	0.4	*Penicillium roqueforti* 16726 Stored for 72h at 2-8°C
Multicomponent foods or meal components	Potato salad Mayonnaise based chilled salads	T36	4.4	4.8	4.6	0.4	Natural contamination

Category	Sample and Type	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Contamination
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	Maple syrup Dry and sugared low moisture aw<0.65	T96	3.6	3.2	3.4	-0.5	*Wallemia sebi* CABI37338 Lyophilised culture, stored for 2 weeks ambient
Fresh produce and fruit	Blueberries Fresh fruit salad and fruit puree	T16	5.7	5.3	5.5	-0.4	Natural contamination
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	Lemon icing Dry and sugared low moisture aw<0.85	T95	8.5	8.1	8.3	-0.4	*Zygosaccharomyces baili* CRA 16125 Lyophilised culture, stored for 2 weeks ambient

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 5 data points from a total of 105 data points which were outside of the CLs. The 5 datapoints outside belong to three categories out of the 7 categories tested. Two out of the 5 discrepant samples were naturally contaminated, and 3 were artificially contaminated. The 3 artificially contaminated samples were inoculated with different strains following two artificial contamination protocols. Data from the studies indicates that there is no indication of systematic bias and all points outside the CLs have a log difference within -0.5 and 0.5 log. The scatter plots and log differences show good agreement between reference and alternative methods.

1ml pour plates – 54h incubation

Figure 2a – Scatter plot of the reference method versus alternative method results for Heat processed milk and dairy products

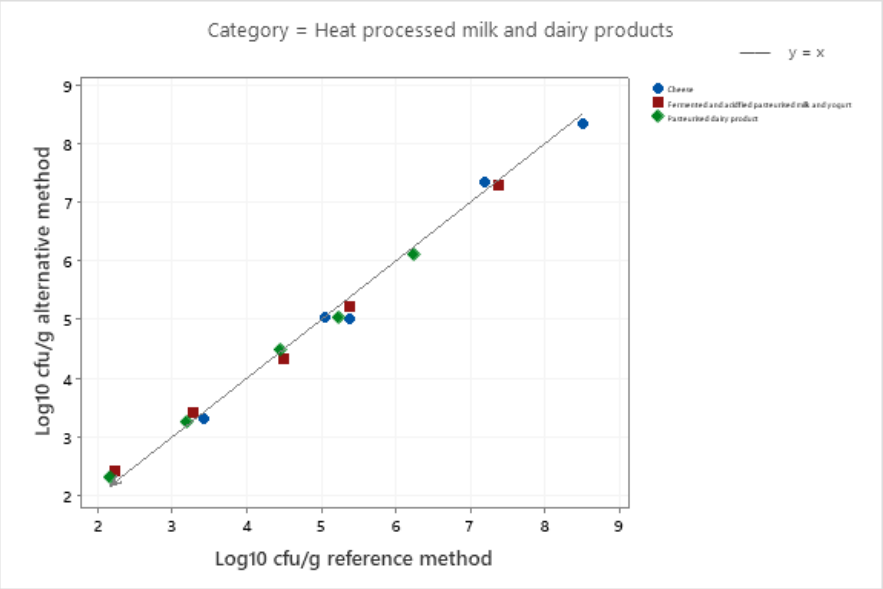


Figure 2b – Scatter plot of the reference method versus alternative method results for Fresh produce and fruits

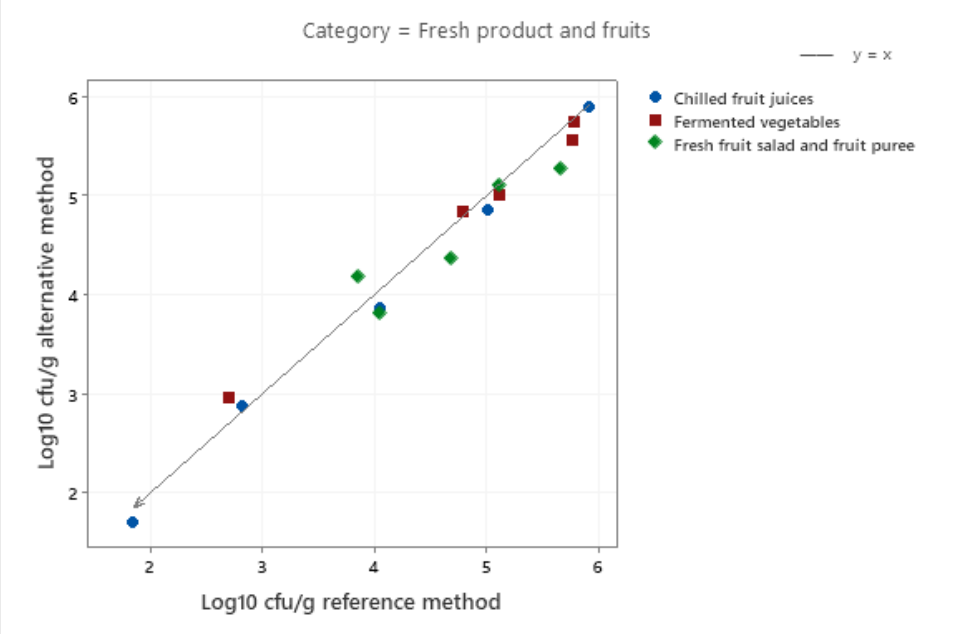


Figure 2c – Scatter plot of the reference method versus alternative method results for Multi component foods or meal components

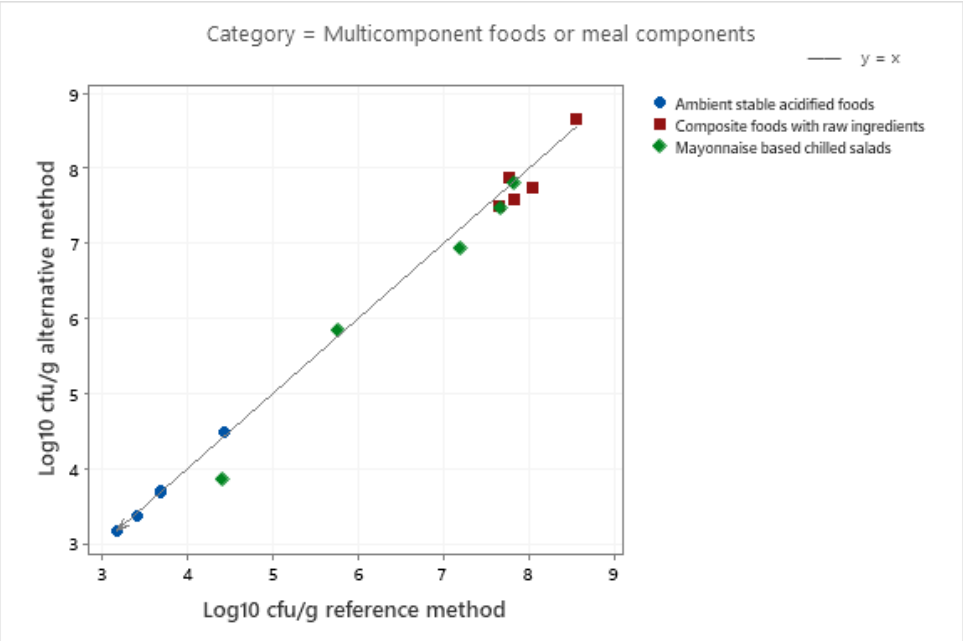


Figure 2d – Scatter plot of the reference method versus alternative method results for Confectionery bakery and eggs (combined category)

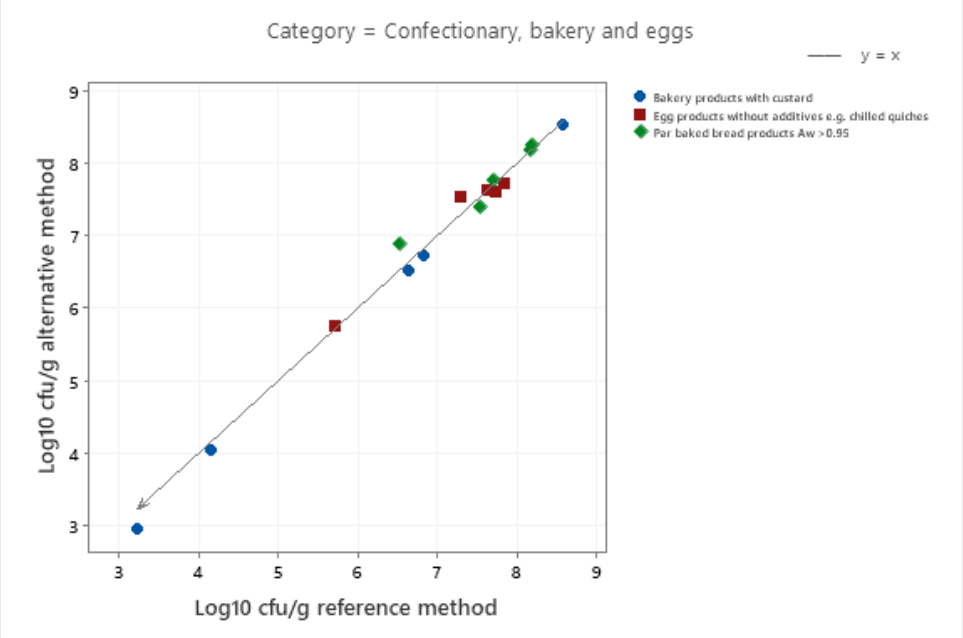


Figure 2e – Scatter plot of the reference method versus alternative method results for RTE/RTRH foods (combined category: meat, poultry fish)

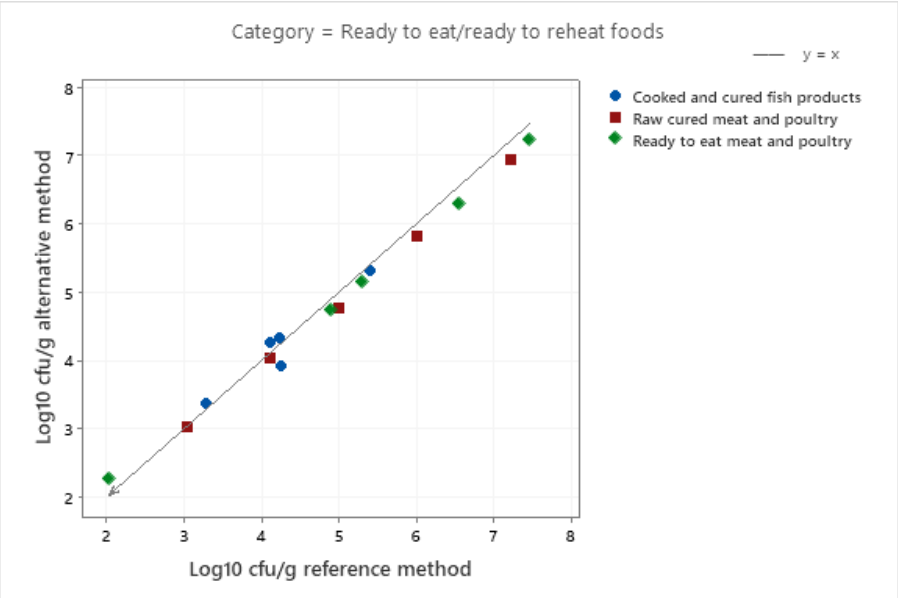


Figure 2f – Scatter plot of the reference method versus alternative method results for Dried cereals, fruits, nuts, seeds and vegetables

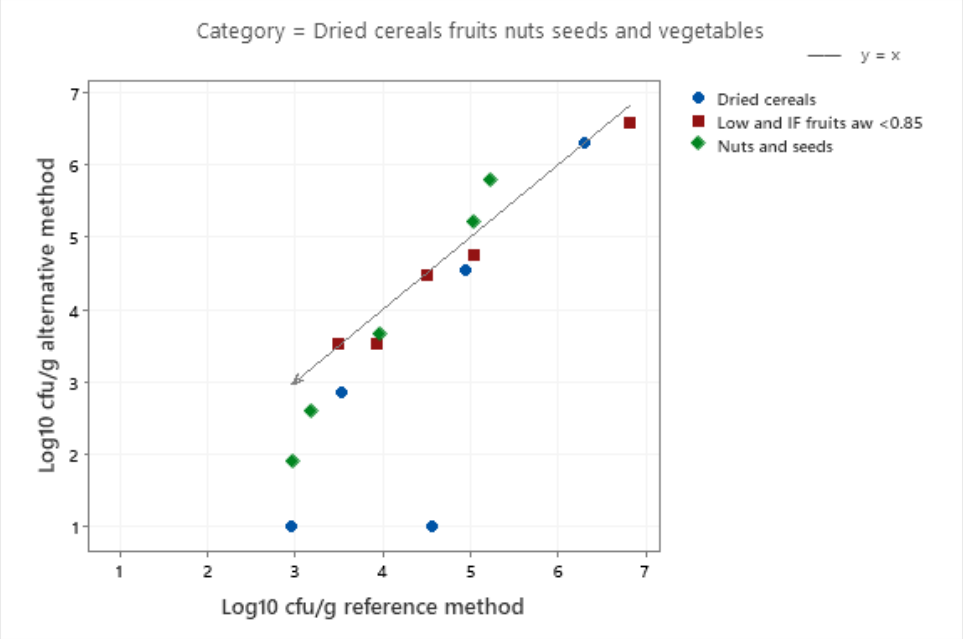


Figure 2g – Scatter plot of the reference method versus alternative method results for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

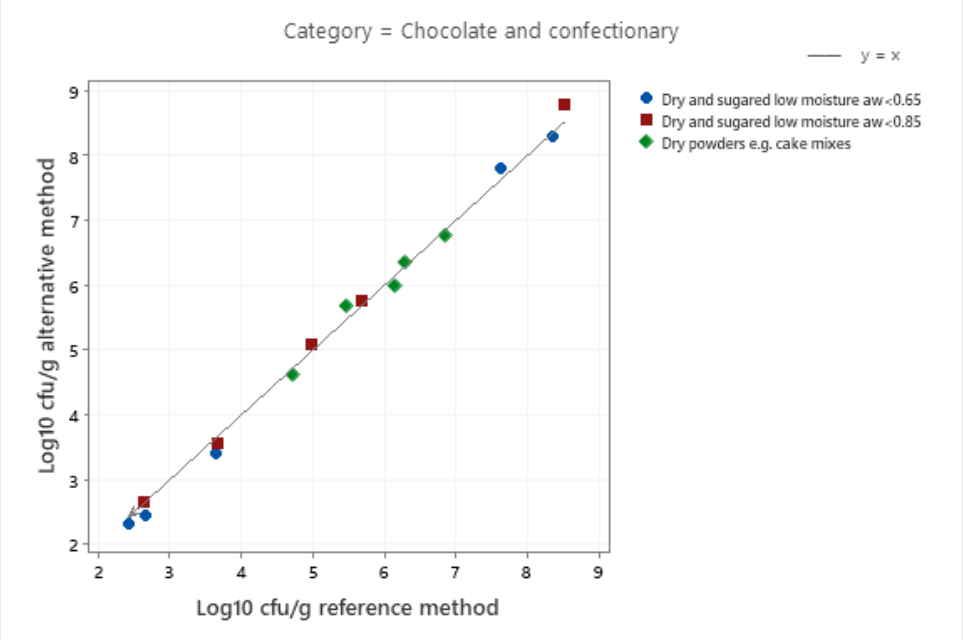
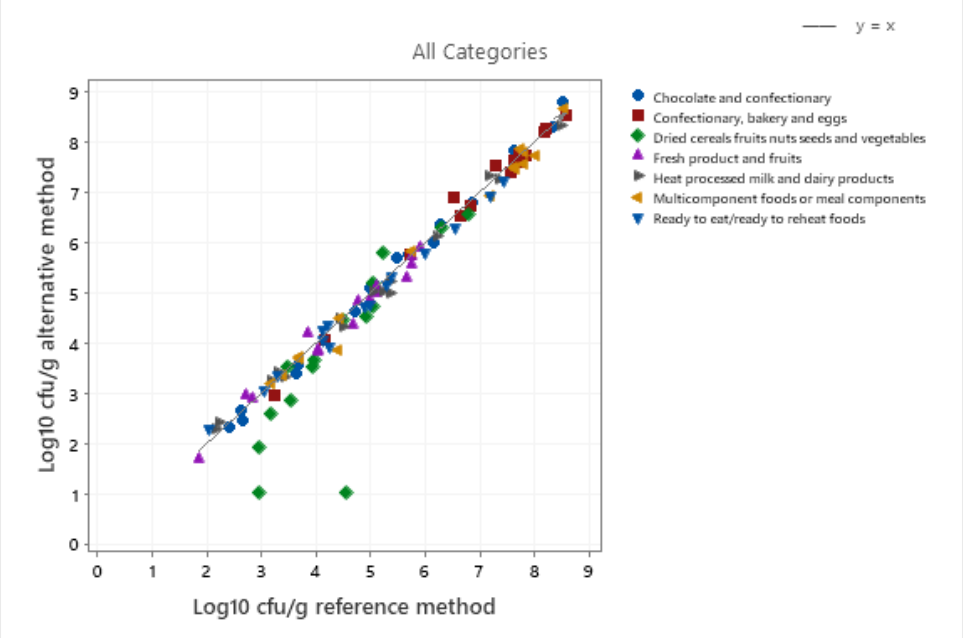


Figure 2h - Scatter plot of the reference method versus alternative method results for all the categories

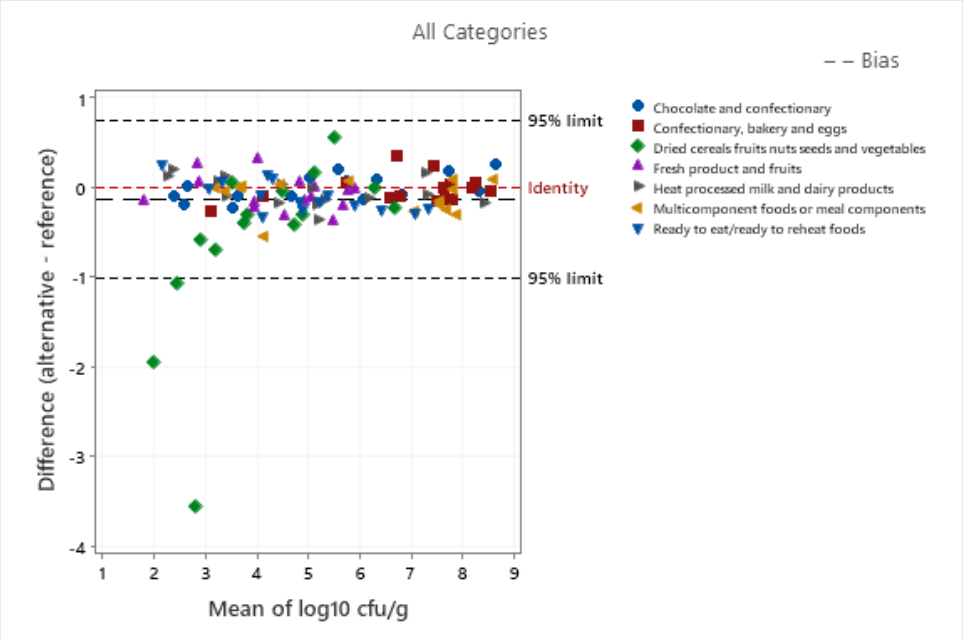


According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plots show no obvious disagreement.

There is a trend towards a larger difference in reference and alternative methods for the dried cereals, fruits, nuts, seeds and vegetables category.

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

Figure 2i – 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 7.

Table 7 - Data which are outside of the accepted limits

Category	Sample and Type	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference Alternative – reference)	Contamination
Dried cereals fruits nuts seeds and vegetables	Unsalted mixed nuts Nuts and seeds	T84	3.0	2.0	2.4	-1.0	*Aspergillus restrictus* ATCC42693 Inoculated using lyophilised culture and stored for 2 weeks ambient
Dried cereals fruits nuts seeds and vegetables	Malt wheats Dried Cereals	T79	3.0	1.0	1.9	-2.0	*Eurotium amstelodami* DSM62629 Inoculated using lyophilised culture and stored for 2 weeks ambient
Dried cereals fruits nuts seeds and vegetables	Wheat biscuits Dried cereals	T80	4.6	1.0	2.8	-3.6	*Eurotium amstelodami* DSM62629 Inoculated using lyophilised culture and stored for 2 weeks ambient

The alternative method results for samples T79 and T80 are below the quantification limit, the datapoints are included in the Bland-Altman analysis and were excluded in the calculated values in Table 8. The results are detailed in Annex E. To meet the ISO 16140-2 statistical analysis, the samples were repeated to investigate the possible reasons for the low counts obtained during the initial analysis.

The repeat analysis followed the same artifical contamination protocol using a different strain for inoculation (*Paecilomyces variotti* CRA 16670). On repeat analysis, the samples gave counts at 54h incubation and the log differences were -0.07 and -0.02 respectively,. Three other samples (T76-T78) analysed in the dried cereals type were also inoculated with *Eurotium amstelodami* DSM62629, however large log differences between methods were not observed with these three samples. The results for the repeated samples and other samples tested in the dried cereals type indicate that the discrepancies seen in T79 and T80 at the 54h incubation are likely to be outlying results caused by strain-matrix interaction and not due to the performance of the alternative method.

A summary of the calculated values per category is provided in Table 8.

Table 8 - Summary of the calculated values per category

Category	n	$\bar{D}$	sD	95% Lower limit	95% Upper limit
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	15	0.11	0.22	-0.37	0.60
Confectionary, bakery and eggs (combined category)	15	-0.07	0.16	-0.43	0.29
Dried cereals fruits nuts seeds and vegetables	15	-0.26	0.45	-1.27	0.74
Fresh product and fruits	15	-0.05	0.13	-0.34	0.23
Heat processed milk and dairy products	15	-0.06	0.11	-0.31	0.18
Multicomponent foods or meal components	15	-0.13	0.28	-0.74	0.49
Ready to eat/ready to reheat foods (combined category: meat, poultry fish)	15	-0.11	0.18	-0.52	0.30
All Categories	**105**	**-0.08**	**0.26**	**-0.60**	**0.44**

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

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 3 data points from a total of 105 data points which were outside of the CLs.

Further analysis of the data suggests a trend towards high differences between the reference and alternative method for the dried cereals, fruits, nuts, seeds, and vegetables category. A negative bias of -0.26 was noted for this low water activity category and a root cause analysis was carried out to investigate the bias. The largest discrepancies in this category were observed with unsalted mixed nuts inoculated with *Aspergillus restrictus* ATCC42696 isolated from dried chillies (-1.21), sunflower seeds inoculated with *Aspergillus restrictus* ATCC42696 isolated from dried chillies (-0.64), apricots inoculated with *Zygosaccharomyces rouxii CRA* 16127 isolated from spoiled fruit (-0.61). The counts recorded for the 3 samples noted above improved with re-incubation for the total 72 hours.

The results of the additional samples tested in the dried cereals, fruits, nuts, seeds, and vegetables category and the root cause analysis of the negative bias observed were reviewed at the MVTC in March 2024. During the meeting, it was agreed that no restrictions were needed on the incubation time of 54h for the pour plates when analysing samples belonging to the dried cereals, fruits, nuts, seeds, and vegetables category.

1ml pour plates – 72h incubation

Figure 2j – Scatter plot of the reference method versus alternative method results for Heat processed milk and dairy products

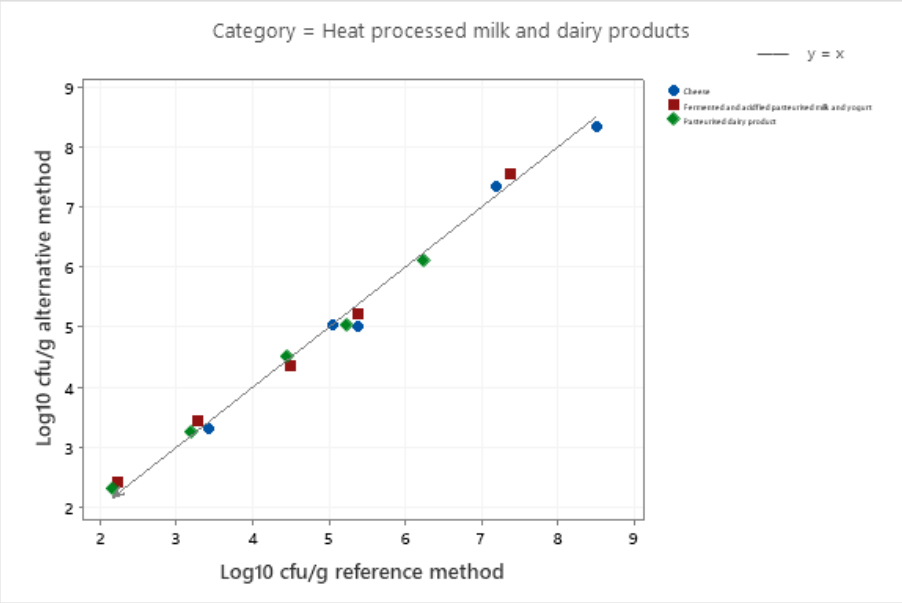


Figure 2k – Scatter plot of the reference method versus alternative method results for Fresh produce and fruits

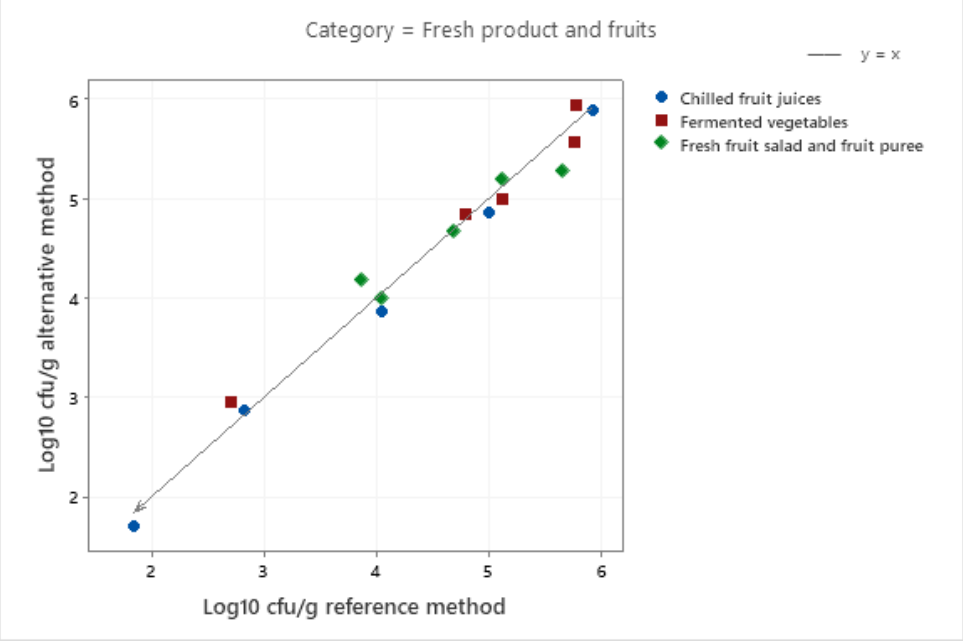


Figure 2l – Scatter plot of the reference method versus alternative method results for Multi component foods or meal components

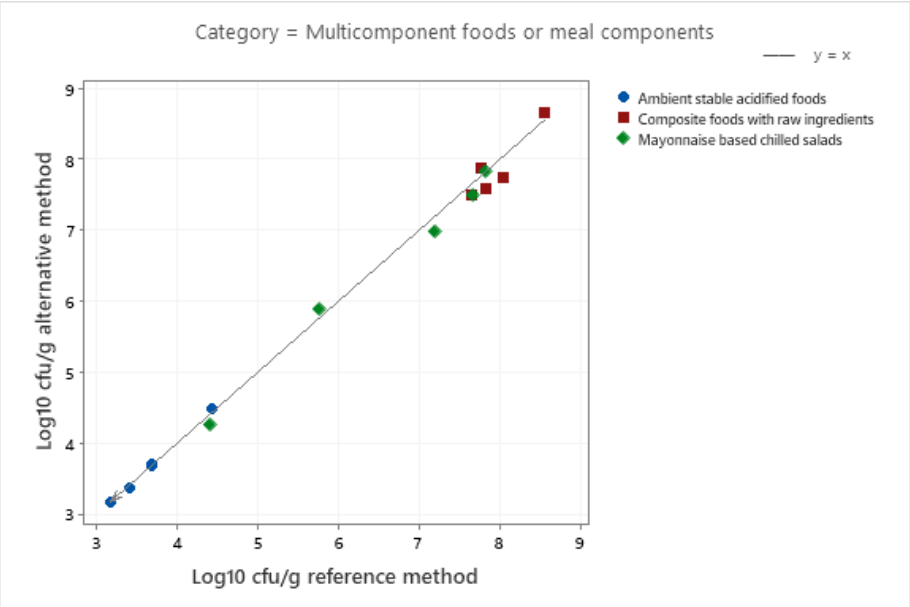


Figure 2m – Scatter plot of the reference method versus alternative method results for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)

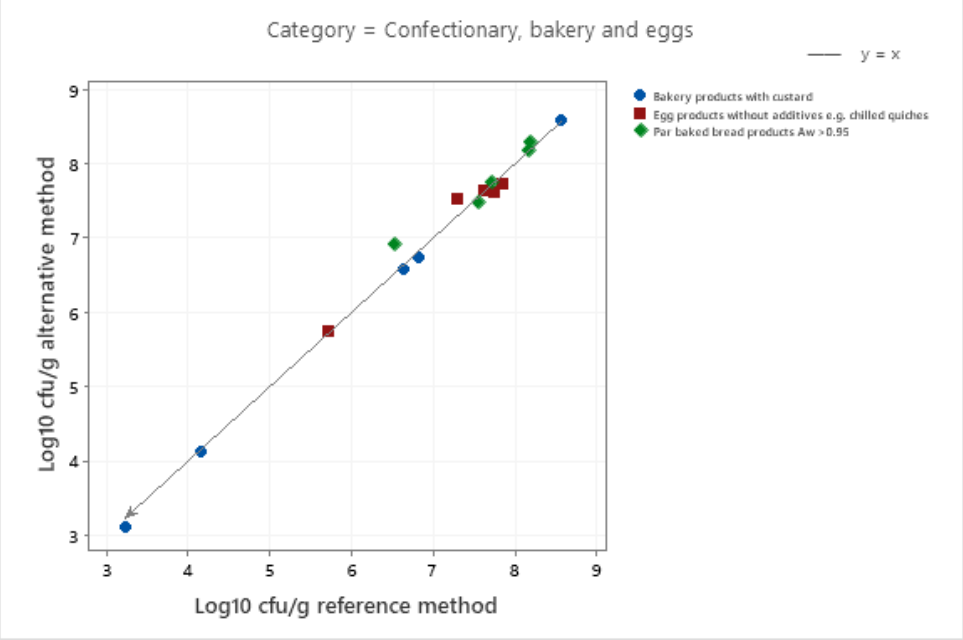


Figure 2n – Scatter plot of the reference method versus alternative method results for RTE/RTRH foods (combined category: meat, poultry and fishery products)

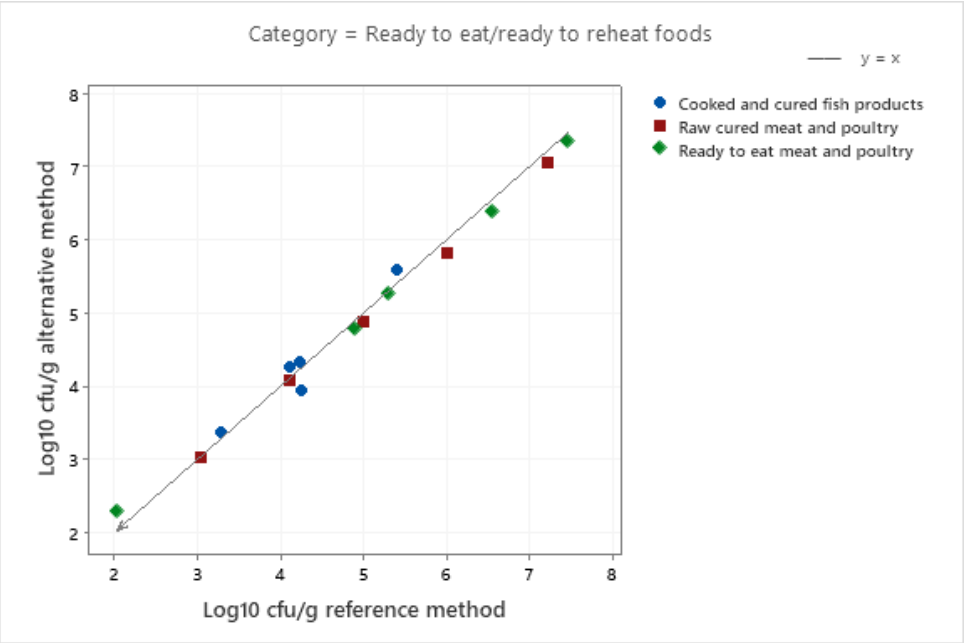


Figure 2o – Scatter plot of the reference method versus alternative method results for Dried cereals, fruits, nuts, seeds and vegetables

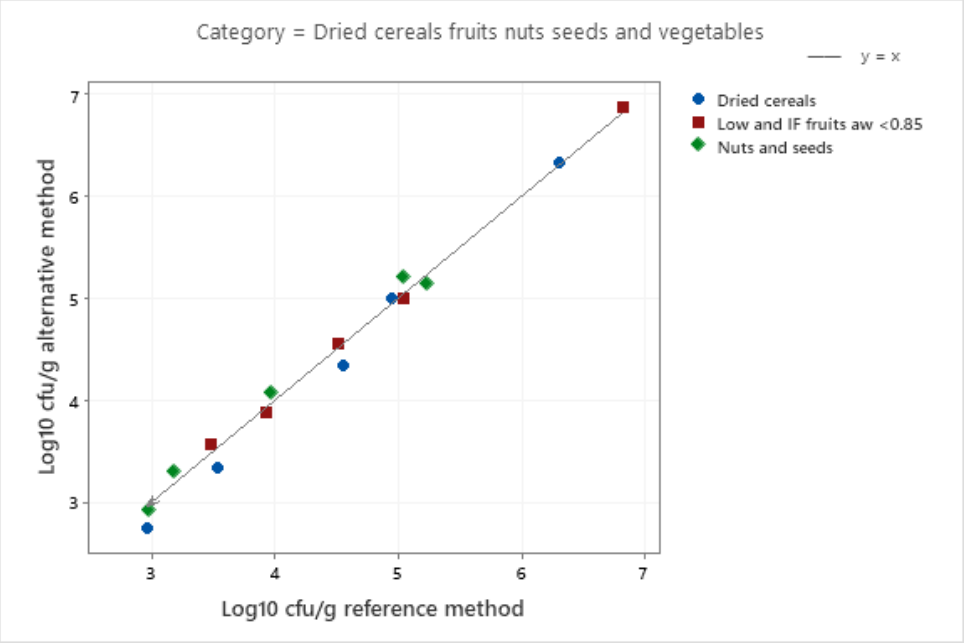


Figure 2p – Scatter plot of the reference method versus alternative method results for Chocolate and confectionary

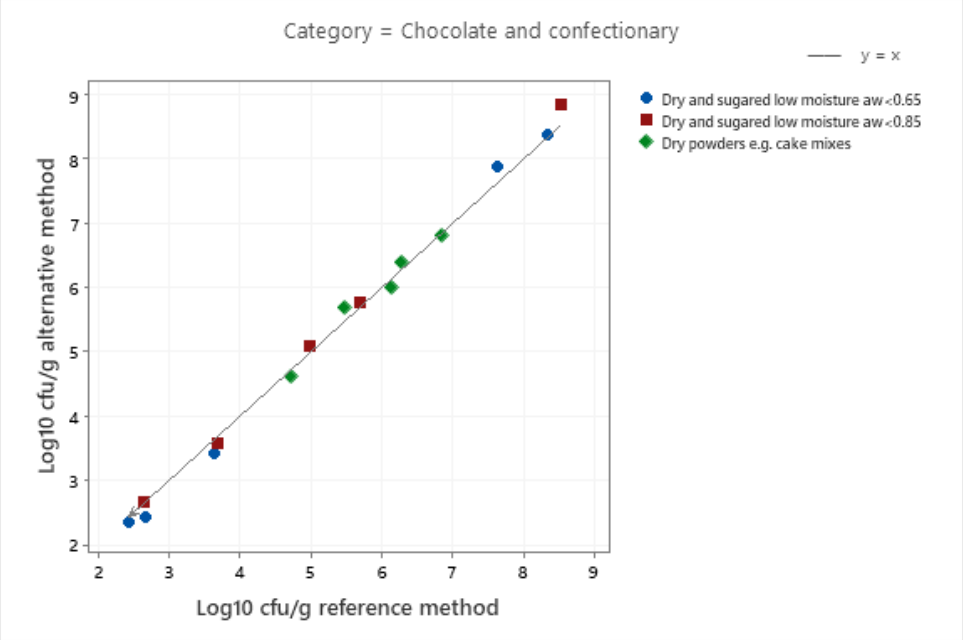
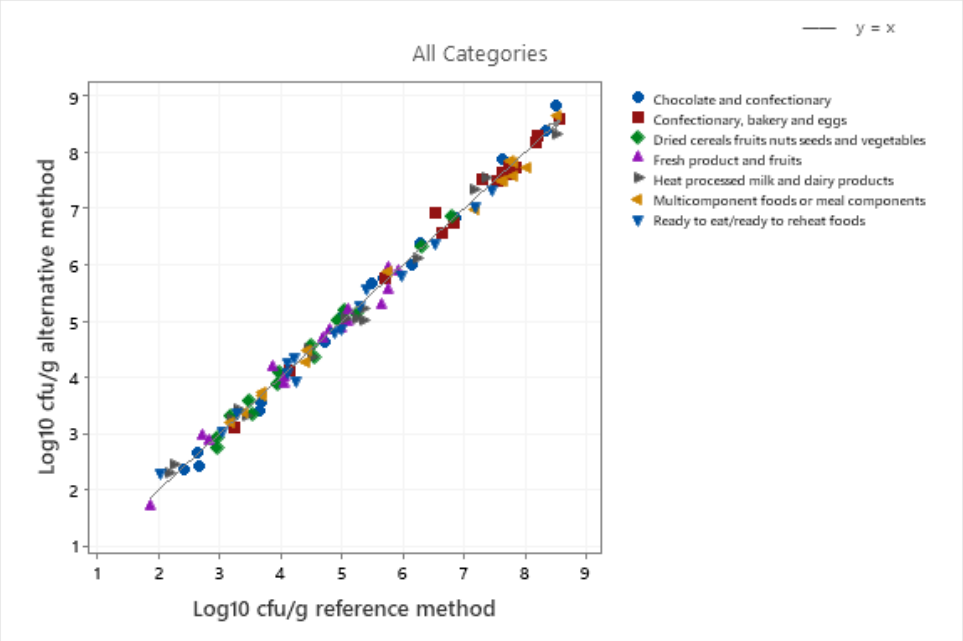


Figure 2q - Scatter plot of the reference method versus alternative method results for all the categories



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 obvious disagreement.

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

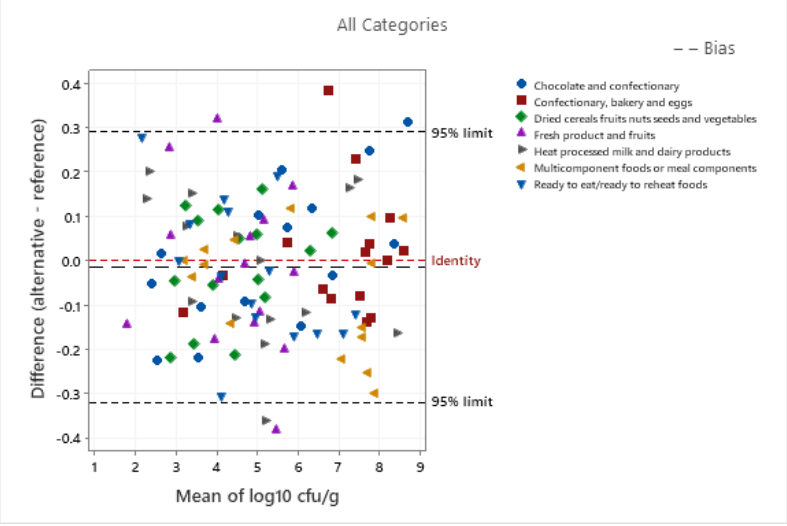
Table 9 - Summary of the calculated values per category

Category.	n	$\bar{D}$	sD	95% Lower limit	95% Upper limit
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	15	0.02	0.16	-0.34	0.38
Confectionary, bakery and eggs (combined category)	15	0.01	0.14	-0.30	0.32
Dried cereals fruits nuts seeds and vegetables	15	-0.01	0.12	-0.29	0.26
Fresh produce and fruits	15	-0.02	0.19	-0.43	0.39
Heat processed milk and dairy products	15	-0.01	0.17	-0.39	0.36
Multicomponent foods or meal components	15	-0.06	0.14	-0.36	0.24
Ready to eat/ready to reheat foods (combined category: meat, poultry fish)	15	-0.03	0.16	-0.38	0.33
All Categories	**105**	**-0.01**	**0.15**	**-0.32**	**0.29**

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

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

Figure 2r – 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 10.

Table 10 - Data which are outside of the accepted limits

Category	**Sample and Type**	**N° Sample**	**Reference method** **Log cfu/g**	**Alternative method** **Log cfu/g**	**Mean** **Log cfu/g**	**Difference** **Alternative – reference**	**Contamination**
Fresh produce and fruits	Apple, banana, strawberry and grape fruit salad Fresh fruit salad and fruit puree	T19	3.9	4.2	4.0	0.3	*Penicillium roqueforti* CRA 16726 stored for 72h at 2-8°C
Fresh produce and fruits	Blueberries Fresh fruit salad and fruit puree	T16	5.7	5.3	5.5	-0.4	Natural contamination
Heat processed milk and dairy products	Mature grated cheddar cheese Cheese	T8	5.4	5.0	5.2	-0.4	*Penicillium roqueforti* CRA 16726 stored for 72h at 2-8°C
Confectionary bakery and eggs (combined category)	Part Baked Rustic Seeded Rolls Par baked bread products Aw >0.95	T57	6.5	6.9	6.7	0.4	Natural contamination
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	Lemon icing Dry and sugared low moisture aw<0.85	T95	8.5	8.8	8.7	0.3	*Zygosaccharomyces baili* CRA 16125 inoculated with a lyophilised culture and stored for 2 weeks ambient

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. In this study there were 5 data points from a total of 105 data points which were outside of the CLs. The 5 datapoints outside the limits belong to four categories out of the 7 categories tested. Two samples with discrepant results were naturally contaminated, and three were artificially contaminated. The 3 artificially contaminated samples were inoculated with different strains following two artificial contamination protocols. Data from the studies suggests that there is no indication of systematic bias and all points outside the CLs have a log difference within -0.4 and 0.4 log. The scatter plots and log differences show good agreement between reference and alternative methods.

3.1.6 Conclusion (RT study)

The relative trueness of the alternative method is satisfied as the data shows good agreement between the reference and alternative methods.

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 is conducted using artificially contaminated samples, using one type per category.

3.2.1 Categories, sample types and strains

Seven food categories were tested with a single batch of two different food types using 6 samples per type.

Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per food type. The following food type/strain pairs were studied (See Table 11):

Table 11 - 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 nougat	Fermented yogurt drink
			Cream cheese
Fresh produce and fruits	Fresh produce	*Debaryomyces hansenii CRA 15969* factory isolate	Vegetabale 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	Pasta salad
			Cous cous salad
Confectionery bakery and eggs (combined category)	Chilled RTE foods	*Aspergillus niger* CRA 16667 isolated from grapes	Quiche
			Custard tart
RTE/RTRH foods (combined category: meat, poultry fish)	Ready to eat meat and poultry	*Penicillium chrysogeum* DSM 848 source unknown	Cooked prawns
			Fish pate
Dried cereals, fruits, nuts seeds and vegetables	Nuts and seeds	*Eurotium amstelodami* CRA 8155 isolated from a mouldy kernel	4 seed mix*
			Toasted seed mix**
Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95)	Dry and sugared low moisture aw<0.85	*Zygosaccharomyces baili* CRA16125 isolated from nougat	Chocolate
			Cream puff

*4 seed mix - sunflower seed (35%), pumpkin seed (35%), sesame seeds, golden flax seed

**Toasted seed mix - toasted brown linseed, toasted pumpkin seed, toasted sunflower seed

Preparation of samples was done as a bulk inoculation. For all matrices with a water activity >0.95, a 100g sample was inoculated with 1ml of appropriate dilution of inoculating strain and homogenised by hand massaging or stomaching to evenly distribute the inoculum. The 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis. For all matrices with a water acitivity <0.95, a 100g sample was inoculated with 1g of lypholised culture and shaken to distribute the inoculum. The 100g samples were inoculated and stored at ambient for 2 weeks prior to analysis. The inoculation of samples with moulds were carried out using mould spores.

Five separate 10g test portions were removed from the bulk sample and mixed with 90ml PSD or appropriate diluent and enumerated on both methods.

All results were tabulated, calculated and interpreted according to ISO 16140-2. An incubation time of 54 hours and 72 hours was used for the study and both are detailed in this report.

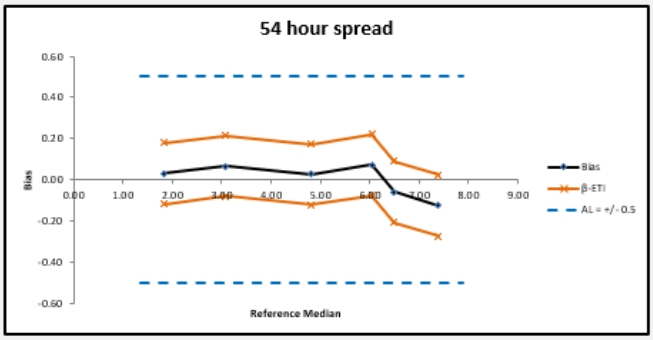
3.2.2 Calculations and interpretation of accuracy profile study
The summary tables (in log CFU/g) are provided in Annex D. The statistical results and the accuracy profiles are provided Figure 3a-f for 0.1ml spread plates with 54-hour incubation, Figure 3g-l for 0.1ml spread plate with 72-hour incubation and 4a-f for 1ml pour plates with 54-hour incubation and Figures 4g-l for 1ml pour plates with 72-hour incubation.

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

OPYM 0.1ml spread 54h incubation

Figure 3a – Accuracy profile for Heat processed milk and dairy products 54h spread

(Food) Category	Heat processed milk and dairy
(Food) Type	54 hour spread

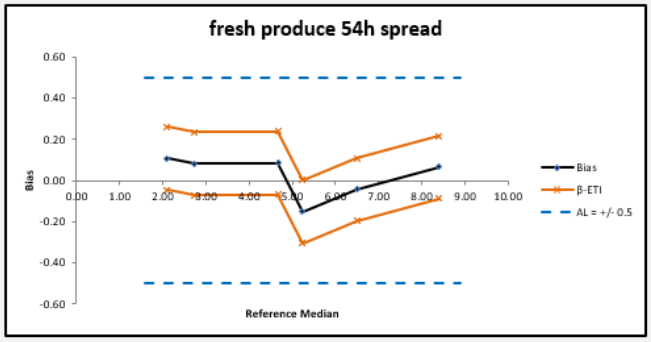


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL±0.5 Acceptable	β-ETI compared to final AL Acceptable
4	1.85	0.030	-0.119	0.179	YES	YES
1	3.08	0.067	-0.082	0.216	YES	YES
2	4.82	0.026	-0.123	0.174	YES	YES
3	6.04	0.073	-0.076	0.221	YES	YES
5	6.49	-0.060	-0.209	0.089	YES	YES
6	7.38	-0.125	-0.274	0.024	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.103	YES	+/- 0.500

Figure 3b – Accuracy profile for Fresh produce and fruits 54h spread

(Food) Category	Fruits and vegetables
(Food) Type	fresh produce 54h spread

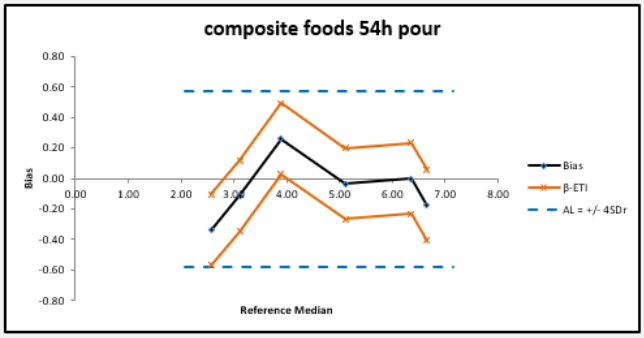


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
10 a-e	2.10	0.107	-0.046	0.260	YES	YES
9 a-e	2.72	0.082	-0.071	0.235	YES	YES
7 a-e	4.66	0.085	-0.068	0.238	YES	YES
11 a-e	5.23	-0.151	-0.304	0.002	YES	YES
8 a-e	6.49	-0.044	-0.197	0.109	YES	YES
12 a-e	8.40	0.064	-0.089	0.217	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.095	0.106	YES	+/- 0.500

Figure 3c – Accuracy profile for Multi component foods or meal components 54h spread

(Food) Category	multicomponent foods and
(Food) Type	composite foods 54h pour

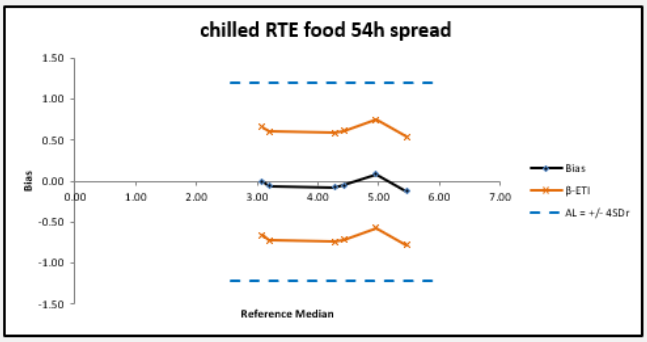


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16 a-e	2.57	-0.338	-0.570	-0.105	NO	YES
13 a-e	3.11	-0.114	-0.346	0.118	YES	YES
17 a-e	3.89	0.260	0.027	0.492	YES	YES
14 a-e	5.11	-0.035	-0.267	0.198	YES	YES
18 a-e	6.36	0.000	-0.232	0.232	YES	YES
15 a-e	6.65	-0.176	-0.409	0.056	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.161	NO	+/- 0.576

Figure 3d – Accuracy profile for Confectionery bakery and eggs (combined category) *54h spread*

(Food) Category	confectionary bakery and eggs
(Food) Type	chilled RTE food 54h spread

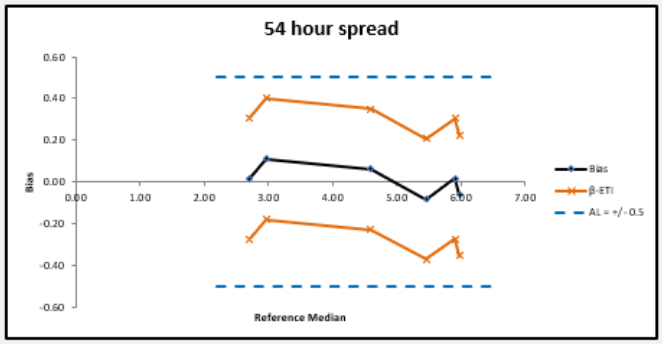


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19 a-e	3.08	0.000	-0.661	0.661	NO	YES
24 a-e	3.20	-0.058	-0.719	0.603	NO	YES
21 a-e	4.28	-0.075	-0.736	0.587	NO	YES
22 a-e	4.43	-0.051	-0.712	0.610	NO	YES
20 a-e	4.95	0.087	-0.574	0.748	NO	YES
23 a-e	5.46	-0.120	-0.781	0.541	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.302	0.458	NO	+/- 1.208

Figure 3e – Accuracy profile for RTE/RTRH foods (combined category: meat, poultry and fish) 54h spread

(Food) Category	RTE RTRH foods
(Food) Type	54 hour spread

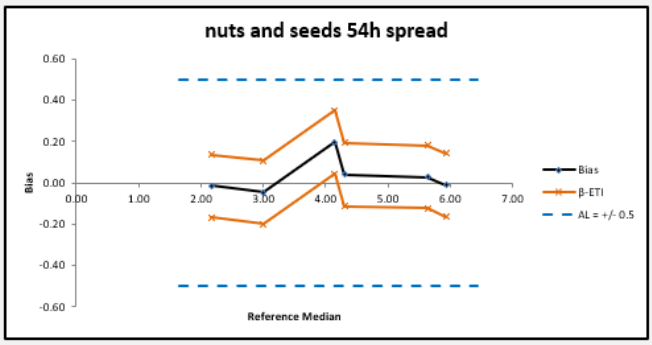


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25	2.71	0.017	-0.273	0.307	YES	YES
28	2.37	0.111	-0.179	0.401	YES	YES
29	4.60	0.061	-0.229	0.351	YES	YES
26	5.46	-0.082	-0.372	0.208	YES	YES
27	5.31	0.016	-0.275	0.306	YES	YES
30	5.38	-0.064	-0.354	0.226	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.200	0.201	NO	+/- 0.500

Figure 3f – Accuracy profile for Dried cereals, fruits, nuts, seeds and vegetables 54h spread

(Food) Category	dried cereals nuts seed and veg
(Food) Type	nuts and seeds 54h spread

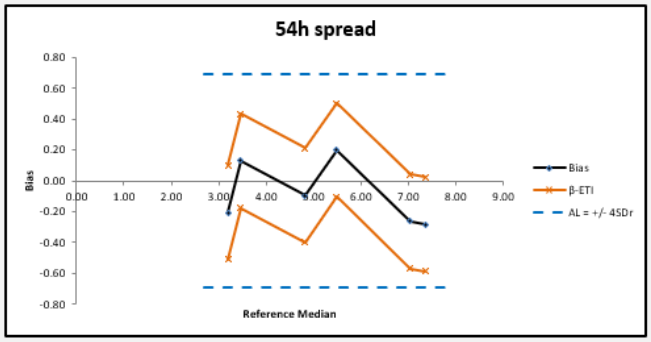


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
31 a-e	2.16	-0.015	-0.168	0.138	YES	YES
34 a-e	3.00	-0.046	-0.199	0.107	YES	YES
35 a-e	4.15	0.196	0.043	0.349	YES	YES
32 a-e	4.30	0.041	-0.112	0.194	YES	YES
36 a-e	5.63	0.029	-0.124	0.182	YES	YES
33 a-e	5.93	-0.010	-0.163	0.143	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.143	0.106	NO	+/- 0.500

Figure 3g – Accuracy profile for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95) 54h spread

(Food) Category	Chocolate, bakery and
(Food) Type	54h spread



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
40	3.20	-0.204	-0.509	0.100	NO	YES
37	3.46	0.129	-0.176	0.433	YES	YES
38	4.83	-0.094	-0.398	0.211	YES	YES
39	5.49	0.193	-0.106	0.503	NO	YES
41	7.04	-0.263	-0.568	0.041	NO	YES
42	7.36	-0.283	-0.587	0.022	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.172	0.211	NO	+/- 0.688

In this study the following categories met the AL of 0.5log

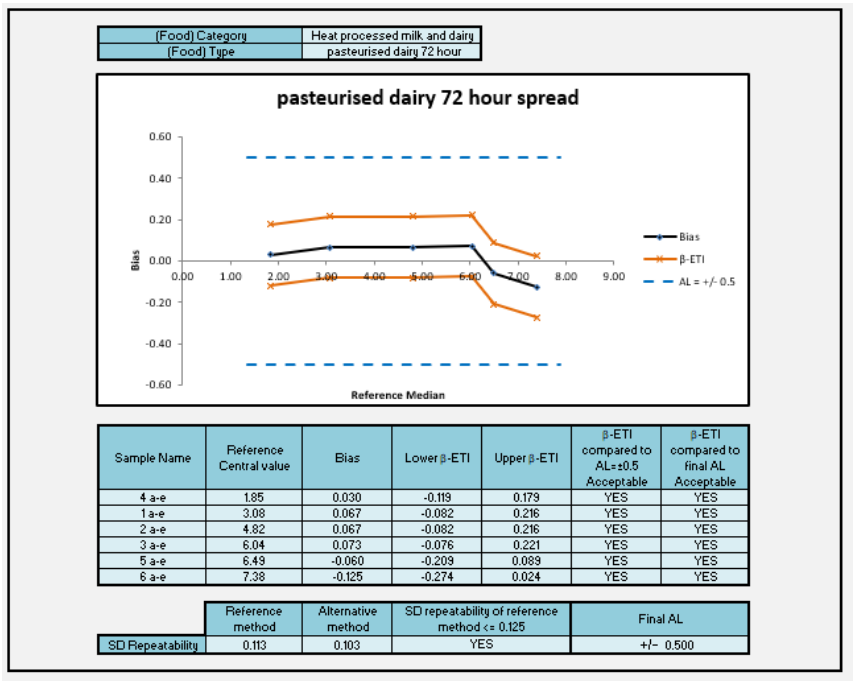
- Heat processed milk and dairy products
- Fruits and vegetables
- RTE/RTRH foods
- Dried cereals fruits nut seeds and vegetables

In this study, Multi component foods or meal components, chocolate and confectionary and confectionary, bakery and eggs required the new AL to be calculated, of ± 0.576, ± 0.688 ±1.208 respectively.

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

OPYM 0.1ml spread 72h incubation

Figure 3h – Accuracy profile for Heat processed milk and dairy products 72h spread

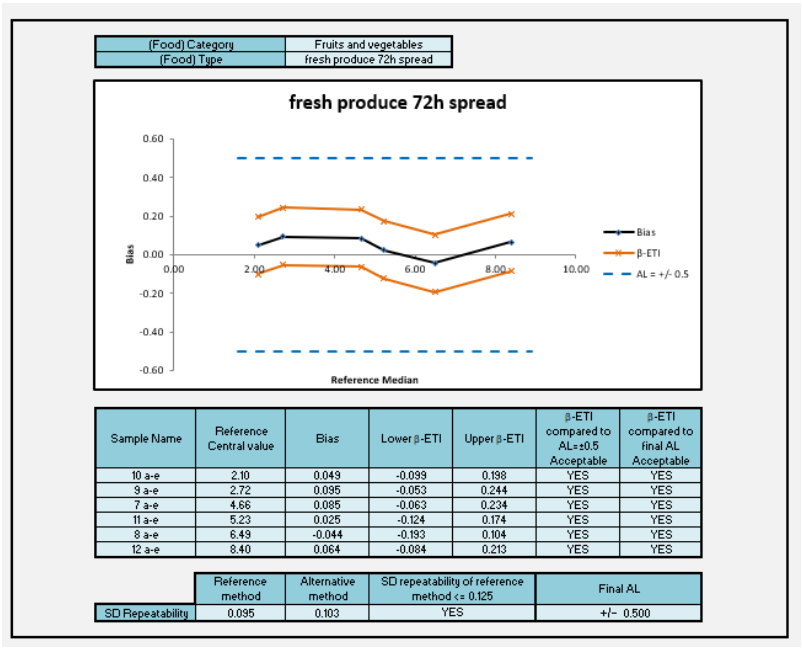


(Food) Category	Heat processed milk and dairy
(Food) Type	pasteurised dairy 72 hour

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
4 a-e	1.85	0.030	-0.119	0.179	YES	YES
1 a-e	3.08	0.067	-0.082	0.216	YES	YES
2 a-e	4.82	0.067	-0.082	0.216	YES	YES
3 a-e	6.04	0.073	-0.076	0.221	YES	YES
5 a-e	6.49	-0.060	-0.209	0.089	YES	YES
6 a-e	7.38	-0.125	-0.274	0.024	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.103	YES	+/- 0.500

Figure 3i – Accuracy profile for Fresh produce and fruits 72h spread

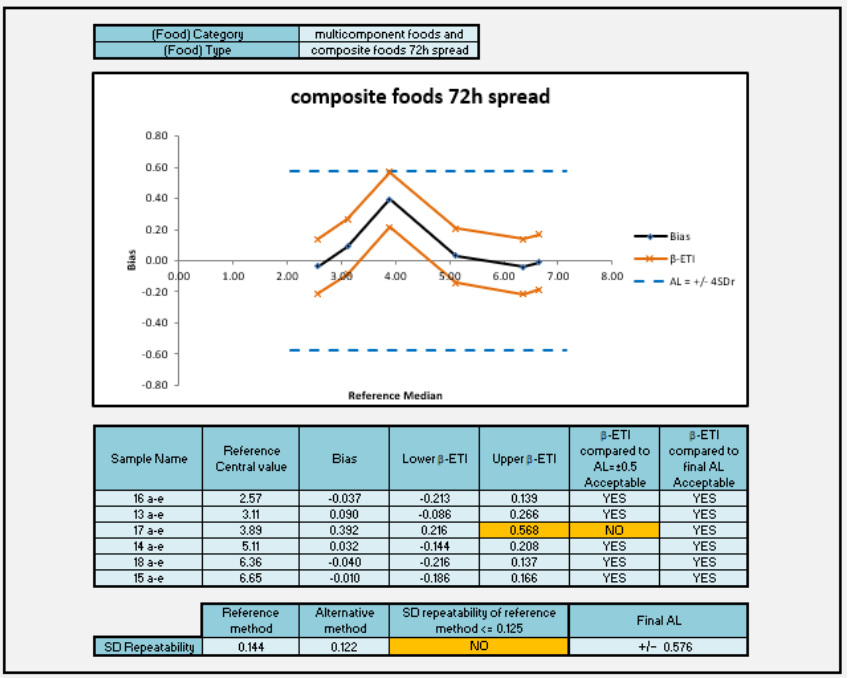


(Food) Category	Fruits and vegetables
(Food) Type	fresh produce 72h spread

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
10 a-e	2.10	0.049	-0.099	0.198	YES	YES
9 a-e	2.72	0.095	-0.053	0.244	YES	YES
7 a-e	4.66	0.085	-0.063	0.234	YES	YES
11 a-e	5.23	0.025	-0.124	0.174	YES	YES
8 a-e	6.49	-0.044	-0.193	0.104	YES	YES
12 a-e	8.40	0.064	-0.084	0.213	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.095	0.103	YES	+/- 0.500

Figure 3j – Accuracy profile for Multi component foods or meal components 72h spread

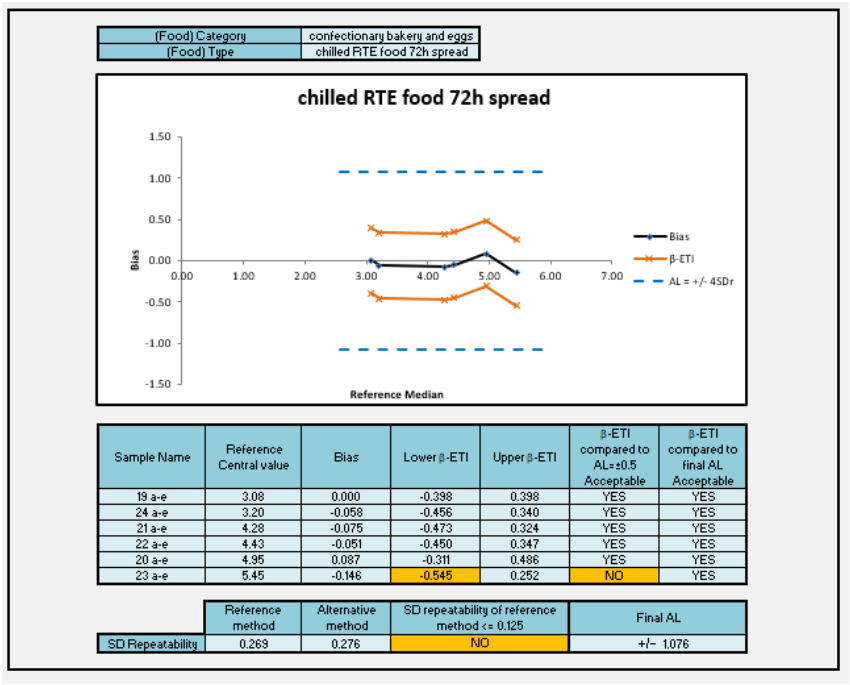


(Food) Category	multicomponent foods and
(Food) Type	composite foods 72h spread

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16 a-e	2.57	-0.037	-0.213	0.139	YES	YES
13 a-e	3.11	0.090	-0.086	0.266	YES	YES
17 a-e	3.89	0.392	0.216	0.568	NO	YES
14 a-e	5.11	0.032	-0.144	0.208	YES	YES
18 a-e	6.36	-0.040	-0.216	0.137	YES	YES
15 a-e	6.65	-0.010	-0.186	0.166	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.122	NO	+/- 0.576

Figure 3k – Accuracy profile for Confectionery bakery and eggs (combined category) *72h spread*

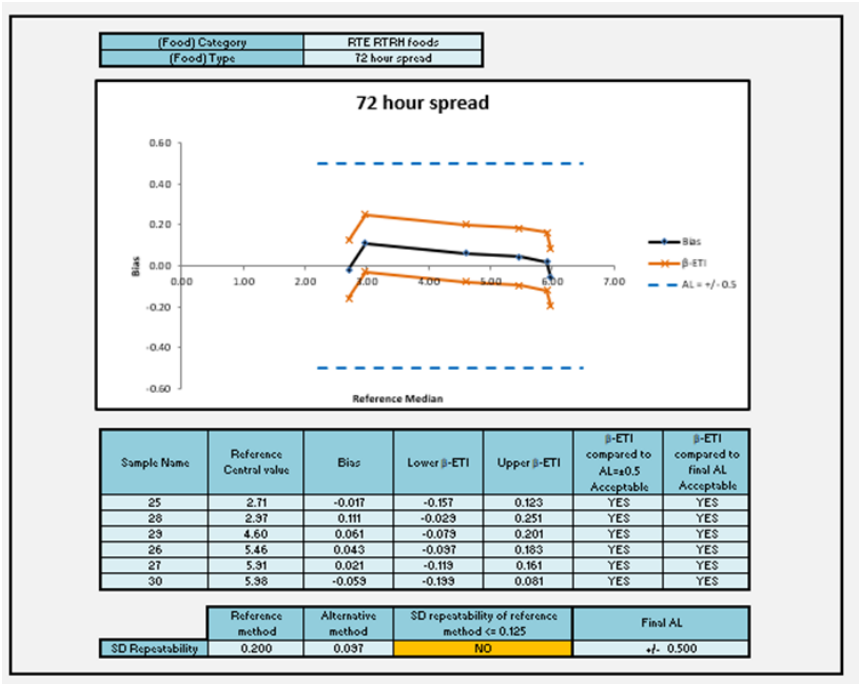


(Food) Category	confectionary bakery and eggs
(Food) Type	chilled RTE food 72h spread

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19 a-e	3.08	0.000	-0.398	0.398	YES	YES
24 a-e	3.20	-0.058	-0.456	0.340	YES	YES
21 a-e	4.28	-0.075	-0.473	0.324	YES	YES
22 a-e	4.43	-0.051	-0.450	0.347	YES	YES
20 a-e	4.95	0.087	-0.311	0.486	YES	YES
23 a-e	5.45	-0.146	-0.545	0.252	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.269	0.276	NO	+/- 1.076

Figure 3l – Accuracy profile for RTE/RTRH foods (combined category: meat, poultry and fish) 72h spread

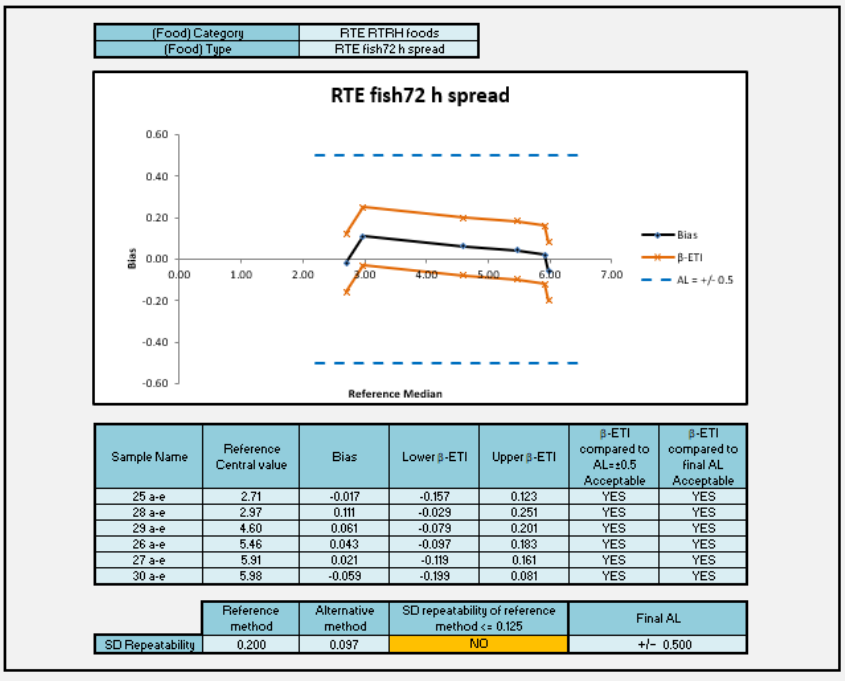


(Food) Category	RTE RTRH foods
(Food) Type	72 hour spread

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	2.71	-0.017	-0.157	0.123	YES	YES
28	2.97	0.111	-0.029	0.251	YES	YES
29	4.60	0.061	-0.079	0.201	YES	YES
26	5.46	0.043	-0.097	0.183	YES	YES
27	5.91	0.021	-0.119	0.161	YES	YES
30	5.98	-0.059	-0.199	0.081	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.200	0.097	NO	+/- 0.500

Figure 3m– Accuracy profile for Dried cereals, fruits, nuts, seeds and vegetables 72h spread

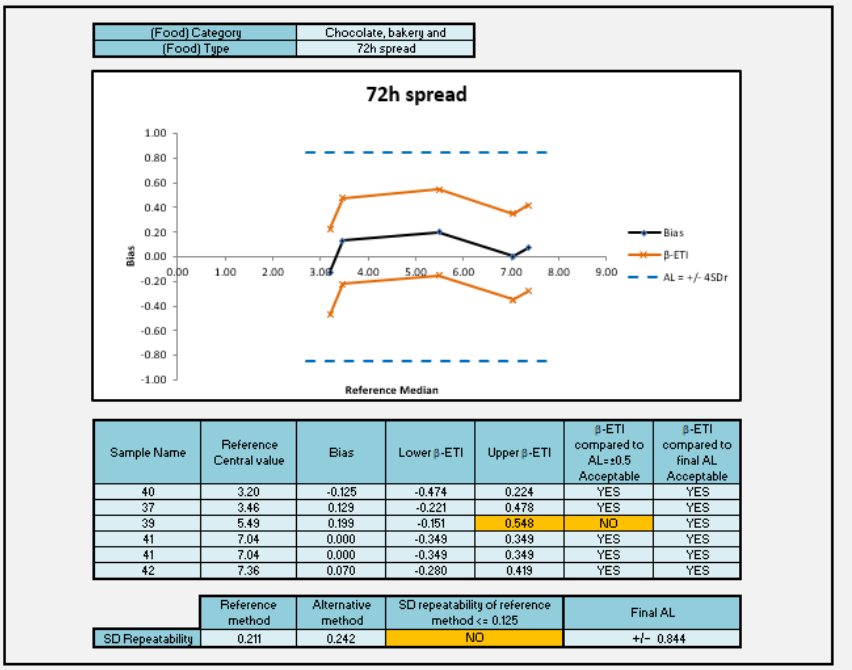


(Food) Category	RTE RTRH foods
(Food) Type	RTE fish72 h spread

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25 a-e	2.71	-0.017	-0.157	0.123	YES	YES
28 a-e	2.97	0.111	-0.029	0.251	YES	YES
29 a-e	4.60	0.061	-0.079	0.201	YES	YES
26 a-e	5.46	0.043	-0.097	0.183	YES	YES
27 a-e	5.91	0.021	-0.119	0.161	YES	YES
30 a-e	5.98	-0.059	-0.199	0.081	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.200	0.097	NO	+/- 0.500

Figure 3n– Accuracy profile for Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95) 72h spread



(Food) Category	Chocolate, bakery and
(Food) Type	72h spread

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
40	3.20	-0.125	-0.474	0.224	YES	YES
37	3.46	0.129	-0.221	0.478	YES	YES
39	5.49	0.199	-0.151	0.548	NO	YES
41	7.04	0.000	-0.349	0.349	YES	YES
41	7.04	0.000	-0.349	0.349	YES	YES
42	7.36	0.070	-0.280	0.419	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.211	0.242	NO	+/- 0.844

In this study the following categories met the AL of 0.5log

- Heat processed milk and dairy products
- Fruits and vegetables
- RTE/RTRH foods
- Dried cereals fruits nut seeds and vegetables

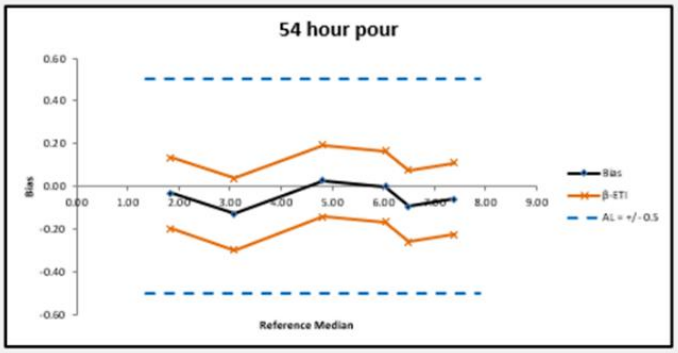
In this study, Multi component foods or meal components, chocolate and confectionary and confectionary, bakery and eggs required the new AL to be calculated, of ± 0.576, ± 0.844 and ±1.208 respectively.

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

OPYM 1ml pour 54h incubation

Figure 4a – Accuracy profile for Heat processed milk and dairy products 54h pour

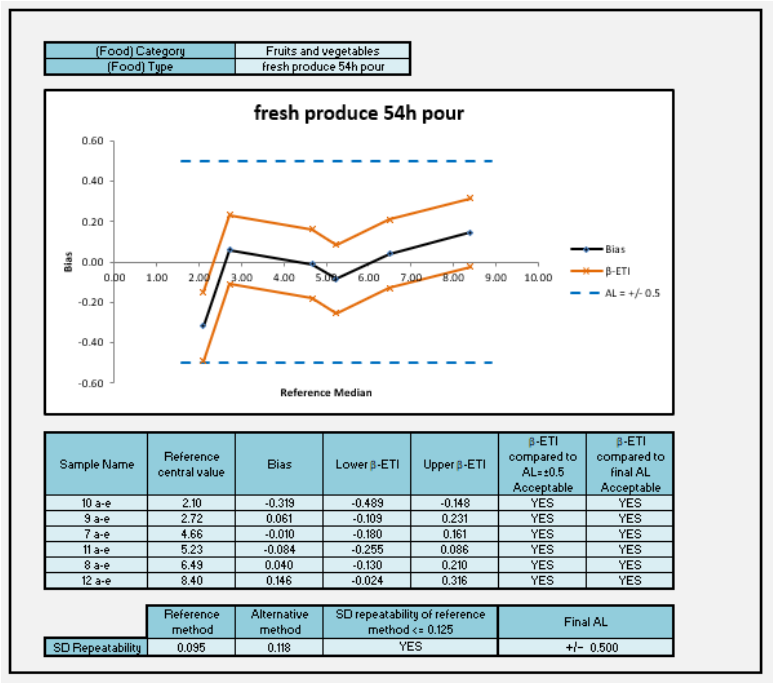
(Food) Category	Heat processed milk and dairy
(Food) Type	54 hour pour



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
4	1.85	-0.032	-0.200	0.135	YES	YES
1	3.08	-0.123	-0.237	0.038	YES	YES
2	4.82	0.026	-0.142	0.193	YES	YES
3	6.04	0.000	-0.167	0.167	YES	YES
5	6.43	-0.093	-0.261	0.074	YES	YES
6	7.38	-0.058	-0.225	0.109	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.116	YES	+/- 0.500

Figure 4b – Accuracy profile for Fruits and vegetables 54h pour

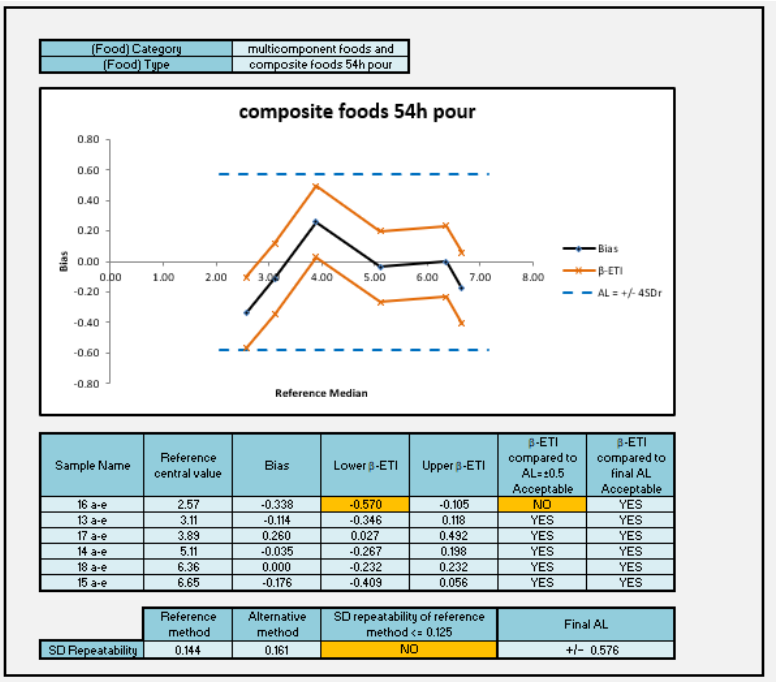


(Food) Category	Fruits and vegetables
(Food) Type	fresh produce 54h pour

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
10 a-e	2.10	-0.319	-0.489	-0.148	YES	YES
9 a-e	2.72	0.061	-0.109	0.231	YES	YES
7 a-e	4.66	-0.010	-0.180	0.161	YES	YES
11 a-e	5.23	-0.084	-0.255	0.086	YES	YES
8 a-e	6.49	0.040	-0.130	0.210	YES	YES
12 a-e	8.40	0.146	-0.024	0.316	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.095	0.118	YES	+/- 0.500

Figure 4c – Accuracy profile for Multi component foods or meal components 54h pour

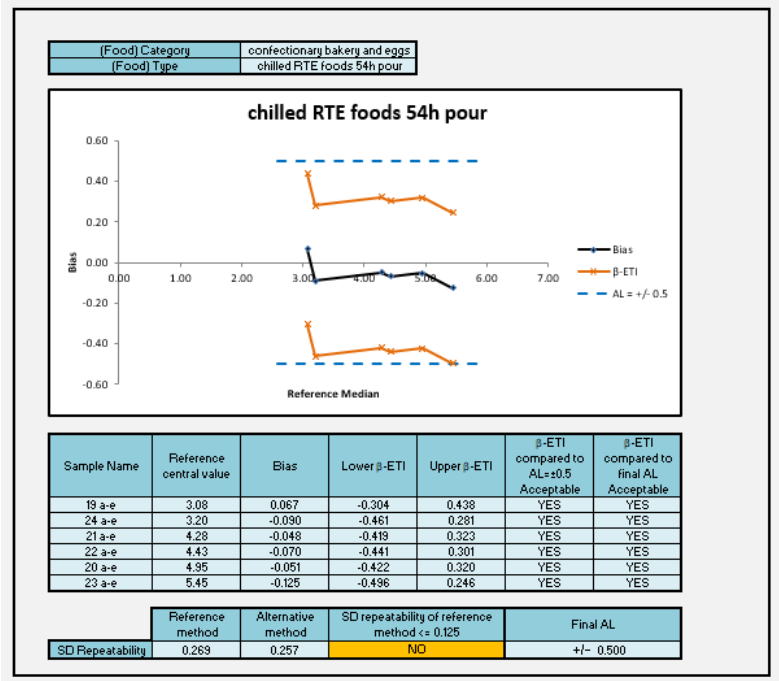


(Food) Category	multicomponent foods and
(Food) Type	composite foods 54h pour

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16 a-e	2.57	-0.338	-0.570	-0.105	NO	YES
13 a-e	3.11	-0.114	-0.346	0.118	YES	YES
17 a-e	3.89	0.260	0.027	0.492	YES	YES
14 a-e	5.11	-0.035	-0.267	0.198	YES	YES
18 a-e	6.36	0.000	-0.232	0.232	YES	YES
15 a-e	6.65	-0.176	-0.409	0.056	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.161	NO	+/- 0.576

Figure 4d – Accuracy profile for Confectionery bakery and eggs 54h pour

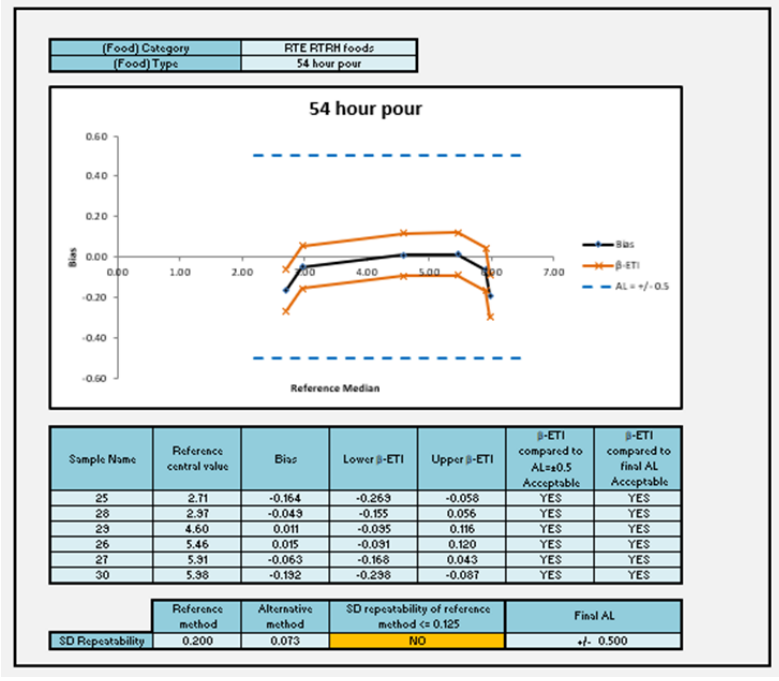


(Food) Category	confectionary bakery and eggs
(Food) Type	chilled RTE foods 54h pour

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19 a-e	3.08	0.067	-0.304	0.438	YES	YES
24 a-e	3.20	-0.090	-0.461	0.281	YES	YES
21 a-e	4.28	-0.048	-0.419	0.323	YES	YES
22 a-e	4.43	-0.070	-0.441	0.301	YES	YES
20 a-e	4.95	-0.051	-0.422	0.320	YES	YES
23 a-e	5.45	-0.125	-0.496	0.246	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.269	0.257	NO	+/- 0.500

Figure 4e – Accuracy profile for RTE/RTRH foods 54h pour

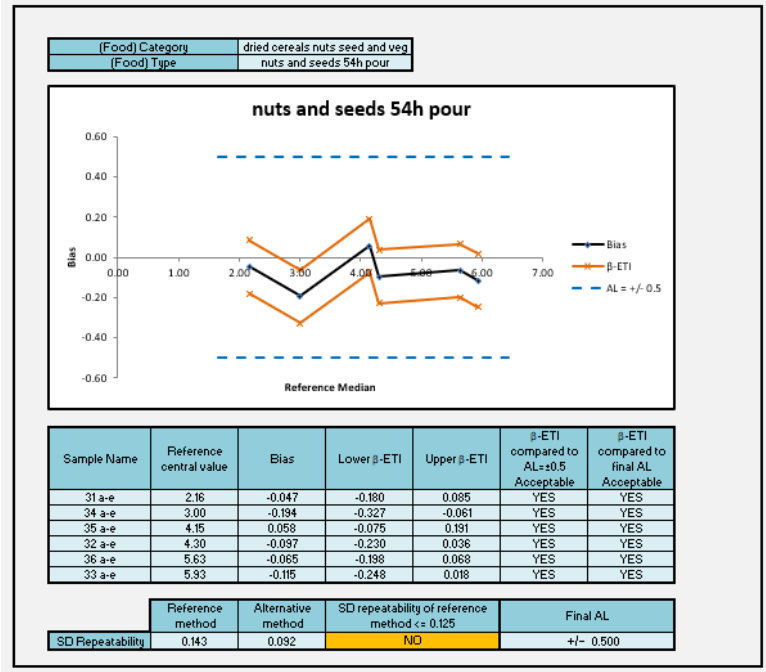


(Food) Category	RTE RTRH foods
(Food) Type	54 hour pour

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	2.71	-0.164	-0.269	-0.058	YES	YES
28	2.37	-0.043	-0.155	0.056	YES	YES
29	4.60	0.011	-0.095	0.116	YES	YES
26	5.46	0.015	-0.091	0.120	YES	YES
27	5.31	-0.063	-0.168	0.043	YES	YES
30	5.38	-0.132	-0.238	-0.087	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.200	0.073	NO	+/- 0.500

Figure 4f – Accuracy profile for Dried cereals fruits nut seeds and vegetables 54h pour

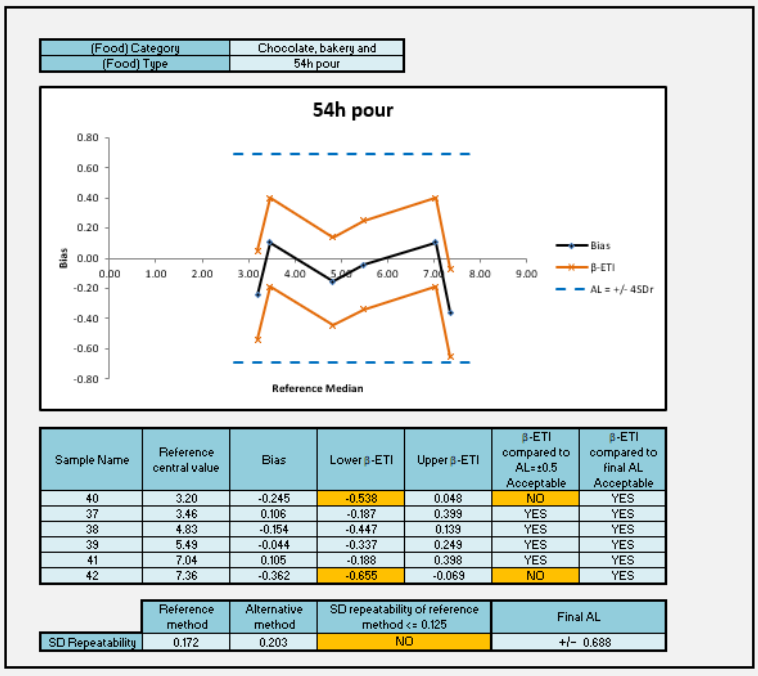


(Food) Category	dried cereals nuts seed and veg
(Food) Type	nuts and seeds 54h pour

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
31 a-e	2.16	-0.047	-0.180	0.085	YES	YES
34 a-e	3.00	-0.194	-0.327	-0.061	YES	YES
35 a-e	4.15	0.058	-0.075	0.191	YES	YES
32 a-e	4.30	-0.097	-0.230	0.036	YES	YES
36 a-e	5.63	-0.065	-0.198	0.068	YES	YES
33 a-e	5.93	-0.115	-0.248	0.018	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.143	0.092	NO	+/- 0.500

Figure 4g – Accuracy profile for chocolate and confectionary 54h pour



(Food) Category	Chocolate, bakery and
(Food) Type	54h pour

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
40	3.20	-0.245	-0.538	0.048	NO	YES
37	3.46	0.106	-0.187	0.399	YES	YES
38	4.83	-0.154	-0.447	0.139	YES	YES
39	5.49	-0.044	-0.337	0.249	YES	YES
41	7.04	0.105	-0.188	0.398	YES	YES
42	7.36	-0.362	-0.655	-0.069	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.172	0.203	NO	+/- 0.688

In this study the following categories met the AL of 0.5log

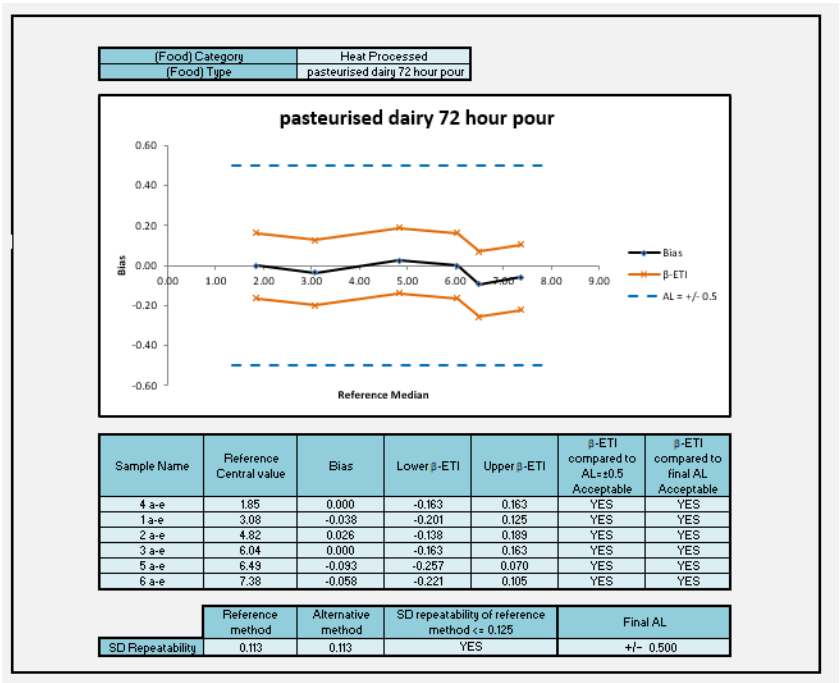
- Heat processed milk and dairy products
- Fruits and vegetables
- RTE/RTRH foods
- Dried cereals fruits nut seeds and vegetables

In this study, Multi component foods or meal components, chocolate and confectionary and confectionary, bakery and eggs required the new AL to be calculated, of ± 0.576, ± 0.688 and ±1.208 respectively.

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

OPYM 1ml pour 72h incubation

Figure 4h – Accuracy profile for Heat processed milk and dairy products 72h pour

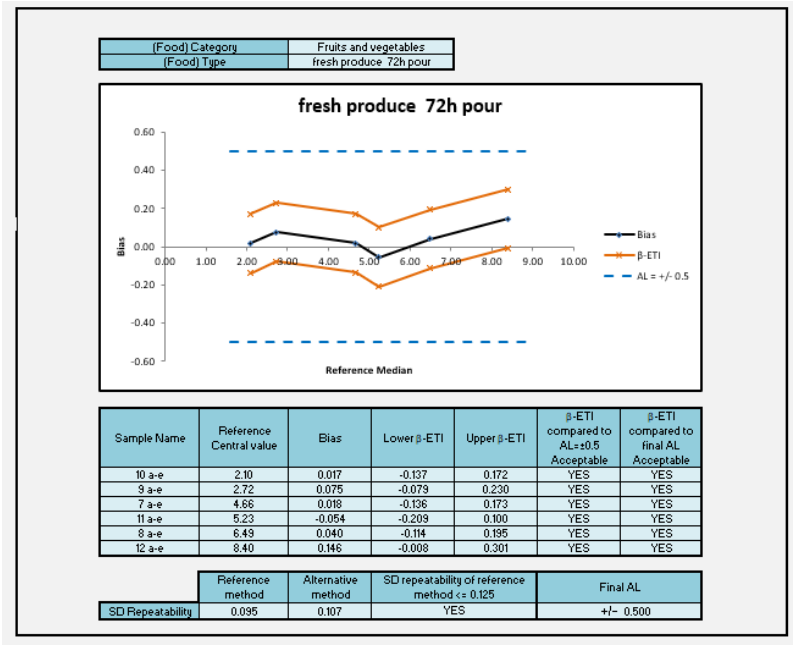


(Food) Category	Heat Processed
(Food) Type	pasteurised dairy 72 hour pour

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
4 a-e	1.85	0.000	-0.163	0.163	YES	YES
1 a-e	3.08	-0.038	-0.201	0.125	YES	YES
2 a-e	4.82	0.026	-0.138	0.189	YES	YES
3 a-e	6.04	0.000	-0.163	0.163	YES	YES
5 a-e	6.49	-0.093	-0.257	0.070	YES	YES
6 a-e	7.38	-0.058	-0.221	0.105	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.113	YES	+/- 0.500

Figure 4i – Accuracy profile for Fresh produce and fruits 72h pour

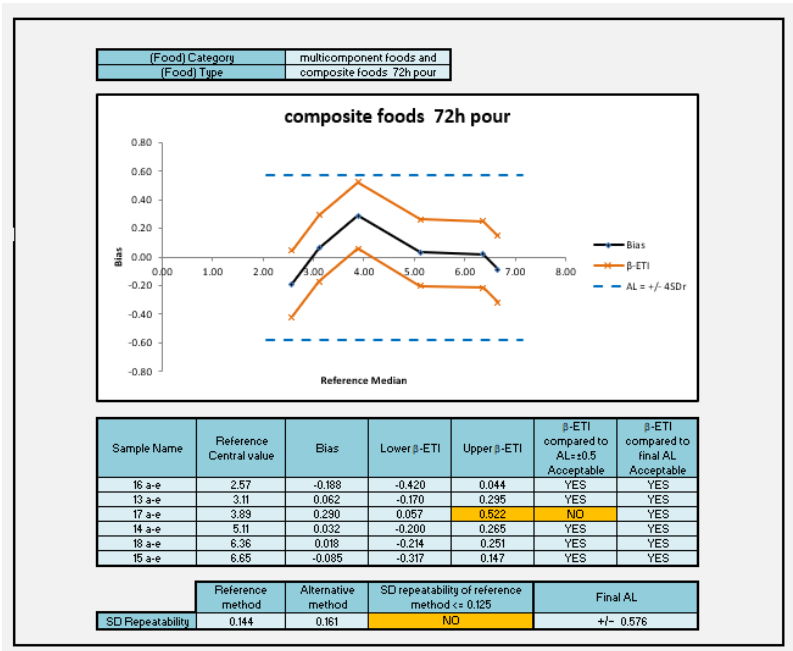


(Food) Category	Fruits and vegetables
(Food) Type	fresh produce 72h pour

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
10 a-e	2.10	0.017	-0.137	0.172	YES	YES
9 a-e	2.72	0.075	-0.079	0.230	YES	YES
7 a-e	4.66	0.018	-0.136	0.173	YES	YES
11 a-e	5.23	-0.054	-0.209	0.100	YES	YES
8 a-e	6.49	0.040	-0.114	0.195	YES	YES
12 a-e	8.40	0.146	-0.008	0.301	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.095	0.107	YES	+/- 0.500

Figure 4j – Accuracy profile for Multi component foods or meal components 72h pour



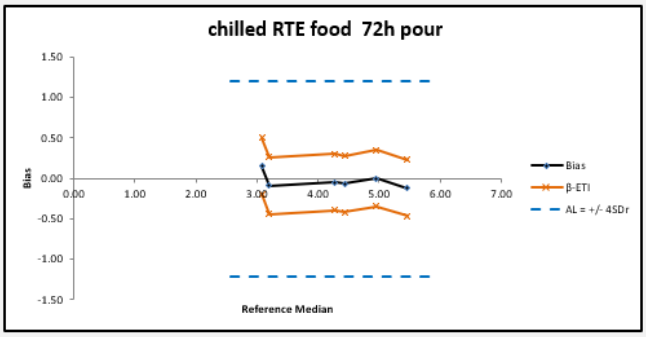
(Food) Category	multicomponent foods and
(Food) Type	composite foods 72h pour

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16 a-e	2.57	-0.188	-0.420	0.044	YES	YES
13 a-e	3.11	0.062	-0.170	0.295	YES	YES
17 a-e	3.89	0.290	0.057	0.522	NO	YES
14 a-e	5.11	0.032	-0.200	0.265	YES	YES
18 a-e	6.36	0.018	-0.214	0.251	YES	YES
15 a-e	6.65	-0.085	-0.317	0.147	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.144	0.161	NO	+/- 0.576

Figure 4k – Accuracy profile for Confectionery bakery and eggs (combined category) *72h pour*

(Food) Category	confectionary bakery and eggs
(Food) Type	chilled RTE food 72h pour

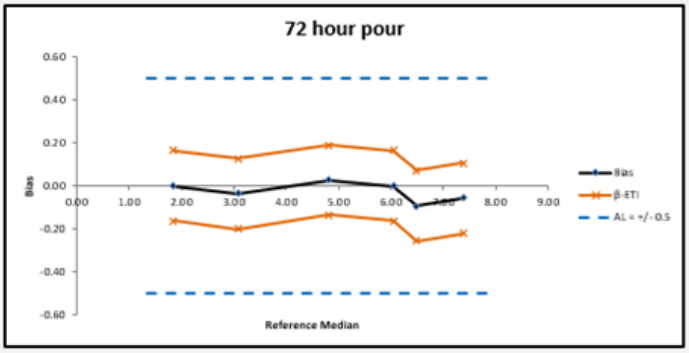


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19 a-e	3.08	0.151	-0.198	0.501	NO	YES
24 a-e	3.20	-0.090	-0.440	0.259	YES	YES
21 a-e	4.28	-0.048	-0.398	0.301	YES	YES
22 a-e	4.43	-0.070	-0.419	0.280	YES	YES
20 a-e	4.95	0.000	-0.349	0.349	YES	YES
23 a-e	5.46	-0.120	-0.469	0.229	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.302	0.242	NO	+/- 1.208

Figure 4l – Accuracy profile for RTE/RTRH foods (combined category: meat, poultry and fishery products) 72h pour

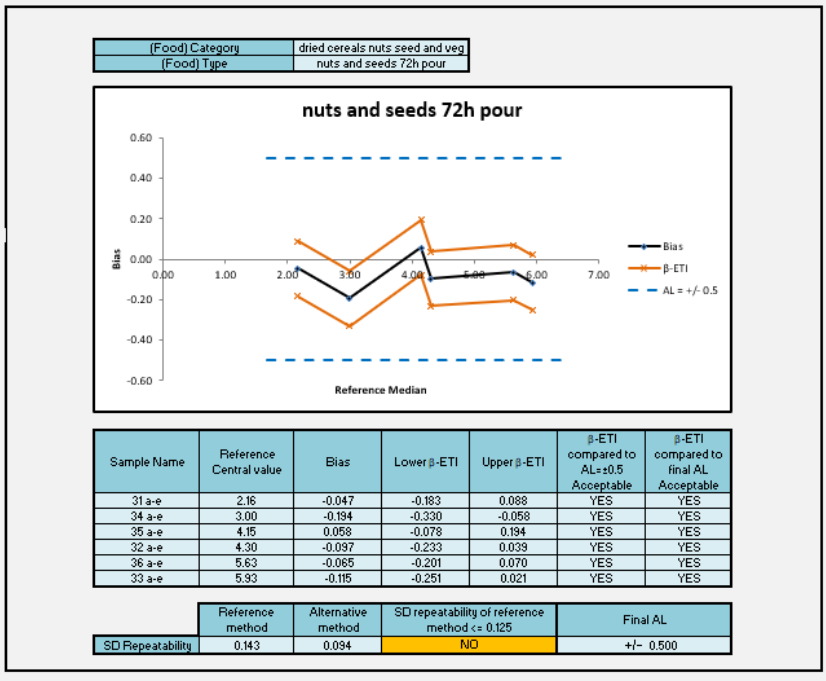
(Food) Category	Heat Processed
(Food) Type	72 hour pour



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
4	1.85	0.000	-0.163	0.163	YES	YES
1	3.08	-0.038	-0.201	0.125	YES	YES
2	4.82	0.026	-0.138	0.189	YES	YES
3	6.04	0.000	-0.163	0.163	YES	YES
5	6.49	-0.093	-0.257	0.070	YES	YES
6	7.38	-0.058	-0.221	0.105	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.113	YES	+/- 0.500

Figure 4m – Accuracy profile for Dried cereals, fruits, nuts seeds and vegetables 72h pour

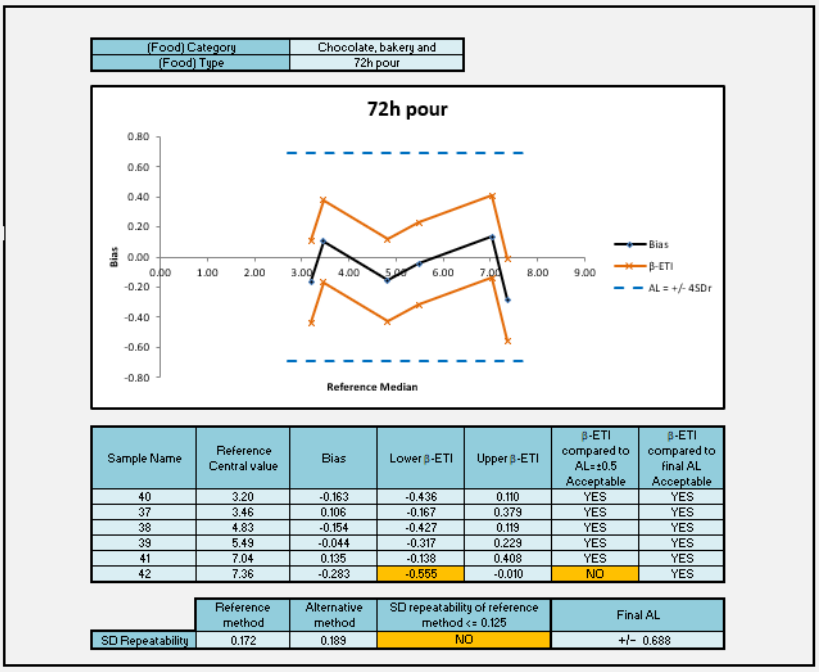


(Food) Category	dried cereals nuts seed and veg
(Food) Type	nuts and seeds 72h pour

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
31 a-e	2.16	-0.047	-0.183	0.088	YES	YES
34 a-e	3.00	-0.194	-0.330	-0.058	YES	YES
35 a-e	4.15	0.058	-0.078	0.194	YES	YES
32 a-e	4.30	-0.097	-0.233	0.039	YES	YES
36 a-e	5.63	-0.065	-0.201	0.070	YES	YES
33 a-e	5.93	-0.115	-0.251	0.021	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.143	0.094	NO	+/- 0.500

Figure 4n – Accuracy profile for chocolate and confectionary 72h pour



(Food) Category	Chocolate, bakery and
(Food) Type	72h pour

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
40	3.20	-0.163	-0.436	0.110	YES	YES
37	3.46	0.106	-0.167	0.379	YES	YES
38	4.83	-0.154	-0.427	0.119	YES	YES
39	5.49	-0.044	-0.317	0.229	YES	YES
41	7.04	0.135	-0.138	0.408	YES	YES
42	7.36	-0.283	-0.555	-0.010	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.172	0.189	NO	+/- 0.688

In this study the following categories met the AL of 0.5log

- Heat processed milk and dairy products
- Fresh produce and fruits
- RTE/RTRH foods (combined category: meat, poultry fish)
- Dried cereals, fruits, nuts, seeds and vegetables

In this study, Multi component foods or meal components, Chocolate, bakery products and confectionary (low moisture aw ≤ 0.95) and confectionary, bakery and eggs (combined category) required the new AL to be calculated, of ± 0.576, ± 0.688 and ±1.208 respectively.

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 pure cultures of yeasts and moulds were tested in the inclusivity study. For this study, 30 yeast isolates and 20 mould isolates were used. Each test was performed once with the alternative method (incubated for 54h and 72h), the reference method and a non-selective agar. The performance of the 2 plating volumes available for the alternative method 0.1ml spread and 1ml pour were assessed in the inclusivity study.

Each strain used was grown overnight in a non-selective broth and diluted so that the inoculum level is at least 100 times greater than the minimum level for quantification of the alternative method being validated. When using a plate method as the alternative method, the inoculum level shall obtain a countable number on the plate.

In this study 2 reference methods were available; ISO 21527:2008 parts 1 and 2. The 50 isolates were plated onto the most appropriate reference method, depending on their growth preferences. All osmophilic yeasts and moulds highlighted in bold were plated onto DG18 agar (ISO 21527:2008 part 2). The isolates expected to be present in foods with Aw >0.95 were plated onto DRBCA (ISO 21527:2008 part 1).

Exclusivity

30 pure cultures of (non-target) microorganisms tested in the exclusivity study. Each test was performed once with the alternative method (incubated for 54h and 72h), the reference method and a non-selective agar. The exclusivity panel was plated out onto both reference media DRBCA and DG18 agar.

Each strain was grown overnight in a non-selective broth and diluted to a level similar to the greatest level of contamination expected to occur in any of the categories being used.

3.3.2 Results

The summary of the raw data for inclusivity is shown in Annex F.

Inclusivity

A total of 50 strains were tested for inclusivity. All 50 isolates showed a positive result with both incubation times (54h and 72) and plating volumes (0.1ml spread and 1ml pour) on the alternative method. The results for the alternative method were in agreement with the results obtained for the reference method, all 50 isolates tested gave the anticipated result.

Exclusivity

A total of 30 isolates were tested for exclusivity. 28 of these strains showed a negative result on the reference and/or alternative methods. Of the remaining 2 isolates, 1 strain showed a positive result on the reference and alternative methods: *Pseudomonas fluorescens* (CRA 7504).

Burkholderia cepacia (CRA 16982) was positive on DRBCA only, but negative on OPYM.

Further analysis was carried out on the 2 isolates giving unexpected results. MALDI analysis was performed using the Bruker MALDI-TOF system on the isolates and the results are shown in the table below.

Strain	**CRA Code**	**Growth on agar**	**MALDI Score**	**MALDI interpretation**
Pseudomonas fluorescens	7504	OPYM spread, OPYM pour, DRBCA, DG18	1.89	Low confidence identification
Burkholderia cepacia	16982	DRBCA	2.37	High confidence identification

The identification of the strains agreed with the assumed identification.

3.3.3 Conclusion
The alternative One Plate Yeast and Mould detection method is selective and specific using both plating volumes (0.1ml spread and 1ml pour) at the two incubation times (54h and 72h).

3.4 Limit of quantification (LOQ)

The LOQ applies only to instrumental methods. It does not apply to methods based on counting visible colonies. It may also not apply to instrumental methods where it is not possible to get blank samples e.g. instrumental methods for total plate counts.

The alternate method is based on visible colonies therefore the LOQ was not calculated for the alternative method in this study.

3.4.1 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The alternative method One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for relative trueness;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for accuracy profile;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould is selective and specific.

4 Interlaboratory study

The aim of the interlaboratory study is to compare the performance of the alternative method to the reference method by different collaborators using identical samples.

4.1 Introduction

An interlaboratory study was performed on two occasions: October 2023 and January 2024.

The initial ILS was performed in October 2023, and experienced issues with stability of both yeast and mould strains. A root cause analysis to investigate the issues experienced in the interlaboratory study performed in October 2023 is detailed in Section 4.3.1.

During the second ILS performed in January 2024, an issue was experienced with the low level samples. One set of low level samples (sample code S5) achieved the expected level of 10^2 cfu/g and the other duplicate low level sample (sample code S1) recieved low or no counts. ISO 16140-2 requires that there are 3 inoculation levels tested in duplicate. Due to issues observed in one sample of the low level results, the January 2024 data does not meet the ISO 16140-2 requirements.

As both interlaboratory studies were carried out using the same food matrix, a proposal to use a combined datset consisting of the low level samples produced in the October 2023 ILS and medium and high level samples produced in the January 2024 ILS, was presented to the committee in March 2024. Details of the proposal are outlined in section 4.5 for reference. After reviewing the proposal, the MVTC agreed to accept this dataset as meeting the ISO 16140-2 requirements.

This interlaboratory study report details the following:

- October 2023 root cause analysis
- January 2024 results and root cause analysis
- Rationale for combining two datasets
- Selection of data in the merged dataset
- Analysis of the merged dataset
- Conclusions of the interlaboratory study

4.2 Interlaboratory study – October 2023

4.2.1 Stability

In advance of the interlaboratory study, samples were inoculated and stored for 5 and 7 days to test for the stability of the strain in the matrix.

The matrix for the interlaboratory study was pasta salad, and the inoculating strain was *Candida sojae* CRA 16138 isolated from soft drinks and *Penicillum rubens* DSM 848.

In order to screen for natural contamination of yeasts and moulds in the matrix, the reference method was performed on five portions (10g) before the inoculation. All the results were negative.

Duplicate samples inoculated at four levels (10^2, 10^3, 10^5, 10^6 cfu/g) were tested for the enumeration of yeast and moulds after 5 and 7 days of storage at 2-8°C. Table 12 shows the data obtained.

Table 12. Yeast and mould stability in the matrix

Day	Storage conditions	Alternative method (log cfu/g) – 54-hour count								Reference method (log cfu/g)							
		Level 1		Level 2		Level 3		Level 4		Level 1		Level 2		Level 3		Level 4	
		a	B	a	B	a	b	A	b	a	b	a	b	a	b	a	b
Day 0	N/A	2.8	2.9	3.7	3.7	5.4	5.3	6.4	6.4	2.7	2.9	3.8	3.8	5.5	5.5	6.4	6.5
Day 5	Storage at 2-8°C	2.8	2.7	3.8	3.6	5.8	5.7	7.3	7.1	2.5	2.7	3.9	3.5	5.9	5.6	7.1	7.2
Day 7		2.7	2.7	3.4	3.3	5.8	5.7	7.0	6.5	2.7	2.7	3.4	3.4	5.8	5.7	7.1	7.1

The data shows good stability under the storage regime tested.

4.2.2 Interlaboratory Study Design

Pasta salad samples were co-inoculated with *Candida sojae* CRA 16138 isolated from soft drinks and *Penicillum rubens* DSM 848. 7 samples were tested and inoculated according to Table 13.

Table 13. Inoculation levels for sample

Sample code	Level	Contamination level (cfu/g)
S1	Low	10^2
S2	Medium	10^4
S3	High	10^6
S4	Blank	-
S5	Low	10^2
S6	Medium	10^4
S7	High	10^6

Participants were using the pour plate format of One Plate Yeast and Mould agar.

Samples were sent to 20 labs.

Collaborative study laboratories and the expert laboratory carried out the analyses on 9th October 2023.

4.2.3 Root cause analysis – October 2023

The interlaboratory study carried out in October 2023 experienced two issues:

- Very low or no mould counts in all samples
- High levels of variation of yeast levels both between labs and between replicate samples

Low mould counts

Very low mould counts were observed in all samples and in all laboratories. There are two potential reasons for this, shown in Table 14.

Table 14. Root cause analysis for low levels of mould observed in October 2023 ILS

Identification number	**Potential cause for low mould counts**	**Rationale**
1	Instability of the mould in the sample	Stability results do not support this conclusion.

2	Inoculation of the mould in the sample	There are several options for this: • Level of the organism inoculated • Viability of the organism • Preparation of the inoculum (use of spores etc)

A review of the possible reasons for low mould counts suggests that are most likely to be due to the inoculation of mould into the sample. The following procedure was used to prepare the mould for inoculation:

Mould was grown on an MEA plate for 10 days at 25°C. A mould colony was then added to MEB and incubated for 5 days at 25°C. This was then vortexed and plated onto MEA and incubated for 5 days at 25°C to assess the levels. The MEB was stored chilled for 5 days until inoculated into the sample.

The procedure used to prepare the mould isolate for inoculation has several issues:

- Spores were not used – which are known to be more stable in a food matrix
- Mould was stored in the MEB at chilled for 5 days after growth – which could affect the level of mould inoculated into the samples.
- Vortexing to mix the mould inoculum could cause damage

It is unclear which of these issues were the root cause of the low mould levels, however, using mould spores or a different method of mould preparation could potentially eliminate these issues.

Large variations in yeast counts

There were large variations in yeast counts observed between replicates at the same level of contamination in the October 2023 interlaboratory study.

There are several potential causes for the large variation observed in yeast counts, these are shown in Table 15.

Table 15. *Root cause analysis for October 2023 ILS*

Identification number	**Potential cause for variation in yeast counts**	**Rationale**
1	Natural contamination of the matrix that is variable between samples	Samples were taken from different batches of pasta salad; some may have more natural contamination than others.

Identification number	Potential cause for variation in yeast counts	Rationale
2	Instability of yeast in the matrix	Stability results do not show die-off over storage time. Many counts are higher than inoculated level.
3	Growth of yeast in the matrix	Stability results do not show growth over the storage time. Many counts are higher than inoculated level, although this is not consistent.
4	Interaction of yeast and mould due to co-inoculation	Stability results do not support this conclusion. Previous interlaboratory studies have been carried out by the expert lab using similar strains with no issue.
5	Yeast is stressed in the matrix due to transport conditions	Samples were stored for 7 days before testing. Potentially an increased risk with stress for internationally shipped parcels due to equipment used in security checks.
6	Issues with sample inoculation	Potential differences in the inoculation of samples (e.g. preparation of inoculum, time between inoculation of samples causing the level of yeast in the broth to increase or decrease before inoculating into the sample).

The conclusion of the root cause analysis is that the large variation between duplicate samples is more likely to be due to either (option 1) natural contamination of the matrix that is variable between samples or (option 6) issues with sample inoculation.

The impact of option 1 or option 6 on the validity of the low-level results is minimal as labs with large variation between samples have been excluded from the data analysis in the combined dataset.

4.3 Interlaboratory study - January 2024

4.3.1 Stability study

In advance of the interlaboratory study, samples were inoculated and stored for 7 days to test for the stability of the strain in the matrix.

The matrix for the interlaboratory study was pasta salad, and the inoculating strain was *Candida sojae* CRA 16138 isolated from soft drinks.

In order to screen for natural contamination of yeasts and moulds in the matrix, the reference method was performed on five portions (10g) before the inoculation. All the results were negative.

Duplicate samples inoculated at four levels (10^2, 10^3, 10^5, 10^6 cfu/g) were tested for the enumeration of yeast after 7 days of storage at ≤-18°C. Frozen samples were thawed under controlled conditions prior to analysis. Table 16 shows the data obtained.

Table 16. Yeast stability in the matrix

Day	Storage conditions	Alternative method (log cfu/g) – 54-hour count								Reference method (log cfu/g)							
		Level 1		Level 2		Level 3		Level 4		Level 1		Level 2		Level 3		Level 4	
		a	b	a	b	a	b	A	b	A	b	a	b	A	b	a	b
Day 0	N/A	2.5	2.7	3.6	3.4	5.4	5.3	6.4	6.4	2.6	2.7	3.6	3.5	5.5	5.5	6.4	6.5
Day 7	Storage at ≤-18°C	2.8	2.8	3.4	3.3	5.4	5.4	6.5	6.3	2.7	2.7	3.4	3.4	5.5	5.4	6.4	6.5

The data shows good stability under the storage regime tested.

4.3.2 Interlaboratory study design

Pasta salad samples were inoculated with *Candida sojae* CRA 16138, isolated from soft drinks. 7 samples were tested and inoculated according to Table 17.

Table 17. Inoculation levels for sample

Sample code	Level	Contamination level (cfu/g)
S1	Low	10^2
S2	Medium	10^4
S3	High	10^6

Sample code	Level	Contamination level (cfu/g)
S4	Blank	-
S5	Low	10^2
S6	Medium	10^4
S7	High	10^6

Participants were using the spread plate format of One Plate Yeast and Mould agar.

Samples were sent to 18 labs. Data has been included from 13 labs, due to issues with shipment and temperature in the remaining 5 labs.

Collaborative study laboratories and the expert laboratory carried out the analyses on 15th January.

4.3.3 Calculation and summary of data
A summary of the data obtained by the collaborative laboratories is shown in Table 18.

Table 18. Summary of the results of the interlaboratory study per analyte level

Collaborator	**Level**	**Reference method (Log cfu/g)**		**Alternative method (Log cfu/g) – 54h incubation**		**Alternative method (Log cfu/g) – 72h incubation**	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
1	Low	1.3*	2.5	<1	2.3	<1	2.3
2	Low	2.7	2.8	2.9	2.7	2.9	2.7
3	Low	1.6	2.5	1.8	2.4	1.8	2.4
4	Low	<1	2.2	<1	2.3	<1	2.3
5	Low	<1	2.4	<1	2.3	<1	2.3
6	Low	<1	2.4	<1	2.3	<1	2.3
7	Low	<1	2.4	<1	2.5	<1	2.5
8	Low	<1	2.3	<1	2.3	<1	2.3
9	Low	<1	2.5	<1	2.3	<1	2.5
14	Low	<1	2.6	<1	2.6	<1	2.7
16	Low	<1	2.6	<1	2.7	2.7	2.7
17	Low	1.3*	2.4	<1	2.5	<1	2.7
18	Low	<1	2.5	<1	2.4	<1	2.6
1	Med	4.4	4.3	4.3	4.3	<1	2.4
2	Med	4.9	4.9	4.9	4.9	4.9	4.9
3	Med	4.4	4.6	4.5	4.7	4.5	4.8
4	Med	4.6	4.5	4.6	4.2	4.6	4.4
5	Med	4.3	4.4	3.9	4.4	4.0	4.4

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 54h incubation		Alternative method (Log cfu/g) – 72h incubation	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
6	Med	4.7	4.1	4.7	4.0	4.7	4.0
7	Med	4.4	4.4	4.6	4.5	4.6	4.5
8	Med	4.5	4.4	4.5	4.4	4.5	4.4
9	Med	4.6	4.6	4.5	4.6	4.6	4.5
14	Med	4.7	4.5	4.4	4.5	4.4	4.6
16	Med	4.8	4.8	4.7	5.0	4.7	5.0
17	Med	4.7	4.5	4.5	4.5	4.5	4.5
18	Med	4.5	4.5	4.4	4.2	4.5	4.2
1	High	6.4	6.2	6.4	6.1	6.4	6.1
2	High	7.0	6.9	7.1	6.9	7.1	6.9
3	High	6.6	6.5	6.4	6.5	6.4	6.5
4	High	6.6	6.2	6.6	6.2	6.6	6.2
5	High	6.4	6.6	6.4	6.5	6.4	6.6
6	High	6.4	6.6	6.5	6.4	6.5	6.4
7	High	6.8	6.7	6.7	6.5	6.7	6.5
8	High	6.5	6.5	6.5	6.4	6.5	6.4
9	High	6.4	6.5	6.4	6.0	6.5	6.5
14	High	6.9	6.8	6.8	6.8	6.8	6.8
15	High	6.8	6.8	6.8	6.8	6.9	6.8
16	High	6.9	6.9	6.8	6.8	6.8	6.8
17	High	6.6	6.7	6.6	6.6	6.7	6.5
18	High	6.5	6.5	6.5	6.3	5.5	6.3
1	Blank	<1		<1		<1	
2	Blank	<1		<1		<1	
3	Blank	<1		<1		<1	
4	Blank	<1		<1		<1	
5	Blank	<1		<1		<1	
6	Blank	<1		<1		<1	
7	Blank	<1		<1		<1	
8	Blank	<1		<1		<1	
9	Blank	<1		<1		<1	
14	Blank	<1		<1		<1	
16	Blank	<1		<1		<1	
17	Blank	<1		<1		<1	
18	Blank	<1		<1		<1	

*Estimated, less than 4 colonies per plate

During the ILS performed in January 2024, an issue was experienced with the low-level samples. One set of low-level samples (Sample S5) achieved the expected level of 10^2 cfu/g and the other duplicate low-level

sample (S1) received low or no counts. ISO 16140-2 requires that there are 3 inoculation levels tested in duplicate, due to issues observed in one sample of the low-level results, the January 2024 data alone does not meet the ISO 16140-2 requirements. A root cause analysis was performed.

4.3.4 Root cause analysis

The low level samples were inoculated at 600 cfu/g and a single inoculum was prepared to inoculate the duplicate samples.

There are several potential explanations for the low or no counts observed in sample S1, these are shown in Table 19, alongside the rationale for each reason.

Table 19. Root cause analysis for January 2024 ILS

Identification number	**Potential cause for low counts in low-level sample**	**Rationale**
1	Die-off of yeast in the samples over the storage period	Stability results do not support this conclusion and very little die-off was seen in the other inoculation levels. Due to the inoculation level of 600 cfu/g, to achieve 0 counts, a die-off of 1.8 logs would be necessary. This is a significant die-off, which is unlikely.
2	Not all the samples were inoculated	S1 was inoculated last of the samples inoculated.
3	The inoculum was not thoroughly mixed	Laboratory records show that S5 was inoculated before S1. If the inoculum was not thoroughly mixed, then proportionally more yeast could have been inoculated into S5 samples. This option is possible; however, all samples were inoculated using the same method. It would therefore be expected that there would be larger differences between replicate 1 and replicate 2 of the other levels tested.
4	Inoculum	Laboratory records show that S5 was inoculated before S1.
5	Issues in plating samples/reading results	Incubation time and temperature were correct. There were no issues with other samples plated. Many of the labs have accreditation in the methods tested. Results obtained from the participants were consistent with Expert Lab data.

Results of the root cause analysis indicate that the low or no counts observed in sample S1 were potentially caused by an issue with inoculation of the sample. The most likely cause was either option 2: some samples were not inoculated or option 4: inoculum die-off between preparation and inoculation.

The impact of option 2 or option 4 on the validity of the medium and high-level results is minimal as the variation between replicates at the medium and high level was within the expectation for an interlaboratory study. The results show that the issues with inoculation are experienced in the low-level samples only.

Initial analysis of the medium and high-level samples from the January 2024 study showed that the results met ISO 16140-2 interlaboratory study criteria. Although the low level sample S5 shows good agreement between methods the single sample at this level of contamination did not meet the requirements of ISO 16140-2.

4.4 Combination of interlaboratory datasets

An investigation was performed to determine if the results obtained from both interlaboratory studies could be combined to generate the full data set required to meet the ISO 16140-2 criteria for ILS studies. The investigation covered study organisation, sample preparation and analysis as well as data analysis of the results were collated for the two studies.

Both interlaboratory studies were carried out using the same food matrix, Tomato and Basil pasta salad purchased from the same supplier. To assess the potential for data from the two studies to be combined, a comparison of the parameters used for each of the studies was carried out. A summary of the study details for the two interlaboratory studies are listed in Table 20 below.

Table 20. Details of the two interlaboratory studies performed

Set up date for pariticpants	Samples proposed for combination	Laboratories involved	Laboratories used for calculations	Countries involved (countries in bold were used for the analysis)	Alternative method protocol used	Inoculation	Storage of samples before testing (and on arrival)
16th October 2023	Low level	20	10	Ireland, **England, Italy**, **Poland, France, Spain,** Denmark	1ml pour plate	Co-inoculation *Candida sojae* CRA 16138 and *Penicillum rubens* DSM 848 Only yeast data was used due to instability of mould	2-8°C
15th January 2024	Medium and High level	18	12	**Ireland, England**, Italy, Poland, **Slovakia, Austria, France**	0.1ml spread plate	*Candida sojae* CRA 16138	≤-10°C

In addtion to the same matrix, the yeast isolate used for sample inoculation was kept constant for the two studies. Some changes in study design were made to the January 2024 ILS and these were approved by the MVTC in October 2023. The changes were:

- Plating format of the samples from 1ml pour plate to 0.1ml spread plate
- Use of *Candida sojae* only for sample inoculation, rather than co-inoculation with the mould isolate *Penicillum rubens* DSM 848.

In addition, the samples analysed in the study carried out in January were stored frozen before shipping and frozen at the sites.

Fourteen out of the twenty-four laboratories took part in both studies, which ensured further consistency between the analysis carried out in the two ILS. Furthermore, data from 6 labs were considered acceptable for inclusion in both studies.

Initial analysis of the data from the January 2024 study showed that the medium and high levels met the requirements for the ILS. The medium and high levels in the January 2024 study were at 10^4 and 10^6 cfu per g respectively. To ensure that the full dataset covered a minimum 3 log span required for ISO 16140 studies, samples at a level of 10^3 cfu per g or less were needed. Further analysis was carried out on the suitability of the data generated from the October 2023 study for inclusion in the analysis. Due to the die off experienced in the samples, the medium levels from the October 2023 study were at 10^3 cfu per g. Defined criteria were applied to select the participants included in the combined dataset for each level and these are detailed in section 4.6 below.

The investigation concluded that the impact of the differences in interlaboratory studies is minimal and that data from both studies to be combined to create a single dataset for analysis. After review, the MVTC in March 2024 approved the proposal to combine defined parts of the dataset from the October 2023 and January 2024.

4.5 Selection of participant data for the merged dataset

The data taken forward for analysis from both studies was selected using defined criteria to ensure that the results were chosen following an unbiased approach.

4.5.1 Selection of labs from January 2024

The data for the medium and high-level samples in the combined dataset is taken from the January 2024 ILS.

Samples were sent to eighteen laboratories and after analysis of the sample receipt data, thirteen laboratories were used for analysis. Three of the eighteen laboratories participating in the study did not receive the samples due to issues with customs (Labs 10, 12 and 13). One laboratory received damaged plates (Lab 11) so could not carry out the analysis. Lab 15 was held in customs and the temperature of the water blank on receipt was 12°C which is above the temperature criteria specified. The temperature measured during transit by the temperature probe was adequate, although the samples were defrosted on arrival. Due to the issues in shipment, it was decided to exclude Lab 15 from the data analysis. The remaining thirteen labs were delivered on time and in appropriate conditions, temperatures during shipment and at receipt were correct.

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 21.

Table 21 - Sample temperatures at receipt – January 2024 ILS

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured by water blank (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
1	N/A – temperature probe was left to run, so average temperature is not accurate	N/A – water blank frozen	1.1°C	11/01/2024 11:40	15/01/2024 9:20
3	-10.1°C	N/A – water blank frozen	4.2°C	11/01/2024 9:45	15/01/2024
4	-15.9°C	N/A – water blank frozen	0.2°C	11/01/2024 14:00	15/01/2024 12:00
5	-15.8°C	4.6°C	-5.0°C	11/01/2024 11:55	15/01/2024
6	-12.8°C	N/A – water blank frozen	-0.1°C	11/01/2024 10:00	15/01/2024 12:20
7	-16.3°C	N/A – water blank frozen	-4.2°C	10/01/2024 13:00	15/01/2024 8:00
8	-12.0°C	N/A – water blank frozen	0.1°C	11/01/2024 10:30	15/01/2024 10:50
9	-19.9°C	N/A – water blank frozen	0.6°C	11/01/2024 11:30	15/10/2024 11:30
14	0.1°C	N/A – water blank frozen	2.9°C	11/01/2024 14:30	15/01/2024
15	-10.8°C	12°C	2.5°C Samples defrosted on arrival	15/01/2024 10:30	15/01/2024 11:00

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured by water blank (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
16	-4°C	N/A – water blank frozen	3.6°C	10/01/2024 9:44	15/01/2024
17	-16.1°C	0.9°C	2.1°C	10/01/2024 12:00	15/01/2024
18	-14.5°C	-2.7°C	0.2°C	11/01/2024 10:00	15/01/2024

4.5.2 Selection of labs from October 2023

The data for the low-level samples in the combined dataset is taken from the October 2023 ILS. The data was taken from the medium-level samples, which were inoculated at 10^3 cfu/g.

20 labs were included in the ILS, and 3 of these labs did not return their results. Out of the remaining 17 labs, the following selection criteria was applied:

- Temperature conditions of the package received by the labs was ≤8°C
- Incubation times and temperatures were correct
- Zero counts on the blank, uninoculated sample
- Duplicate samples from the same level (i.e. inoculated with the same inoculum) producing 10^2-10^3 cfu/g

10 labs met the criteria specified and were included in the low-level samples results in the analysis.

7 labs had high variation between duplicate samples (2-3 logs difference) and so were excluded from the analysis.

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 22.

Table 22 - Sample temperatures at receipt

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured by water blank (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
6	3.4°C	6.5°C	2.5°C	12/10/2023 15:11	15/10/2023
7	2.0°C	4.6°C	2.3°C	12/10/2023 12:00	15/10/2023
8	N/A – temperature probe data missing	1.7°C	N/A – temperature probe data missing	12/10/2023 11:55	15/10/2023
9	4.2°C	2°C	1.9°C	12/10/2023 8:59	15/10/2023
10	3.8°C	1.1°C	2.3°C	12/10/2023 11:00	15/10/2023
11	3.5°C	-0.4°C	3.5°C	12/10/2023 10:00	15/10/2023
12	4.3°C	6.9°C	6.5°C	12/10/2023 17:40	15/10/2023
16	3.0°C	7.2°C	4.5°C	12/10/2023 14:30	15/10/2023
20	4.1°C	3.7°C	5.8°C	12/10/2023 17:10	15/10/2023

For the 10 labs used in the data analysis: the samples were delivered on time and in appropriate conditions, temperatures during shipment and at receipt were correct.

4.5.3 Exclusions from the merged dataset

Between both interlaboratory studies, a total of 24 labs participated.14 labs participated in both studies (October 2023, January 2024). 6 labs were used in the data analysis for both studies.

The reasons for exclusion of datasets in the merged dataset are shown in Table 23.

Table 23. Participants used for analysis – exclusions from ILS

Lab identification number	Participation in ILS	Data included for analysis of ILS	Reason excluded from ILS 1	Reason excluded from ILS 2
1	1,2	2	Temperature conditions (9.8°C on arrival). 2 logs difference between	-

Lab identification number	Participation in ILS	Data included for analysis of ILS	Reason excluded from ILS 1	Reason excluded from ILS 2
			replicates. Incorrect incubation time was used.	
2	1,2	N/A	Data not returned	Data not returned
3	1,2	2	Temperature conditions (11°C on arrival). 2 logs difference between replicates. Incorrect incubation time was used	-
4	1	N/A	2 logs between replicates	-
5	1,2	1,2	-	-
6	1,2	1,2	-	-
7	1,2	1,2	-	-
8	1	1	-	-
9	1,2	1,2	-	-
10	1,2	1,2	-	-
11	2	2	-	-
12	1,2	1	-	Parcel did not arrive; tests were not completed
13	1,2	1	-	Parcel did not arrive; tests were not completed
14	1	N/A	3 logs difference between replicates. Temperature was 8.4°C on arrival.	-
15	1,2	N/A	3 logs difference between replicates	Parcel did not arrive; tests were not completed
16	1,2	N/A	3 logs difference between replicates. Temperature abuse in transit, samples reached >10°C and were then frozen.	Parcel did not arrive; tests were not completed
17	2	2	-	-
18	1,2	2	3 logs difference between replicates.	Temperature was 12°C on arrival, so data was excluded.
19	2	2	-	-
20	1,2	1,2	-	-
21	2	2	-	-
22	1	1	-	-
23	1	N/A	Did not return results	-
24	1	N/A	Did not return results	-

Key: ILS 1 = October 2023, ILS 2 = January 2024

4.6 Analysis of the merged interlaboratory study dataset

4.6.1 Expert laboratory dataset

The results obtained by the expert laboratory are given in Table 24.

Table 24 – Results obtained by the expert lab.

Level	Date of analysis	Reference method	Alternative method – 54h	Reference method – 72h
Blank	15/10/2024 and 15/01/2024	<1	<1	<1
Low	15/10/2024	3.3	3.3	3.3
Low	15/10/2024	3.3	3.4	3.4
Medium	15/01/2024	5.0	4.8	4.8
Medium	15/01/2024	4.7	4.7	4.8
High	15/01/2024	6.9	6.7	6.8
High	15/01/2024	6.9	6.7	6.8

4.6.2 Results obtained by the collaborative laboratories

The combined dataset is shown in Table 25.

Table 25. Summary of the results of the interlaboratory study per analyte level

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 54h incubation		Alternative method (Log cfu/g) – 72h incubation	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
6	Low	3.2	3.3	3.0	3.1	3.1	3.2
7	Low	3.0	4.1	3.2	3.3	3.3	3.4
8	Low	3.1	3.1	3.2	3.4	3.2	3.4
9	Low	3.5	3.5	3.2	3.2	3.2	3.2
10	Low	3.1	2.7	3.2	3.1	3.3	3.1
11	Low	3.5	3.3	3.4	3.3	3.4	3.3
12	Low	3.4	3.3	3.8	3.0	3.8	3.1

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 54h incubation		Alternative method (Log cfu/g) – 72h incubation	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
14	Low	3.6	3.2	2.6	2.7	2.6	2.7
16	Low	3.3	3.3	3.4	3.3	3.4	3.3
20	Low	3.1	3.1	3.3	3.1	3.3	3.1
1	Med	4.4	4.3	4.3	4.3	4.3	4.4
2	Med	4.9	4.9	4.9	4.9	4.9	4.9
3	Med	4.4	4.6	4.5	4.7	4.5	4.8
4	Med	4.6	4.5	4.6	4.2	4.6	4.4
5	Med	4.3	4.4	3.9	4.4	4.0	4.4
6	Med	4.7	4.1	4.7	4.0	4.7	4.0
7	Med	4.4	4.4	4.6	4.5	4.6	4.5
8	Med	4.5	4.4	4.5	4.4	4.5	4.4
9	Med	4.6	4.6	4.5	4.6	4.6	4.5
14	Med	4.7	4.5	4.4	4.5	4.4	4.6
15	Med	4.9	4.7	4.7	4.8	4.7	4.8
16	Med	4.8	4.8	4.7	5.0	4.7	5.0
17	Med	4.7	4.5	4.5	4.5	4.5	4.5
18	Med	4.5	4.5	4.4	4.2	4.5	4.2
1	High	6.4	6.2	6.4	6.1	6.4	6.1
2	High	7.0	6.9	7.1	6.9	7.1	6.9
3	High	6.6	6.5	6.4	6.5	6.4	6.5
4	High	6.6	6.2	6.6	6.2	6.6	6.2
5	High	6.4	6.6	6.4	6.5	6.4	6.6
6	High	6.4	6.6	6.5	6.4	6.5	6.4
7	High	6.8	6.7	6.7	6.5	6.7	6.5
8	High	6.5	6.5	6.5	6.4	6.5	6.4
9	High	6.4	6.5	6.4	6.0	6.5	6.5
14	High	6.9	6.8	6.8	6.8	6.8	6.8
15	High	6.8	6.8	6.8	6.8	6.9	6.8
16	High	6.9	6.9	6.8	6.8	6.8	6.8
17	High	6.6	6.7	6.6	6.6	6.7	6.5
18	High	6.5	6.5	6.5	6.3	5.5	6.3
1	Blank	<1		<1		<1	
2	Blank	<1		<1		<1	
3	Blank	<1		<1		<1	
4	Blank	<1		<1		<1	
5	Blank	<1		<1		<1	
6	Blank	<1		<1		<1	
7	Blank	<1		<1		<1	
8	Blank	<1		<1		<1	
9	Blank	<1		<1		<1	

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 54h incubation		Alternative method (Log cfu/g) – 72h incubation	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
14	Blank	<1		<1		<1	
15	Blank	<1		<1		<1	
16	Blank	<1		<1		<1	
17	Blank	<1		<1		<1	
18	Blank	<1		<1		<1	

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 accuracy profile plot is shown in Figures 5 and 6 and the statistical analysis of the data shown in Tables 26 and 27.

Figure 5. Accuracy profile of OPYM from the ILS – 54h incubation

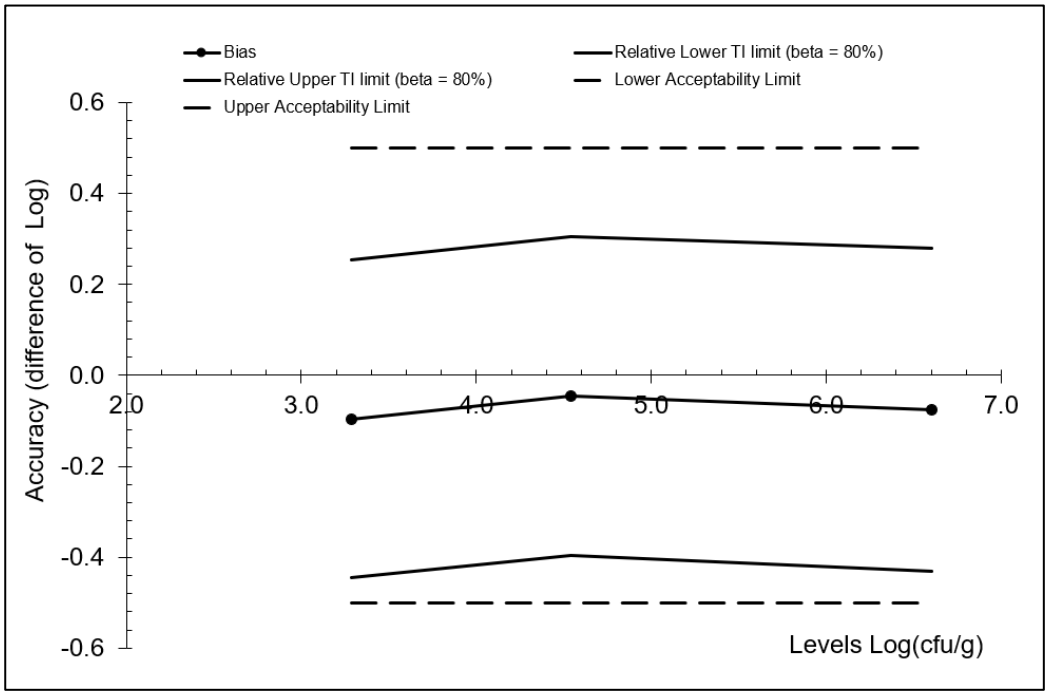


Figure 6. Accuracy profile of OPYM from the ILS – 72h incubation

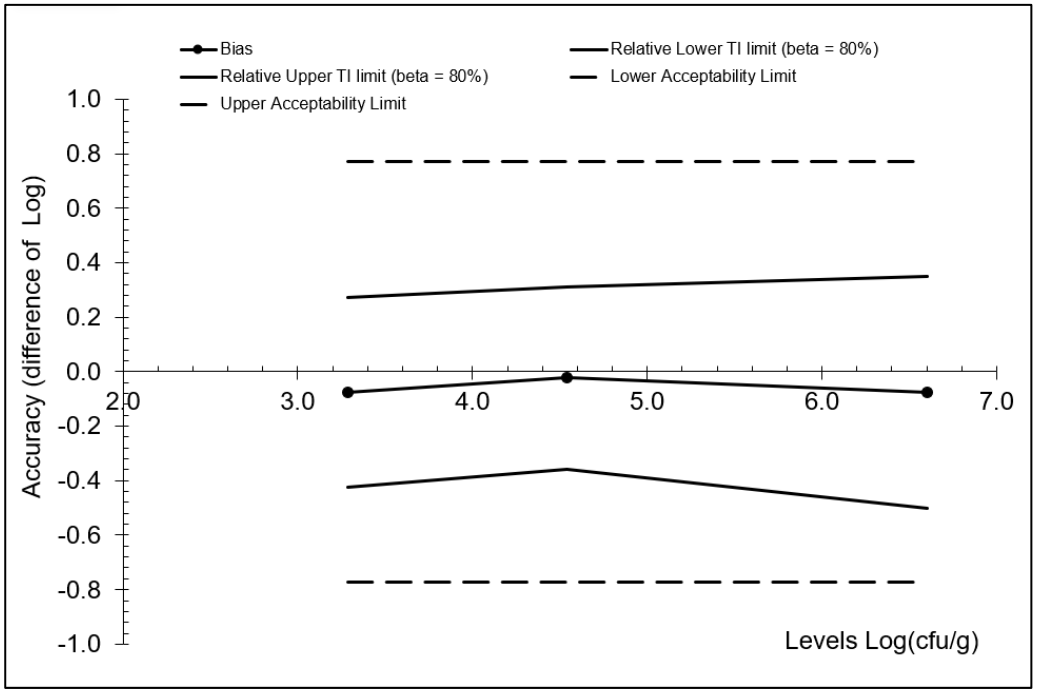


Table 25. Statistical analysis of the ILS data according to the ISO spreadsheet – 54h incubation[1]

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	3.287	4.541	6.600			
Number of participants (K)	10	13	13	10	13	13
Average for alternative method	3.210	4.519	6.524	3.287	4.541	6.600
Repeatability standard deviation (sr)	0.180	0.203	0.205	0.287	0.127	0.115
Between-labs standard deviation (sL)	0.175	0.140	0.233	0.000	0.143	0.178
Reproducibility standard deviation (sR)	0.251	0.247	0.311	0.287	0.191	0.212
Corrected number of dof	14.700	22.089	18.300	18.947	18.380	16.045
Coverage factor	1.391	1.354	1.369			
Interpolated Student t	1.342	1.321	1.330			
Tolerance interval standard deviation	0.2602	0.2534	0.3197			
Lower TI limit	2.861	4.184	6.099			
Upper TI limit	3.559	4.854	6.949			
Bias	-0.077	-0.022	-0.076			
Relative Lower TI limit (beta = 80%)	-0.426	-0.357	-0.501			
Relative Upper TI limit (beta = 80%)	0.273	0.313	0.350			
Lower Acceptability Limit	-0.77	-0.77	-0.77			
Upper Acceptability Limit	0.77	0.77	0.77			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.234					

TRUE
FALSE

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

Table 26. Statistical analysis of the ILS data according to the ISO spreadsheet – 72h incubation[2]

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	3.287	4.541	6.600			
Number of participants (K)	10	13	13	10	13	13
Average for alternative method	3.210	4.519	6.524	3.287	4.541	6.600
Repeatability standard deviation (sr)	0.180	0.203	0.205	0.287	0.127	0.115
Between-labs standard deviation (sL)	0.175	0.140	0.233	0.000	0.143	0.178
Reproducibility standard deviation (sR)	0.251	0.247	0.311	0.287	0.191	0.212
Corrected number of dof	14.700	22.089	18.300	18.947	18.380	16.045
Coverage factor	1.391	1.354	1.369			
Interpolated Student t	1.342	1.321	1.330			
Tolerance interval standard deviation	0.2602	0.2534	0.3197			
Lower TI limit	2.861	4.184	6.099			
Upper TI limit	3.559	4.854	6.949			
Bias	-0.077	-0.022	-0.076			
Relative Lower TI limit (beta = 80%)	-0.426	-0.357	-0.501			
Relative Upper TI limit (beta = 80%)	0.273	0.313	0.350			
Lower Acceptability Limit	-0.77	-0.77	-0.77			
Upper Acceptability Limit	0.77	0.77	0.77			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.234					

TRUE
FALSE

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

[1] Low level results were produced using a 1ml pour plate technique for the alternative method. Medium and high level results were produced using a 0.1ml spread plate technique for the alternative method.
[2] Low level results were produced using a 1ml pour plate technique for the alternative method. Medium and high level results were produced using a 0.1ml spread plate technique for the alternative method.

4.7 Conclusions of the interlaboratory study

The combined data meets the requirements of ISO 16140-2 and was approved for analysis by the MVTC. All interlaboratory study results show good agreement between the reference and alternative methods for both incubation times analysed in the study. In addition, the data obtained in the ILS is consistent with the results observed in the Method Comparison Study.

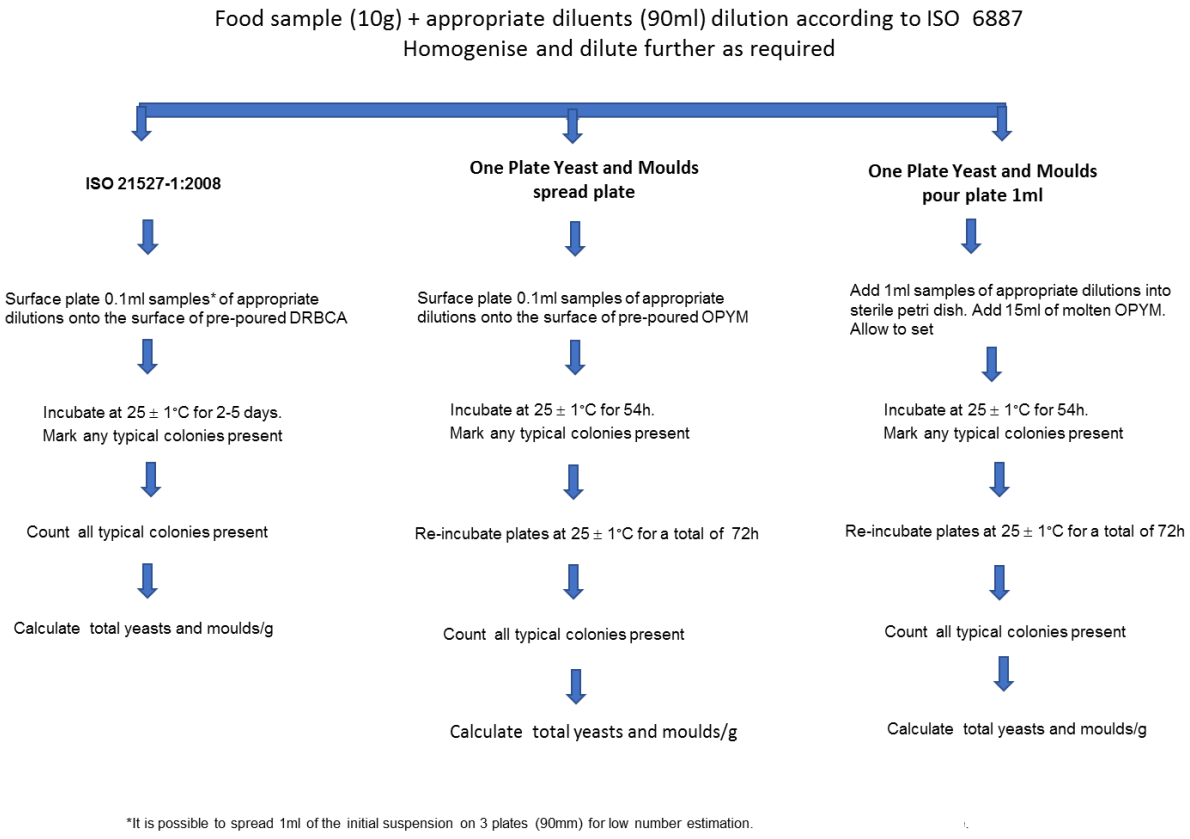
Overall, the conclusions of the study are:

- The alternative method One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for relative trueness;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould shows satisfactory results for accuracy profile;
- The alternative One Plate Yeast and Mould for enumeration of Yeast and Mould is selective and specific.
- The combined data meets the requirements of ISO 16140-2 and was approved for analysis by the MVTC. All interlaboratory study results show good agreement between the reference and alternative methods for both incubation times analysed in the study.
- The alternative method, OPYM, shows comparable performance to the reference method, ISO 21527:2008 parts 1 and 2 for the enumeration of yeasts and moulds in a broad range of foods for both plating formats (1ml pour plate and 0.1ml spread plate) at the 2 time points tested (54 h and 72 h).

11 June 2024

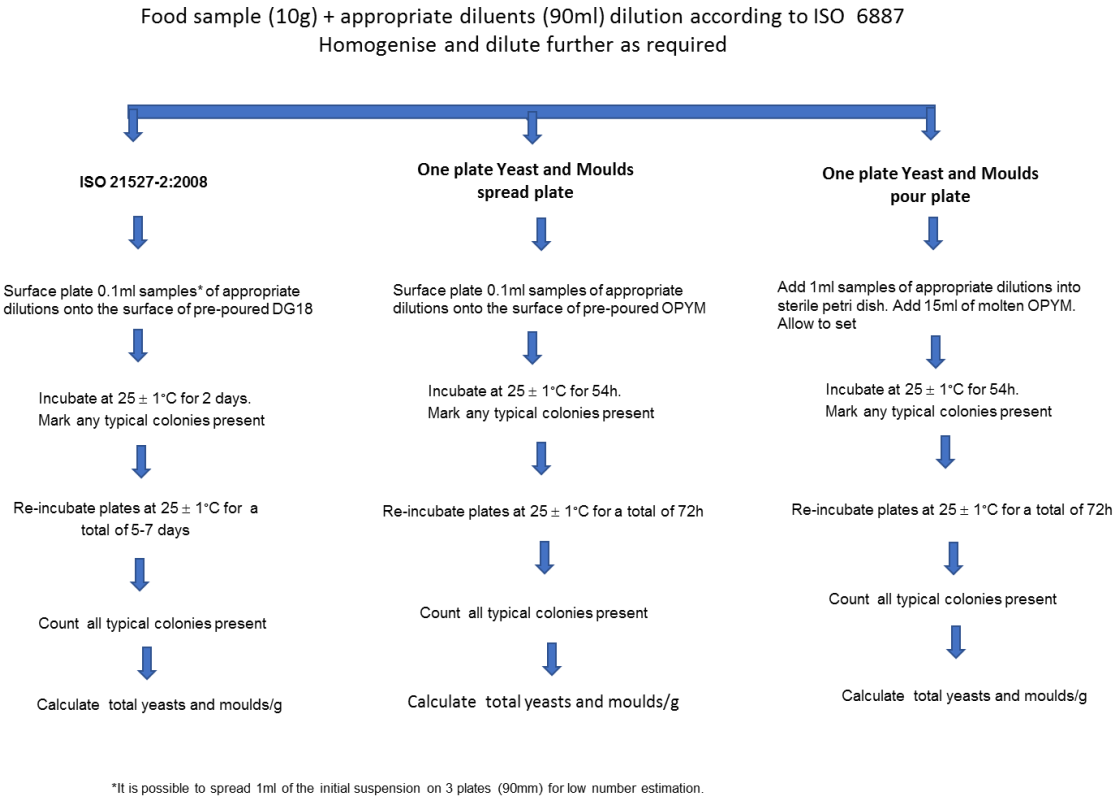
Alice Foxall
Project Manager – Molecular Microbiology and Methods

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



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

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



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

ANNEX C: Calculation and interpretation of relative trueness

OPYM - 54h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Heat processed milk and dairy products						
Pasteurised dairy product	british whole milk	**T1**	6.23	6.04	6.14	-0.19
Pasteurised dairy product	vanillalicious milkshake	**T2**	5.23	5.26	5.24	0.02
Pasteurised dairy product	british double cream	**T3**	4.45	4.34	4.39	-0.10
Pasteurised dairy product	soured cream	**T4**	3.18	3.32	3.25	0.15
Pasteurised dairy product	ice cream	**T5**	2.16	2.26	2.21	0.09
Cheese	blue stilton cheese	T6	7.18	7.40	7.29	0.22
Cheese	cottage cheese, fat free	**T7**	3.41	3.28	3.35	-0.14
Cheese	British Mature grated cheddar cheese	**T8**	5.36	5.04	5.20	-0.32
Cheese	Dairy soft cheese	T9	8.51	8.49	8.50	-0.01
Cheese	grated four cheese mix	**T10**	5.04	5.08	5.06	0.04
Fermented and acidfied pasteurised milk and yogurt	peach yogurt drink	**T11**	5.36	5.41	5.39	0.05
Fermented and acidfied pasteurised milk and yogurt	strawberry yogurt	**T12**	4.49	4.48	4.48	-0.01
Fermented and acidfied pasteurised milk and yogurt	mango yogurt	**T13**	3.28	3.53	3.41	0.25
Fermented and acidfied pasteurised milk and yogurt	apricot yogurt	**T14**	2.23	2.41	2.32	0.18
Fermented and acidfied pasteurised milk and yogurt	kiwi yogurt	**T15**	7.36	7.53	7.45	0.17
Fresh produce and fruits						
Fresh fruit salad and fruit puree	blueberries	T16	5.66	5.23	5.45	-0.43
Fresh fruit salad and fruit puree	strawberries	T17	4.68	4.32	4.50	-0.36
Fresh fruit salad and fruit puree	berry fruit salad	T18	5.11	5.15	5.13	0.03
Fresh fruit salad and fruit puree	apple banana strawberry and grape	**T19**	3.86	4.08	3.97	0.22
Fresh fruit salad and fruit puree	melon kiwi and strawberry	T20	4.04	3.86	3.95	-0.18
Chilled fruit juices	apple and Raspberry juice	T21	1.85	1.78	1.81	-0.07

OPYM - 54h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Chilled fruit juices	pure orange	T22	2.82	2.89	2.85	0.07
Chilled fruit juices	apple and pear juice	T23	4.04	3.94	3.99	-0.10
Chilled fruit juices	multivitamin boost fruit juice	T24	5.00	4.85	4.92	-0.15
Chilled fruit juices	pure orange and mango fruit juice	T25	5.91	5.96	5.94	0.05
Fermented vegetables	sauerkraut with carrot	T26	2.70	2.65	2.68	-0.05
Fermented vegetables	Raw Kimchi	T27	5.77	6.04	5.91	0.27
Fermented vegetables	Pitted Green Olives	T28	5.76	5.74	5.75	-0.02
Fermented vegetables	Pitted Black Olives	T29	4.79	4.75	4.77	-0.04
Fermented vegetables	Kimchi Spicy	T30	5.11	5.08	5.10	-0.03
Multicomponent foods or meal components						
Composite foods with raw ingredients	ham sandwich no mayo	T31	7.64	7.40	7.52	-0.25
Composite foods with raw ingredients	seafood cocktail sandwich	T32	8.04	8.04	8.04	0.00
Composite foods with raw ingredients	red leicester ploughman's sandwich	T33	8.56	8.58	8.57	0.02
Composite foods with raw ingredients	pomodorino tomato and sweet pepper salad	T34	7.83	7.72	7.78	-0.11
Composite foods with raw ingredients	mediterranean salad	T35	7.76	7.79	7.77	0.02
Mayonnaise based chilled salads	potato salad	T36	4.40	4.30	4.35	-0.10
Mayonnaise based chilled salads	potato salad	T37	7.20	7.04	7.12	-0.16
Mayonnaise based chilled salads	potato and egg salad	T38	5.76	5.76	5.76	0.00
Mayonnaise based chilled salads	ham egg and coleslaw salad	T39	7.66	7.60	7.63	-0.06
Mayonnaise based chilled salads	coleslaw	T40	7.83	7.85	7.84	0.01
Ambient stable acidified foods	mayonnaise squeezy	**T41**	4.43	4.59	4.51	0.16
Ambient stable acidified foods	sweet chilli sauce	**T42**	3.69	3.54	3.62	-0.15
Ambient stable acidified foods	tartare sauce	**T43**	3.18	3.20	3.19	0.03
Ambient stable acidified foods	bbq sauce	**T44**	3.40	3.68	3.54	0.28

OPYM - 54h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Ambient stable acidified foods	tomato ketchup	**T45**	3.69	3.41	3.55	-0.28
Confectionary, bakery and eggs						
Bakery products with custard	Fresh Custard Slices	T46	8.57	8.56	8.56	-0.01
Bakery products with custard	Egg custard tarts	**T47**	3.23	3.15	3.19	-0.08
Bakery products with custard	custard tart	**T48**	4.15	4.11	4.13	-0.03
Bakery products with custard	Portuguese custard tart	T49	6.83	6.86	6.84	0.03
Bakery products with custard	Pastel de nata	T50	6.63	6.59	6.61	-0.04
Egg products without additives e.g. chilled quiches	broccoli and tomato quiche	T51	7.30	7.49	7.40	0.19
Egg products without additives e.g. chilled quiches	crustless spinach & feta quiche	T52	7.62	7.70	7.66	0.08
Egg products without additives e.g. chilled quiches	crustless broccoli and tomato quiche	T53	5.71	5.76	5.74	0.06
Egg products without additives e.g. chilled quiches	crustless quiche lorraine	T54	7.85	7.98	7.91	0.13
Egg products without additives e.g. chilled quiches	quiche lorraine	T55	7.74	7.79	7.76	0.04
Par baked bread products Aw >0.95	Part Bakes Rustic White Rolls x4	T56	7.72	7.76	7.74	0.04
Par baked bread products Aw >0.95	Part Bakes Rustic Seeded Rolls x4	T57	6.53	6.88	6.71	0.35
Par baked bread products Aw >0.95	Brown Baguetts Bake at Home x2	T58	8.20	8.26	8.23	0.05
Par baked bread products Aw >0.95	Bake at Home White Petit Pains x6	T59	7.56	7.40	7.48	-0.16
Par baked bread products Aw >0.95	Bake at Home Stonebaked White baguettes	T60	8.18	8.18	8.18	0.00
Ready to eat/ready to reheat foods						
Ready to eat meat and poultry	Chicken stuffing fridge raiders	T61	7.46	7.11	7.29	-0.35
Ready to eat meat and poultry	Copernicus Toruriska	**T62**	2.02	2.32	2.17	0.30
Ready to eat meat and poultry	Berklinki chicken	T63	6.54	6.72	6.63	0.17
Ready to eat meat and poultry	Bratwurst	**T64**	5.30	5.04	5.17	-0.26
Ready to eat meat and poultry	Sliced turkey	**T65**	4.89	4.92	4.91	0.04
Cooked and cured fish products	Cooked prawns	T66	5.40	5.23	5.31	-0.17

OPYM - 54h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Cooked and cured fish products	Smoked mackarel flakes	T67	3.28	3.23	3.25	-0.05
Cooked and cured fish products	sardines in brine	T68	4.26	4.18	4.22	-0.08
Cooked and cured fish products	skinless + boneless mackrel	T69	4.11	4.11	4.11	0.00
Cooked and cured fish products	sardines with sea salt	T70	4.23	4.30	4.27	0.07
Raw cured meat and poultry	Galican chorizo	T71	3.04	3.04	3.04	0.00
Raw cured meat and poultry	Spicy chorizo slices	T72	4.11	4.00	4.06	-0.11
Raw cured meat and poultry	pizza pepperoni slices	T73	5.00	5.11	5.06	0.11
Raw cured meat and poultry	prosciutto crudo and salami platter	T74	7.20	7.18	7.19	-0.03
Raw cured meat and poultry	salami platter	T75	6.00	6.11	6.05	0.12
Dried cereals fruits nuts seeds and vegetables						
Dried cereals	rice snaps	**T76**	6.30	6.04	6.17	-0.26
Dried cereals	corn flakes	**T77**	4.94	5.00	4.97	0.06
Dried cereals	low fat special flakes	**T78**	3.53	3.34	3.44	-0.19
Dried cereals	malt wheats	**T79**	2.96	2.78	2.87	-0.18
Dried cereals	wheat biscuits	**T111**	2.89	2.11	2.50	-0.78
Nuts and seeds	wholefoods 4 seed mix	**T81**	5.23	5.18	5.20	-0.05
Nuts and seeds	toasted 3 seed mix	**T82**	3.96	3.81	3.89	-0.15
Nuts and seeds	sunflower seeds	**T83**	3.18	2.83	3.00	-0.35
Nuts and seeds	unsalted mixed nuts	**T84**	2.96	2.69	2.83	-0.27
Nuts and seeds	cashew nuts	**T85**	5.04	4.93	4.99	-0.11
Low and IF fruits aw <0.85	sultanas	**T86**	6.81	6.96	6.89	0.15
Low and IF fruits aw <0.85	raisins	**T87**	4.51	4.64	4.57	0.14
Low and IF fruits aw <0.85	apricots	**T88**	3.93	4.04	3.99	0.11
Low and IF fruits aw <0.85	dried mixed fruit	**T89**	3.48	3.28	3.38	-0.20
Low and IF fruits aw <0.85	organic apricots	**T90**	5.04	5.04	5.04	0.00

OPYM - 54h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Chocolate and confectionary						
Dry and sugared low moisture aw<0.85	ready to roll fonant icing	**T91**	5.68	5.49	5.59	-0.19
Dry and sugared low moisture aw<0.85	marzipan	**T92**	4.98	4.88	4.93	-0.10
Dry and sugared low moisture aw<0.85	vanilla frosting	**T93**	3.67	3.71	3.69	0.04
Dry and sugared low moisture aw<0.85	chocolate frosting	**T94**	2.64	2.46	2.55	-0.17
Dry and sugared low moisture aw<0.85	lemon icing	**T95**	8.52	8.11	8.32	-0.40
Dry and sugared low moisture aw<0.65	maple syrup	**T96**	3.63	3.18	3.40	-0.46
Dry and sugared low moisture aw<0.65	golden syrup	**T97**	2.65	2.46	2.56	-0.19
Dry and sugared low moisture aw<0.65	raspberry syrup	**T98**	8.34	8.04	8.19	-0.30
Dry and sugared low moisture aw<0.65	black treacle	**T99**	2.41	2.11	2.26	-0.30
Dry and sugared low moisture aw<0.65	honey	**T100**	7.63	7.46	7.55	-0.17
Dry powders e.g. cake mixes	choc chip cookie mix	**T101**	6.85	6.71	6.78	-0.14
Dry powders e.g. cake mixes	choc cake mix	**T102**	6.28	6.26	6.27	-0.02
Dry powders e.g. cake mixes	vanilla cupcake mix	**T103**	6.15	6.20	6.18	0.06
Dry powders e.g. cake mixes	lemon drizzle mix	**T104**	5.48	5.45	5.46	-0.03
Dry powders e.g. cake mixes	victoria sponge mix	**T105**	4.72	4.76	4.74	0.04

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Heat processed milk and dairy products						
Pasteurised dairy product	british whole milk	**T1**	6.23	6.04	6.14	-0.19

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Pasteurised dairy product	vanillalicious milkshake	**T2**	5.23	5.26	5.24	0.02
Pasteurised dairy product	british double cream	**T3**	4.45	4.34	4.39	-0.10
Pasteurised dairy product	soured cream	**T4**	3.18	3.32	3.25	0.15
Pasteurised dairy product	ice cream	**T5**	2.16	2.30	2.23	0.14
Cheese	blue stilton cheese	T6	7.18	7.40	7.29	0.22
Cheese	cottage cheese, fat free	**T7**	3.41	3.32	3.37	-0.09
Cheese	British Mature grated cheddar cheese	**T8**	5.36	5.08	5.22	-0.28
Cheese	Dairy soft cheese	T9	8.51	8.49	8.50	-0.01
Cheese	grated four cheese mix	**T10**	5.04	5.08	5.06	0.04
Fermented and acidfied pasteurised milk and yogurt	peach yogurt drink	**T11**	5.36	5.41	5.39	0.05
Fermented and acidfied pasteurised milk and yogurt	strawberry yogurt	**T12**	4.49	4.48	4.48	-0.01
Fermented and acidfied pasteurised milk and yogurt	mango yogurt	**T13**	3.28	3.41	3.35	0.14
Fermented and acidfied pasteurised milk and yogurt	apricot yogurt	**T14**	2.23	2.41	2.32	0.18
Fermented and acidfied pasteurised milk and yogurt	kiwi yogurt	**T15**	7.36	7.53	7.45	0.17
Fresh produce and fruits						
Fresh fruit salad and fruit puree	blueberries	T16	5.66	5.26	5.46	-0.41
Fresh fruit salad and fruit puree	strawberries	T17	4.68	4.45	4.56	-0.23
Fresh fruit salad and fruit puree	berry fruit salad	T18	5.11	5.15	5.13	0.03
Fresh fruit salad and fruit puree	apple banana strawberry and grape	**T19**	3.86	4.26	4.06	0.40
Fresh fruit salad and fruit puree	melon kiwi and strawberry	T20	4.04	3.95	4.00	-0.09
Chilled fruit juices	apple and Raspberry juice	T21	1.85	1.78	1.81	-0.07
Chilled fruit juices	pure orange	T22	2.82	2.89	2.85	0.07
Chilled fruit juices	apple and pear juice	T23	4.04	3.97	4.01	-0.07
Chilled fruit juices	multivitamin boost fruit juice	T24	5.00	4.85	4.92	-0.15
Chilled fruit juices	pure orange and mango fruit juice	T25	5.91	5.92	5.92	0.01

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Fermented vegetables	sauerkraut with carrot	T26	2.70	2.69	2.69	-0.01
Fermented vegetables	Raw Kimchi	T27	5.77	6.04	5.91	0.27
Fermented vegetables	Pitted Green Olives	T28	5.76	5.76	5.76	0.01
Fermented vegetables	Pitted Black Olives	T29	4.79	4.76	4.77	-0.03
Fermented vegetables	Kimchi Spicy	T30	5.11	5.08	5.10	-0.03
Multicomponent foods or meal components						
Composite foods with raw ingredients	ham sandwich no mayo	T31	7.64	7.60	7.62	-0.04
Composite foods with raw ingredients	seafood cocktail sandwich	T32	8.04	8.08	8.06	0.04
Composite foods with raw ingredients	red leicester ploughman's sandwich	T33	8.56	8.64	8.60	0.09
Composite foods with raw ingredients	pomodorino tomato and sweet pepper salad	T34	7.83	7.74	7.79	-0.09
Composite foods with raw ingredients	mediterranean salad	T35	7.76	7.99	7.88	0.22
Mayonnaise based chilled salads	potato salad	T36	4.40	4.79	4.60	0.39
Mayonnaise based chilled salads	potato salad	T37	7.20	7.04	7.12	-0.16
Mayonnaise based chilled salads	potato and egg salad	T38	5.76	5.77	5.77	0.01
Mayonnaise based chilled salads	ham egg and coleslaw salad	T39	7.66	7.62	7.64	-0.04
Mayonnaise based chilled salads	coleslaw	T40	7.83	7.85	7.84	0.02
Ambient stable acidified foods	mayonnaise squeezy	**T41**	4.43	4.59	4.51	0.16
Ambient stable acidified foods	sweet chilli sauce	**T42**	3.69	3.54	3.62	-0.15
Ambient stable acidified foods	tartare sauce	**T43**	3.18	3.20	3.19	0.03
Ambient stable acidified foods	bbq sauce	**T44**	3.40	3.68	3.54	0.28
Ambient stable acidified foods	tomato ketchup	**T45**	3.69	3.41	3.55	-0.28
Confectionary, bakery and eggs						
Bakery products with custard	Fresh Custard Slices	T46	8.57	8.56	8.56	-0.01
Bakery products with custard	Egg custard tarts	**T47**	3.23	3.20	3.22	-0.03

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Bakery products with custard	custard tart	**T48**	4.15	4.15	4.15	0.00
Bakery products with custard	Portuguese custard tart	T49	6.83	6.86	6.84	0.03
Bakery products with custard	Pastel de nata	T50	6.63	6.63	6.63	0.00
Egg products without additives e.g. chilled quiches	broccoli and tomato quiche	T51	7.30	7.49	7.40	0.19
Egg products without additives e.g. chilled quiches	crustless spinach & feta quiche	T52	7.62	7.70	7.66	0.08
Egg products without additives e.g. chilled quiches	crustless broccoli and tomato quiche	T53	5.71	5.76	5.74	0.06
Egg products without additives e.g. chilled quiches	crustless quiche lorraine	T54	7.85	7.98	7.91	0.13
Egg products without additives e.g. chilled quiches	quiche lorraine	T55	7.74	7.79	7.76	0.04
Par baked bread products Aw >0.95	Part Bakes Rustic White Rolls x4	T56	7.72	7.79	7.76	0.07
Par baked bread products Aw >0.95	Part Bakes Rustic Seeded Rolls x4	T57	6.53	6.46	6.50	-0.07
Par baked bread products Aw >0.95	Brown Baguetts Bake at Home x2	T58	8.20	8.34	8.27	0.14
Par baked bread products Aw >0.95	Bake at Home White Petit Pains x6	T59	7.56	7.61	7.58	0.06
Par baked bread products Aw >0.95	Bake at Home Stonebaked White baguettes	T60	8.18	8.20	8.19	0.03
Ready to eat/ready to reheat foods						
Ready to eat meat and poultry	Chicken stuffing fridge raiders	T61	7.46	7.18	7.32	-0.29
Ready to eat meat and poultry	Copernicus Toruriska	**T62**	2.02	2.32	2.17	0.30
Ready to eat meat and poultry	Berklinki chicken	T63	6.54	6.72	6.63	0.17
Ready to eat meat and poultry	Bratwurst	**T64**	5.30	5.08	5.19	-0.22
Ready to eat meat and poultry	Sliced turkey	**T65**	4.89	4.92	4.91	0.04
Cooked and cured fish products	Cooked prawns	T66	5.40	5.59	5.49	0.19
Cooked and cured fish products	Smoked mackarel flakes	T67	3.28	3.23	3.25	-0.05
Cooked and cured fish products	sardines in brine	T68	4.26	4.18	4.22	-0.08
Cooked and cured fish products	skinless + boneless mackrel	T69	4.11	4.11	4.11	0.00
Cooked and cured fish products	sardines with sea salt	T70	4.23	4.30	4.27	0.07

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Raw cured meat and poultry	Galican chorizo	T71	3.04	3.04	3.04	0.00
Raw cured meat and poultry	Spicy chorizo slices	T72	4.11	4.11	4.11	0.00
Raw cured meat and poultry	pizza pepperoni slices	T73	5.00	5.11	5.06	0.11
Raw cured meat and poultry	prosciutto crudo and salami platter	T74	7.20	7.18	7.19	-0.03
Raw cured meat and poultry	salami platter	T75	6.00	6.11	6.05	0.12
Dried cereals fruits nuts seeds and vegetables						
Dried cereals	rice snaps	**T76**	6.30	6.08	6.19	-0.22
Dried cereals	corn flakes	**T77**	4.94	5.00	4.97	0.06
Dried cereals	low fat special flakes	**T78**	3.53	3.63	3.58	0.10
Dried cereals	malt wheats	**T79**	2.96	2.82	2.89	-0.14
Dried cereals	wheat biscuits	**T80**	4.56	4.40	4.48	-0.16
Nuts and seeds	wholefoods 4 seed mix	**T81**	5.23	5.28	5.25	0.05
Nuts and seeds	toasted 3 seed mix	**T82**	3.96	4.04	4.00	0.08
Nuts and seeds	sunflower seeds	**T83**	3.18	3.23	3.20	0.05
Nuts and seeds	unsalted mixed nuts	**T84**	2.96	3.11	3.04	0.15
Nuts and seeds	cashew nuts	**T85**	5.04	4.93	4.99	-0.11
Low and IF fruits aw <0.85	sultanas	**T86**	6.81	6.96	6.89	0.15
Low and IF fruits aw <0.85	raisins	**T87**	4.51	4.67	4.59	0.17
Low and IF fruits aw <0.85	apricots	**T88**	3.93	4.15	4.04	0.22
Low and IF fruits aw <0.85	dried mixed fruit	**T89**	3.48	3.40	3.44	-0.08
Low and IF fruits aw <0.85	organic apricots	**T90**	5.04	5.04	5.04	0.00
Chocolate and confectionary						
Dry and sugared low moisture aw<0.85	ready to roll fonant icing	**T91**	5.68	5.64	5.66	-0.04
Dry and sugared low moisture aw<0.85	marzipan	**T92**	4.98	4.92	4.95	-0.05
Dry and sugared low moisture aw<0.85	vanilla frosting	**T93**	3.67	3.72	3.70	0.05

OPYM - 72h spread						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Dry and sugared low moisture aw<0.85	chocolate frosting	**T94**	2.64	2.46	2.55	-0.17
Dry and sugared low moisture aw<0.85	lemon icing	**T95**	8.52	8.11	8.32	-0.40
Dry and sugared low moisture aw<0.65	maple syrup	**T96**	3.63	3.18	3.40	-0.46
Dry and sugared low moisture aw<0.65	golden syrup	**T97**	2.65	2.58	2.62	-0.07
Dry and sugared low moisture aw<0.65	raspberry syrup	**T98**	8.34	8.18	8.26	-0.17
Dry and sugared low moisture aw<0.65	black treacle	**T99**	2.41	2.23	2.32	-0.18
Dry and sugared low moisture aw<0.65	honey	**T100**	7.63	7.46	7.55	-0.17
Dry powders e.g. cake mixes	choc chip cookie mix	**T101**	6.85	6.72	6.78	-0.14
Dry powders e.g. cake mixes	choc cake mix	**T102**	6.28	6.26	6.27	-0.02
Dry powders e.g. cake mixes	vanilla cupcake mix	**T103**	6.15	6.20	6.18	0.06
Dry powders e.g. cake mixes	lemon drizzle mix	**T104**	5.48	5.45	5.46	-0.03
Dry powders e.g. cake mixes	victoria sponge mix	**T105**	4.72	4.76	4.74	0.04

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Heat processed milk and dairy products						
Pasteurised dairy product	british whole milk	**T1**	6.23	6.11	6.17	-0.12
Pasteurised dairy product	vanillalicious milkshake	**T2**	5.23	5.04	5.14	-0.19
Pasteurised dairy product	british double cream	**T3**	4.45	4.49	4.47	0.04
Pasteurised dairy product	soured cream	**T4**	3.18	3.26	3.22	0.08
Pasteurised dairy product	ice cream	**T5**	2.16	2.30	2.23	0.14

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Cheese	blue stilton cheese	T6	7.18	7.34	7.26	0.17
Cheese	cottage cheese, fat free	**T7**	3.41	3.32	3.37	-0.09
Cheese	British Mature grated cheddar cheese	**T8**	5.36	5.00	5.18	-0.36
Cheese	Dairy soft cheese	T9	8.51	8.34	8.42	-0.16
Cheese	grated four cheese mix	**T10**	5.04	5.04	5.04	0.00
Fermented and acidfied pasteurised milk and yogurt	peach yogurt drink	**T11**	5.36	5.23	5.30	-0.13
Fermented and acidfied pasteurised milk and yogurt	strawberry yogurt	**T12**	4.49	4.32	4.41	-0.17
Fermented and acidfied pasteurised milk and yogurt	mango yogurt	**T13**	3.28	3.41	3.35	0.14
Fermented and acidfied pasteurised milk and yogurt	apricot yogurt	**T14**	2.23	2.43	2.33	0.20
Fermented and acidfied pasteurised milk and yogurt	kiwi yogurt	**T15**	7.36	7.28	7.32	-0.08
Fresh produce and fruits						
Fresh fruit salad and fruit puree	blueberries	T16	5.66	5.28	5.47	-0.38
Fresh fruit salad and fruit puree	strawberries	T17	4.68	4.36	4.52	-0.32
Fresh fruit salad and fruit puree	berry fruit salad	T18	5.11	5.11	5.11	0.00
Fresh fruit salad and fruit puree	apple banana strawberry and grape	**T19**	3.86	4.18	4.02	0.32
Fresh fruit salad and fruit puree	melon kiwi and strawberry	T20	4.04	3.81	3.93	-0.23
Chilled fruit juices	apple and Raspberry juice	T21	1.85	1.70	1.77	-0.15
Chilled fruit juices	pure orange	T22	2.82	2.88	2.85	0.06
Chilled fruit juices	apple and pear juice	T23	4.04	3.86	3.95	-0.18
Chilled fruit juices	multivitamin boost fruit juice	T24	5.00	4.86	4.93	-0.14
Chilled fruit juices	pure orange and mango fruit juice	T25	5.91	5.89	5.90	-0.03
Fermented vegetables	sauerkraut with carrot	T26	2.70	2.95	2.83	0.26
Fermented vegetables	Raw Kimchi	T27	5.77	5.74	5.76	-0.03
Fermented vegetables	Pitted Green Olives	T28	5.76	5.56	5.66	-0.20
Fermented vegetables	Pitted Black Olives	T29	4.79	4.84	4.81	0.05

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Fermented vegetables	Kimchi Spicy	T30	5.11	5.00	5.05	-0.12
Multicomponent foods or meal components						
Composite foods with raw ingredients	ham sandwich no mayo	T31	7.64	7.49	7.57	-0.15
Composite foods with raw ingredients	seafood cocktail sandwich	T32	8.04	7.74	7.89	-0.30
Composite foods with raw ingredients	red leicester ploughman's sandwich	T33	8.56	8.65	8.60	0.10
Composite foods with raw ingredients	pomodorino tomato and sweet pepper salad	T34	7.83	7.58	7.71	-0.25
Composite foods with raw ingredients	mediterranean salad	T35	7.76	7.86	7.81	0.10
Mayonnaise based chilled salads	potato salad	T36	4.40	3.85	4.12	-0.55
Mayonnaise based chilled salads	potato salad	T37	7.20	6.94	7.07	-0.26
Mayonnaise based chilled salads	potato and egg salad	T38	5.76	5.84	5.80	0.08
Mayonnaise based chilled salads	ham egg and coleslaw salad	T39	7.66	7.48	7.57	-0.19
Mayonnaise based chilled salads	coleslaw	T40	7.83	7.81	7.82	-0.03
Ambient stable acidified foods	mayonnaise squeezy	**T41**	4.43	4.48	4.45	0.05
Ambient stable acidified foods	sweet chilli sauce	**T42**	3.69	3.68	3.69	-0.01
Ambient stable acidified foods	tartare sauce	**T43**	3.18	3.18	3.18	0.00
Ambient stable acidified foods	bbq sauce	**T44**	3.40	3.36	3.38	-0.04
Ambient stable acidified foods	tomato ketchup	**T45**	3.69	3.72	3.70	0.03
Confectionary, bakery and eggs						
Bakery products with custard	Fresh Custard Slices	T46	8.57	8.53	8.55	-0.04
Bakery products with custard	Egg custard tarts	**T47**	3.23	2.96	3.10	-0.27
Bakery products with custard	custard tart	**T48**	4.15	4.04	4.09	-0.10
Bakery products with custard	Portuguese custard tart	T49	6.83	6.73	6.78	-0.09
Bakery products with custard	Pastel de nata	T50	6.63	6.52	6.58	-0.11
Egg products without additives e.g. chilled quiches	broccoli and tomato quiche	T51	7.30	7.53	7.42	0.23

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Egg products without additives e.g. chilled quiches	crustless spinach & feta quiche	T52	7.62	7.61	7.62	-0.01
Egg products without additives e.g. chilled quiches	crustless broccoli and tomato quiche	T53	5.71	5.75	5.73	0.04
Egg products without additives e.g. chilled quiches	crustless quiche lorraine	T54	7.85	7.72	7.78	-0.13
Egg products without additives e.g. chilled quiches	quiche lorraine	T55	7.74	7.60	7.67	-0.14
Par baked bread products Aw >0.95	Part Bakes Rustic White Rolls x4	T56	7.72	7.76	7.74	0.04
Par baked bread products Aw >0.95	Part Bakes Rustic Seeded Rolls x4	T57	6.53	6.88	6.71	0.35
Par baked bread products Aw >0.95	Brown Baguetts Bake at Home x2	T58	8.20	8.26	8.23	0.05
Par baked bread products Aw >0.95	Bake at Home White Petit Pains x6	T59	7.56	7.40	7.48	-0.16
Par baked bread products Aw >0.95	Bake at Home Stonebaked White baguettes	T60	8.18	8.18	8.18	0.00
Ready to eat/ready to reheat foods						
Ready to eat meat and poultry	Chicken stuffing fridge raiders	T61	7.46	7.23	7.35	-0.23
Ready to eat meat and poultry	Copernicus Toruriska	**T62**	2.02	2.28	2.15	0.26
Ready to eat meat and poultry	Berklinki chicken	T63	6.54	6.30	6.42	-0.24
Ready to eat meat and poultry	Bratwurst	**T64**	5.30	5.15	5.22	-0.15
Ready to eat meat and poultry	Sliced turkey	**T65**	4.89	4.74	4.81	-0.15
Cooked and cured fish products	Cooked prawns	T66	5.40	5.32	5.36	-0.08
Cooked and cured fish products	Smoked mackarel flakes	T67	3.28	3.36	3.32	0.08
Cooked and cured fish products	sardines in brine	T68	4.26	3.93	4.09	-0.33
Cooked and cured fish products	skinless + boneless mackrel	T69	4.11	4.26	4.18	0.14
Cooked and cured fish products	sardines with sea salt	T70	4.23	4.34	4.29	0.11
Raw cured meat and poultry	Galican chorizo	T71	3.04	3.04	3.04	0.00
Raw cured meat and poultry	Spicy chorizo slices	T72	4.11	4.04	4.08	-0.07
Raw cured meat and poultry	pizza pepperoni slices	T73	5.00	4.76	4.88	-0.24
Raw cured meat and poultry	prosciutto crudo and salami platter	T74	7.20	6.93	7.07	-0.27

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Raw cured meat and poultry	salami platter	T75	6.00	5.81	5.90	-0.19
Dried cereals fruits nuts seeds and vegetables						
Dried cereals	rice snaps	**T76**	6.30	6.30	6.30	0.00
Dried cereals	corn flakes	**T77**	4.94	4.53	4.74	-0.41
Dried cereals	low fat special flakes	**T78**	3.53	2.85	3.19	-0.69
Dried cereals	malt wheats	**T110**	3.85	3.79	3.82	-0.07
Dried cereals	wheat biscuits	**T111**	2.92	2.89	2.91	-0.02
Nuts and seeds	wholefoods 4 seed mix	**T81**	5.23	5.79	5.51	0.56
Nuts and seeds	toasted 3 seed mix	**T82**	3.96	3.66	3.81	-0.30
Nuts and seeds	sunflower seeds	**T83**	3.18	2.59	2.88	-0.58
Nuts and seeds	unsalted mixed nuts	**T84**	2.96	1.90	2.43	-1.06
Nuts and seeds	cashew nuts	**T85**	5.04	5.20	5.12	0.16
Low and IF fruits aw <0.85	sultanas	**T86**	6.81	6.58	6.70	-0.23
Low and IF fruits aw <0.85	raisins	**T87**	4.51	4.46	4.48	-0.04
Low and IF fruits aw <0.85	apricots	**T88**	3.93	3.53	3.73	-0.40
Low and IF fruits aw <0.85	dried mixed fruit	**T89**	3.48	3.53	3.50	0.05
Low and IF fruits aw <0.85	organic apricots	**T90**	5.04	4.74	4.89	-0.30
Chocolate and confectionary						
Dry and sugared low moisture aw<0.85	ready to roll fonant icing	**T91**	5.68	5.76	5.72	0.07
Dry and sugared low moisture aw<0.85	marzipan	**T92**	4.98	5.08	5.03	0.10
Dry and sugared low moisture aw<0.85	vanilla frosting	**T93**	3.67	3.57	3.62	-0.10
Dry and sugared low moisture aw<0.85	chocolate frosting	**T94**	2.64	2.65	2.64	0.02
Dry and sugared low moisture aw<0.85	lemon icing	**T95**	8.52	8.77	8.64	0.25
Dry and sugared low moisture aw<0.65	maple syrup	**T96**	3.63	3.40	3.52	-0.24
Dry and sugared low moisture aw<0.65	golden syrup	**T97**	2.65	2.46	2.56	-0.19

OPYM - 54h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Dry and sugared low moisture aw<0.65	raspberry syrup	**T98**	8.34	8.30	8.32	-0.04
Dry and sugared low moisture aw<0.65	black treacle	**T99**	2.41	2.32	2.37	-0.09
Dry and sugared low moisture aw<0.65	honey	**T100**	7.63	7.81	7.72	0.18
Dry powders e.g. cake mixes	choc chip cookie mix	**T101**	6.85	6.78	6.81	-0.07
Dry powders e.g. cake mixes	choc cake mix	**T102**	6.28	6.36	6.32	0.08
Dry powders e.g. cake mixes	vanilla cupcake mix	**T103**	6.15	6.00	6.07	-0.15
Dry powders e.g. cake mixes	lemon drizzle mix	**T104**	5.48	5.68	5.58	0.20
Dry powders e.g. cake mixes	victoria sponge mix	**T105**	4.72	4.62	4.67	-0.09

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Heat processed milk and dairy products						
Pasteurised dairy product	british whole milk	**T1**	6.23	6.11	6.17	-0.12
Pasteurised dairy product	vanillalicious milkshake	**T2**	5.23	5.04	5.14	-0.19
Pasteurised dairy product	british double cream	**T3**	4.45	4.51	4.48	0.06
Pasteurised dairy product	soured cream	**T4**	3.18	3.26	3.22	0.08
Pasteurised dairy product	ice cream	**T5**	2.16	2.30	2.23	0.14
Cheese	blue stilton cheese	T6	7.18	7.34	7.26	0.17
Cheese	cottage cheese, fat free	**T7**	3.41	3.32	3.37	-0.09
Cheese	British Mature grated cheddar cheese	**T8**	5.36	5.00	5.18	-0.36
Cheese	Dairy soft cheese	T9	8.51	8.34	8.42	-0.16

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Cheese	grated four cheese mix	**T10**	5.04	5.04	5.04	0.00
Fermented and acidfied pasteurised milk and yogurt	peach yogurt drink	**T11**	5.36	5.23	5.30	-0.13
Fermented and acidfied pasteurised milk and yogurt	strawberry yogurt	**T12**	4.49	4.36	4.43	-0.13
Fermented and acidfied pasteurised milk and yogurt	mango yogurt	**T13**	3.28	3.43	3.36	0.15
Fermented and acidfied pasteurised milk and yogurt	apricot yogurt	**T14**	2.23	2.43	2.33	0.20
Fermented and acidfied pasteurised milk and yogurt	kiwi yogurt	**T15**	7.36	7.54	7.45	0.18
Fresh produce and fruits						
Fresh fruit salad and fruit puree	blueberries	T16	5.66	5.28	5.47	-0.38
Fresh fruit salad and fruit puree	strawberries	T17	4.68	4.67	4.68	-0.01
Fresh fruit salad and fruit puree	berry fruit salad	T18	5.11	5.20	5.16	0.09
Fresh fruit salad and fruit puree	apple banana strawberry and grape	**T19**	3.86	4.18	4.02	0.32
Fresh fruit salad and fruit puree	melon kiwi and strawberry	T20	4.04	4.00	4.02	-0.04
Chilled fruit juices	apple and Raspberry juice	T21	1.85	1.70	1.77	-0.15
Chilled fruit juices	pure orange	T22	2.82	2.88	2.85	0.06
Chilled fruit juices	apple and pear juice	T23	4.04	3.86	3.95	-0.18
Chilled fruit juices	multivitamin boost fruit juice	T24	5.00	4.86	4.93	-0.14
Chilled fruit juices	pure orange and mango fruit juice	T25	5.91	5.89	5.90	-0.03
Fermented vegetables	sauerkraut with carrot	T26	2.70	2.95	2.83	0.26
Fermented vegetables	Raw Kimchi	T27	5.77	5.94	5.86	0.17
Fermented vegetables	Pitted Green Olives	T28	5.76	5.56	5.66	-0.20
Fermented vegetables	Pitted Black Olives	T29	4.79	4.84	4.81	0.05
Fermented vegetables	Kimchi Spicy	T30	5.11	5.00	5.05	-0.12
Multicomponent foods or meal components						

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Composite foods with raw ingredients	ham sandwich no mayo	T31	7.64	7.49	7.57	-0.15
Composite foods with raw ingredients	seafood cocktail sandwich	T32	8.04	7.74	7.89	-0.30
Composite foods with raw ingredients	red leicester ploughman's sandwich	T33	8.56	8.65	8.60	0.10
Composite foods with raw ingredients	pomodorino tomato and sweet pepper salad	T34	7.83	7.58	7.71	-0.25
Composite foods with raw ingredients	mediterranean salad	T35	7.76	7.86	7.81	0.10
Mayonnaise based chilled salads	potato salad	T36	4.40	4.26	4.33	-0.14
Mayonnaise based chilled salads	potato salad	T37	7.20	6.98	7.09	-0.22
Mayonnaise based chilled salads	potato and egg salad	T38	5.76	5.88	5.82	0.12
Mayonnaise based chilled salads	ham egg and coleslaw salad	T39	7.66	7.49	7.58	-0.17
Mayonnaise based chilled salads	coleslaw	T40	7.83	7.83	7.83	-0.01
Ambient stable acidified foods	mayonnaise squeezy	**T41**	4.43	4.48	4.45	0.05
Ambient stable acidified foods	sweet chilli sauce	**T42**	3.69	3.68	3.69	-0.01
Ambient stable acidified foods	tartare sauce	**T43**	3.18	3.18	3.18	0.00
Ambient stable acidified foods	bbq sauce	**T44**	3.40	3.36	3.38	-0.04
Ambient stable acidified foods	tomato ketchup	**T45**	3.69	3.72	3.70	0.03
Confectionary, bakery and eggs						
Bakery products with custard	Fresh Custard Slices	T46	8.57	8.59	8.58	0.02
Bakery products with custard	Egg custard tarts	**T47**	3.23	3.11	3.17	-0.12
Bakery products with custard	custard tart	**T48**	4.15	4.11	4.13	-0.03
Bakery products with custard	Portuguese custard tart	T49	6.83	6.74	6.78	-0.09
Bakery products with custard	Pastel de nata	T50	6.63	6.57	6.60	-0.07
Egg products without additives e.g. chilled quiches	broccoli and tomato quiche	T51	7.30	7.53	7.42	0.23
Egg products without additives e.g. chilled quiches	crustless spinach & feta quiche	T52	7.62	7.64	7.63	0.02

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Egg products without additives e.g. chilled quiches	crustless broccoli and tomato quiche	T53	5.71	5.75	5.73	0.04
Egg products without additives e.g. chilled quiches	crustless quiche lorraine	T54	7.85	7.72	7.78	-0.13
Egg products without additives e.g. chilled quiches	quiche lorraine	T55	7.74	7.60	7.67	-0.14
Par baked bread products Aw >0.95	Part Bakes Rustic White Rolls x4	T56	7.72	7.76	7.74	0.04
Par baked bread products Aw >0.95	Part Bakes Rustic Seeded Rolls x4	T57	6.53	6.91	6.72	0.38
Par baked bread products Aw >0.95	Brown Baguetts Bake at Home x2	T58	8.20	8.30	8.25	0.10
Par baked bread products Aw >0.95	Bake at Home White Petit Pains x6	T59	7.56	7.48	7.52	-0.08
Par baked bread products Aw >0.95	Bake at Home Stonebaked White baguettes	T60	8.18	8.18	8.18	0.00
Ready to eat/ready to reheat foods						
Ready to eat meat and poultry	Chicken stuffing fridge raiders	T61	7.46	7.34	7.40	-0.12
Ready to eat meat and poultry	Copernicus Toruriska	**T62**	2.02	2.30	2.16	0.28
Ready to eat meat and poultry	Berklinki chicken	T63	6.54	6.38	6.46	-0.16
Ready to eat meat and poultry	Bratwurst	**T64**	5.30	5.28	5.29	-0.02
Ready to eat meat and poultry	Sliced turkey	**T65**	4.89	4.79	4.84	-0.09
Cooked and cured fish products	Cooked prawns	T66	5.40	5.59	5.49	0.19
Cooked and cured fish products	Smoked mackarel flakes	T67	3.28	3.36	3.32	0.08
Cooked and cured fish products	sardines in brine	T68	4.26	3.95	4.10	-0.31
Cooked and cured fish products	skinless + boneless mackrel	T69	4.11	4.26	4.18	0.14
Cooked and cured fish products	sardines with sea salt	T70	4.23	4.34	4.29	0.11
Raw cured meat and poultry	Galican chorizo	T71	3.04	3.04	3.04	0.00
Raw cured meat and poultry	Spicy chorizo slices	T72	4.11	4.08	4.10	-0.03

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Raw cured meat and poultry	pizza pepperoni slices	T73	5.00	4.88	4.94	-0.12
Raw cured meat and poultry	prosciutto crudo and salami platter	T74	7.20	7.04	7.12	-0.16
Raw cured meat and poultry	salami platter	T75	6.00	5.83	5.91	-0.17
Dried cereals fruits nuts seeds and vegetables						
Dried cereals	rice snaps	**T76**	6.30	6.32	6.31	0.02
Dried cereals	corn flakes	**T77**	4.94	5.00	4.97	0.06
Dried cereals	low fat special flakes	**T78**	3.53	3.34	3.44	-0.19
Dried cereals	malt wheats	**T79**	2.96	2.74	2.85	-0.22
Dried cereals	wheat biscuits	**T80**	4.56	4.34	4.45	-0.21
Nuts and seeds	wholefoods 4 seed mix	**T81**	5.23	5.15	5.19	-0.08
Nuts and seeds	toasted 3 seed mix	**T82**	3.96	4.08	4.02	0.12
Nuts and seeds	sunflower seeds	**T83**	3.18	3.30	3.24	0.12
Nuts and seeds	unsalted mixed nuts	**T84**	2.96	2.92	2.94	-0.04
Nuts and seeds	cashew nuts	**T85**	5.04	5.20	5.12	0.16
Low and IF fruits aw <0.85	sultanas	**T86**	6.81	6.88	6.84	0.06
Low and IF fruits aw <0.85	raisins	**T87**	4.51	4.56	4.53	0.05
Low and IF fruits aw <0.85	apricots	**T88**	3.93	3.88	3.90	-0.05
Low and IF fruits aw <0.85	dried mixed fruit	**T89**	3.48	3.57	3.52	0.09
Low and IF fruits aw <0.85	organic apricots	**T90**	5.04	5.00	5.02	-0.04
Chocolate and confectionary						
Dry and sugared low moisture aw<0.85	ready to roll fonant icing	**T91**	5.68	5.76	5.72	0.07
Dry and sugared low moisture aw<0.85	marzipan	**T92**	4.98	5.08	5.03	0.10
Dry and sugared low moisture aw<0.85	vanilla frosting	**T93**	3.67	3.57	3.62	-0.10
Dry and sugared low moisture aw<0.85	chocolate frosting	**T94**	2.64	2.65	2.64	0.02

OPYM - 72h pour						
Type	**Item**	**Sample code**	**log(Ref)**	**log(Alt)**	**Mean**	**Difference**
Dry and sugared low moisture aw<0.85	lemon icing	**T95**	8.52	8.83	8.68	0.31
Dry and sugared low moisture aw<0.65	maple syrup	**T96**	3.63	3.41	3.52	-0.22
Dry and sugared low moisture aw<0.65	golden syrup	**T97**	2.65	2.43	2.54	-0.23
Dry and sugared low moisture aw<0.65	raspberry syrup	**T98**	8.34	8.38	8.36	0.04
Dry and sugared low moisture aw<0.65	black treacle	**T99**	2.41	2.36	2.39	-0.05
Dry and sugared low moisture aw<0.65	honey	**T100**	7.63	7.88	7.76	0.25
Dry powders e.g. cake mixes	choc chip cookie mix	**T101**	6.85	6.82	6.84	-0.03
Dry powders e.g. cake mixes	choc cake mix	**T102**	6.28	6.40	6.34	0.12
Dry powders e.g. cake mixes	vanilla cupcake mix	**T103**	6.15	6.00	6.07	-0.15
Dry powders e.g. cake mixes	lemon drizzle mix	**T104**	5.48	5.68	5.58	0.20
Dry powders e.g. cake mixes	victoria sponge mix	**T105**	4.72	4.62	4.67	-0.09

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ANNEX D: Summary tables accuracy profile study

(Food) Category 1	Dairy
(Food) Type 1	54 hour pour

			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
4 a-e	Cream cheese	low	70	65	65	90	105	85	70	65	50	65
1 a-e	Kefir	low	1400	1400	1100	1200	1100	1200	1000	804	891	791
2 a-e	Kefir	interme diate	66000	84000	74000	65000	57000	64000	73000	70000	45000	76000
3 a-e	Kefir	high	1100000	1300000	1100000	1100000	1600000	1000000	1100000	1200000	1000000	1100000
5 a-e	Cream cheese	interme diate	3300000	1800000	3100000	4300000	2900000	2500000	2000000	2600000	3700000	1700000
6 a-e	Cream cheese	high	25000000	9300000	20000000	24000000	28000000	21000000	9400000	16000000	32000000	26000000

(Food) Category 2	Dairy
(Food) Type 2	72 hour pour

			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
4 a-e	Cream cheese	low	70	65	65	90	105	90	75	70	50	70
1 a-e	Kefir	low	1400	1400	1100	1200	1100	1300	1300	1100	1100	1000
2 a-e	Kefir	interme diate	66000	84000	74000	65000	57000	65000	73000	70000	49000	76000
3 a-e	Kefir	high	1100000	1300000	1100000	1100000	1600000	1000000	1200000	1000000	1100000	1100000
5 a-e	Cream cheese	interme diate	3300000	1800000	3100000	4300000	2900000	2500000	2000000	2700000	3700000	1700000
6 a-e	Cream cheese	high	25000000	9300000	20000000	24000000	28000000	21000000	9500000	16000000	32000000	26000000

(Food) Category 3	Heat processed
(Food) Type 3	54 hour spread

			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
4 a-e	Cream cheese	low	70	65	65	90	105	60	80	75	75	80
1 a-e	Kefir	low	1400	1400	1100	1200	1100	1400	1400	1100	1500	1100
2 a-e	Kefir	interme diate	66000	84000	74000	65000	57000	55000	87000	86000	62000	70000
3 a-e	Kefir	high	1100000	1300000	1100000	1100000	1600000	1300000	1400000	1200000	1000000	1500000
5 a-e	Cream cheese	interme diate	3300000	1800000	3100000	4300000	2900000	3400000	1700000	2500000	3700000	2700000
6 a-e	Cream cheese	high	25000000	9300000	20000000	24000000	28000000	18000000	9700000	16000000	21000000	27000000

(Food) Category 4	Heat processed
(Food) Type 4	72 hour spread

			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
4 a-e	Cream cheese	low	70	65	65	90	105	60	80	75	75	80
1 a-e	Kefir	low	1400	1400	1100	1200	1100	1400	1400	1100	1500	1200
2 a-e	Kefir	interme diate	66000	84000	74000	65000	57000	55000	88000	89000	64000	77000
3 a-e	Kefir	high	1100000	1300000	1100000	1100000	1600000	1300000	1400000	1200000	1100000	1500000
5 a-e	Cream cheese	interme diate	3300000	1800000	3100000	4300000	2900000	3500000	1700000	2500000	3800000	2700000
6 a-e	Cream cheese	high	25000000	9300000	20000000	24000000	28000000	18000000	9700000	17000000	22000000	27000000

(Food) Category 6		Fresh produce										
(Food) Type 6		54 hour pour										
			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
10 a-e	beetroot salad	low	165	125	120	150	125	60	110	60	40	100
9 a-e	vegetable juice	low	530	633	447	467	660	510	480	630	620	610
7 a-e	vegetable juice	interme diate	42000	45000	58000	52000	46000	50000	39000	36000	53000	45000
11 a-e	beetroot salad	interme diate	140000	150000	170000	180000	260000	140000	150000	170000	140000	140000
8 a-e	vegetable juice	high	3200000	3100000	2300000	1900000	3700000	3600000	2100000	2000000	3400000	3900000
12 a-e	beetroot salad	high	250000000	310000000	190000000	320000000	160000000	220000000	430000000	300000000	540000000	350000000

(Food) Category 2		Fresh produce										
(Food) Type 2		72 hour pour										
			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
10 a-e	beetroot salad	low	165	125	120	150	125	80	130	120	170	140
9 a-e	vegetable juice	low	530	633	447	467	660	520	480	660	630	640
7 a-e	vegetable juice	interme diate	42000	45000	58000	52000	46000	51000	42000	36000	56000	48000
11 a-e	beetroot salad	interme diate	140000	150000	170000	180000	260000	140000	150000	170000	150000	140000
8 a-e	vegetable juice	high	3200000	3100000	2300000	1900000	3700000	3900000	2200000	2000000	3400000	4100000
12 a-e	beetroot salad	high	250000000	310000000	190000000	320000000	160000000	220000000	430000000	300000000	540000000	350000000

(Food) Category 8		Fresh produce										
(Food) Type 8		54h spread										
			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
10 a-e	beetroot salad	low	165	125	120	150	125	140	120	240	160	340
9 a-e	vegetable juice	low	530	633	447	467	660	670	610	530	640	770
7 a-e	vegetable juice	interme diate	42000	45000	58000	52000	46000	59000	59000	54000	51000	56000
11 a-e	beetroot salad	interme diate	140000	150000	170000	180000	260000	130000	69000	120000	180000	100000
8 a-e	vegetable juice	high	3200000	3100000	2300000	1900000	3700000	3400000	2500000	2800000	3400000	2800000
12 a-e	beetroot salad	high	250000000	310000000	190000000	320000000	160000000	290000000	350000000	280000000	350000000	290000000

(Food) Category 4		Fresh produce										
(Food) Type 4		72h spread										
			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
10 a-e	beetroot salad	low	165	125	120	150	125	140	120	240	160	140
9 a-e	vegetable juice	low	530	633	447	467	660	670	660	550	660	770
7 a-e	vegetable juice	interme diate	42000	45000	58000	52000	46000	59000	59000	54000	51000	56000
11 a-e	beetroot salad	interme diate	140000	150000	170000	180000	260000	180000	79000	220000	210000	100000
8 a-e	vegetable juice	high	3200000	3100000	2300000	1900000	3700000	3400000	2500000	2800000	3400000	2800000
12 a-e	beetroot salad	high	250000000	310000000	190000000	320000000	160000000	290000000	350000000	280000000	350000000	290000000

(Food) Category 1	bakery and eggs
(Food) Type 1	54 hour pour

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
19 a-e	quiche	low	1200	1400	970	1400	1000	1400	2000	900	1500	760
24 a-e	custard tart	low	1600	1600	1700	1500	1200	1100	1800	1700	1300	700
21 a-e	quiche	interme diate	26000	16000	19000	11000	150000	17000	15000	17000	8000	20000
22 a-e	custard tart	interme diate	23000	27000	35000	32000	25000	15000	27000	29000	19000	23000
20 a-e	quiche	high	110000	650000	40000	90000	70000	400000	70000	80000	100000	80000
23 a-e	custard tart	high	290000	200000	280000	1600000	300000	160000	150000	220000	1800000	210000

(Food) Category 2	bakery and eggs
(Food) Type 2	72 hour pour

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
19 a-e	quiche	low	1200	1400	970	1400	1000	1700	1400	1600	1700	1700
24 a-e	custard tart	low	1600	1600	1700	1500	1200	1100	1800	1700	1300	710
21 a-e	quiche	interme diate	26000	16000	19000	11000	150000	17000	15000	17000	8000	20000
22 a-e	custard tart	interme diate	23000	27000	35000	32000	25000	15000	27000	29000	19000	23000
20 a-e	quiche	high	110000	650000	40000	90000	70000	70000	400000	90000	100000	80000
23 a-e	custard tart	high	290000	200000	280000	1600000	300000	180000	160000	220000	1800000	220000

(Food) Category 3	bakery and eggs
(Food) Type 3	54 hour spread

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
19 a-e	quiche	low	1200	1400	970	1400	1000	1200	1200	1300	1800	1100
24 a-e	custard tart	low	1600	1600	1700	1500	1200	1600	1500	1400	1300	910
21 a-e	quiche	interme diate	26000	16000	19000	11000	150000	17000	15000	16000	9000	180000
22 a-e	custard tart	interme diate	23000	27000	35000	32000	25000	19000	31000	32000	24000	16000
20 a-e	quiche	high	110000	650000	40000	90000	70000	60000	610000	80000	110000	120000
23 a-e	custard tart	high	290000	200000	280000	1600000	300000	320000	200000	220000	1800000	6000

(Food) Category 4	bakery and eggs
(Food) Type 4	72 hour spread

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
19 a-e	quiche	low	1200	1400	970	1400	1000	1200	1200	1200	1800	1100
24 a-e	custard tart	low	1600	1600	1700	1500	1200	1600	1500	1400	1300	910
21 a-e	quiche	interme diate	26000	16000	19000	11000	150000	17000	15000	16000	9000	180000
22 a-e	custard tart	interme diate	23000	27000	35000	32000	25000	20000	31000	32000	24000	16000
20 a-e	quiche	high	110000	650000	40000	90000	70000	60000	610000	80000	110000	120000
23 a-e	custard tart	high	290000	200000	280000	1600000	300000	320000	200000	220000	1800000	200000

(Food) Category 1	RTE RTRH foods
(Food) Type 1	54 hour pour

			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
25 a-e	Cooked prawns	low	440	390	570	660	510	350	320	330	510	450
28 a-e	Fish pate	low	930	760	1100	910	1600	830	750	720	920	890
29 a-e	Fish pate	interme diate	62000	4900	50000	40000	37000	48000	35000	42000	33000	41000
26 a-e	Cooked prawns	interme diate	320000	230000	320000	190000	290000	390000	230000	270000	330000	300000
27 a-e	Cooked prawns	high	820000	670000	980000	1100000	740000	710000	570000	860000	860000	670000
30 a-e	Fish pate	high	1000000	1000000	760000	950000	950000	640000	610000	490000	710000	520000

(Food) Category 2	RTE RTRH foods
(Food) Type 2	72 hour pour

			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
25 a-e	Cooked prawns	low	440	390	570	660	510	360	340	330	510	450
28 a-e	Fish pate	low	930	760	1100	910	1600	830	750	740	950	940
29 a-e	Fish pate	interme diate	62000	4900	50000	40000	37000	48000	35000	42000	34000	41000
26 a-e	Cooked prawns	interme diate	320000	230000	320000	190000	290000	440000	320000	390000	660000	410000
27 a-e	Cooked prawns	high	820000	670000	980000	1100000	740000	760000	570000	860000	890000	690000
30 a-e	Fish pate	high	1000000	1000000	760000	950000	950000	640000	610000	490000	710000	520000

(Food) Category 3	RTE RTRH foods
(Food) Type 3	54 hour spread

			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
25 a-e	Cooked prawns	low	440	390	570	660	510	450	530	460	450	560
28 a-e	Fish pate	low	930	760	1100	910	1600	2000	1500	1100	960	1200
29 a-e	Fish pate	interme diate	62000	4900	50000	40000	37000	57000	32000	46000	56000	41000
26 a-e	Cooked prawns	interme diate	320000	230000	320000	190000	290000	210000	240000	260000	280000	210000
27 a-e	Cooked prawns	high	820000	670000	980000	1100000	740000	850000	660000	910000	1700000	700000
30 a-e	Fish pate	high	1000000	1000000	760000	950000	950000	1000000	820000	830000	770000	820000

(Food) Category 4	RTE RTRH foods
(Food) Type 4	72 hour spread

			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
25 a-e	Cooked prawns	low	440	390	570	660	510	490	530	460	450	580
28 a-e	Fish pate	low	930	760	1100	910	1600	2000	1500	1200	1000	1200
29 a-e	Fish pate	interme diate	62000	4900	50000	40000	37000	58000	33000	46000	56000	41000
26 a-e	Cooked prawns	interme diate	320000	230000	320000	190000	290000	360000	270000	260000	360000	320000
27 a-e	Cooked prawns	high	820000	670000	980000	1100000	740000	860000	710000	910000	1700000	700000
30 a-e	Fish pate	high	1000000	1000000	760000	950000	950000	1000000	820000	830000	770000	830000

(Food) Category 1	multicomponent
(Food) Type 1	54 hour pour

			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 a-e	cous cous salad	low	370	537	155	227	373	250	170	130	130	260
13 a-e	pasta salad	low	1100	2200	1200	1300	1500	1200	1000	650	940	1700
17 a-e	cous cous salad	interme diate	5000	12000	7700	8500	5300	9000	33000	13000	21000	14000
14 a-e	pasta salad	interme diate	120000	140000	130000	130000	120000	120000	120000	120000	130000	120000
18 a-e	cous cous salad	high	2700000	2200000	2400000	1200000	2300000	2300000	2400000	1100000	780000	2600000
15 a-e	pasta salad	high	7600000	3400000	4500000	3500000	5400000	4200000	2800000	3800000	2600000	3000000

(Food) Category 2	multicomponent
(Food) Type 2	72 hour pour

			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 a-e	cous cous salad	low	370	537	155	227	373	370	240	140	150	330
13 a-e	pasta salad	low	1100	2200	1200	1300	1500	1700	1300	1300	1500	1500
17 a-e	cous cous salad	interme diate	5000	12000	7700	8500	5300	9000	33000	14000	21000	15000
14 a-e	pasta salad	interme diate	120000	140000	130000	130000	120000	160000	130000	120000	140000	150000
18 a-e	cous cous salad	high	2700000	2200000	2400000	1200000	2300000	2600000	2400000	1100000	880000	2600000
15 a-e	pasta salad	high	7600000	3400000	4500000	3500000	5400000	5800000	3300000	4600000	2700000	3700000

(Food) Category 3	multicomponent
(Food) Type 3	54 hour spread

			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 a-e	cous cous salad	low	370	537	155	227	373	370	180	180	170	260
13 a-e	pasta salad	low	1100	2200	1200	1300	1500	1900	1600	1200	1700	1600
17 a-e	cous cous salad	interme diate	5000	12000	7700	8500	5300	18000	18000	21000	11000	23000
14 a-e	pasta salad	interme diate	120000	140000	130000	130000	120000	120000	120000	140000	140000	120000
18 a-e	cous cous salad	high	2700000	2200000	2400000	1200000	2300000	2400000	2000000	2500000	920000	2000000
15 a-e	pasta salad	high	7600000	3400000	4500000	3500000	5400000	6500000	5100000	3200000	3000000	4400000

(Food) Category 4	multicomponent
(Food) Type 4	72 hour spread

			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 a-e	cous cous salad	low	370	537	155	227	373	390	340	300	220	500
13 a-e	pasta salad	low	1100	2200	1200	1300	1500	1900	1600	1200	1700	1600
17 a-e	cous cous salad	interme diate	5000	12000	7700	8500	5300	19000	18000	21000	11000	24000
14 a-e	pasta salad	interme diate	120000	140000	130000	130000	120000	120000	150000	140000	140000	120000
18 a-e	cous cous salad	high	2700000	2200000	2400000	1200000	2300000	2500000	2100000	2500000	980000	2000000
15 a-e	pasta salad	high	7600000	3400000	4500000	3500000	5400000	6500000	5100000	3200000	3000000	4400000

(Food) Category 1	dried cereals nuts
(Food) Type 1	54 hour pour

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
31 a-e	3 seed mix	low	150	215	128	145	50	110	120	160	150	130
34 a-e	4 seed mix	low	1000	880	1000	770	1100	800	670	640	490	640
35 a-e	4 seed mix	interme diate	20000	14000	18000	9000	7000	12000	13000	27000	16000	17000
32 a-e	3 seed mix	interme diate	20000	20000	21000	16000	18000	15000	17000	16000	14000	20000
36 a-e	4 seed mix	high	380000	440000	820000	430000	410000	370000	370000	540000	390000	260000
33 a-e	3 seed mix	high	690000	860000	810000	960000	1000000	660000	520000	570000	680000	790000

(Food) Category 2	dried cereals nuts
(Food) Type 2	72 hour pour

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
31 a-e	3 seed mix	low	150	215	128	145	50	120	120	160	150	130
34 a-e	4 seed mix	low	1000	880	1000	770	1100	800	670	640	500	490
35 a-e	4 seed mix	interme diate	20000	14000	18000	9000	7000	12000	13000	28000	16000	17000
32 a-e	3 seed mix	interme diate	20000	20000	21000	16000	18000	15000	17000	16000	15000	20000
36 a-e	4 seed mix	high	380000	440000	820000	430000	410000	370000	370000	560000	390000	270000
33 a-e	3 seed mix	high	690000	860000	810000	960000	1000000	660000	520000	570000	680000	790000

(Food) Category 3	dried cereals nuts
(Food) Type 3	54 hour spread

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
31 a-e	3 seed mix	low	150	215	128	145	50	140	200	180	110	120
34 a-e	4 seed mix	low	1000	880	1000	770	1100	900	900	800	700	1200
35 a-e	4 seed mix	interme diate	20000	14000	18000	9000	7000	22000	32000	23000	21000	22000
32 a-e	3 seed mix	interme diate	20000	20000	21000	16000	18000	23000	17000	22000	11000	23000
36 a-e	4 seed mix	high	380000	440000	820000	430000	410000	340000	460000	620000	550000	280000
33 a-e	3 seed mix	high	690000	860000	810000	960000	1000000	840000	860000	1000000	830000	720000

(Food) Category 4	dried cereals nuts
(Food) Type 4	72 hour spread

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
31 a-e	3 seed mix	low	150	215	128	145	50	90	200	180	110	120
34 a-e	4 seed mix	low	1000	880	1000	770	1100	900	900	800	700	1200
35 a-e	4 seed mix	interme diate	20000	14000	18000	9000	7000	22000	32000	23000	21000	22000
32 a-e	3 seed mix	interme diate	20000	20000	21000	16000	18000	23000	17000	22000	11000	23000
36 a-e	4 seed mix	high	380000	440000	820000	430000	410000	340000	460000	620000	550000	280000
33 a-e	3 seed mix	high	690000	860000	810000	960000	1000000	840000	860000	1000000	830000	720000

MICROVAL® NEN

ANNEX E: Samples not used in calculations

Sample code	Item	Strain	CRA code	origin	OPYM 1ml pour 54 hour				OPYM 0.1ml spread 54 hour				DG18 or DRBCA (reference) 0.1ml spread					
					-1	-2	count cfu/g	log cfu/g	1ml -1	-1	count cfu/g	log cfu/g	1ml -1	1ML -1	-1	-2	count cfu/g	log cfu/g
T79	malt wheats	*Eurotium amstelodami*	DSM62632	Mouldy kernel	0	0	<1.00E+01	1.0	6	0	600	2.8		82	10	0	9.10E+02	3.0
T80	wheat biscuits	*Eurotium amstelodami*	DSM62633	Mouldy kernel	0	0	<1.00E+01	1.0	0	0	10	<1.0	T	T	T	36	3.60E+04	4.6
T84	unsalted mixed nuts	*Aspergillus restrictus*	ATCC42696	Dried chillies	8	2	80	1.9	49	7	490	2.7		104	8	2	920	3.0

ANNEX F: Summary of inclusivity and exclusivity data

Number	Inclusivity Organism	CRA code	Source	OPYM spread - 54h (log cfu/ml)	OPYM pour - 54h (log cfu/ml)	OPYM spread - 72h (log cfu/ml)	OPYM pour - 72h (log cfu/ml)	DG18 reference method (log cfu/ml)	DRBCA - Reference method (log cfu/ml)	Non selective - MEA (log cfu/ml)
1.	*Aureobasidium pullulans*	16148	Soft drinks factory	7.5	7.2	7.5	7.3	7.5	7.5	7.3
2.	*Aspergillus niger*	16667	Grapes	6.5	6.4	6.5	6.4	6.5	6.7	6.4
3.	*Aspergillus brasiliensis*	17002	Plants	6.6	6.4	6.6	6.4	6.6	6.4	6.3
4.	*Aspergillus restrictus*	ATCC42693	Dried chillies	4.6	4.2	4.7	4.3	4.3	not tested	4.3
5.	*Botrytis cineria*	6728	Dried chillies	6.3	5.9	6.4	5.9	<4	6.6	7.5
6.	*Byssochlamys spectabilis*	17025	Factory isolate	6.6	6.4	6.6	6.5	6.5	6.5	6.6
7.	*Byssochlamys fulva*	16668	Pasteurised fruit juice	7.0	6.8	7.0	6.8	6.7	6.7	7.2
8.	*Candida magnoliae*	8611	Strawberry ingredient	8.2	8.1	8.3	8.2	8.2	8.2	8.1
9.	*Candida krussei*	629	Yogurt base	6.7	8.6	7.7	7.6	7.7	7.7	7.6
10.	*Candida sojae*	16138	Soft drinks factory	8.6	8.6	8.6	8.6	8.5	8.6	8.5
11.	*Candida wyomingensis*	16144	Soft drinks factory	6.8	8.8	7.8	7.8	7.8	7.8	7.8

Number	Inclusivity Organism	CRA code	Source	OPYM spread - 54h (log cfu/ml)	OPYM pour - 54h (log cfu/ml)	OPYM spread - 72h (log cfu/ml)	OPYM pour - 72h (log cfu/ml)	DG18 reference method (log cfu/ml)	DRBCA - Reference method (log cfu/ml)	Non selective - MEA (log cfu/ml)
12.	*Clavispora lusitaniae*	15967	Strawberry yogurt	7.8	7.8	7.8	7.9	7.7	7.8	7.8
13.	*Cryptococcus laurenti*	16139	Soft drinks factory	7.1	7.1	7.1	7.2	7.1	7.1	6.7
14.	*Cryptococcus uzbekistanensis*	15967	South African factory	6.5	6.5	6.5	6.5	6.4	6.4	6.4
15.	*Debaryomyces hansenii*	16833	Factory isolate	7.1	7.1	7.1	7.1	7.2	7.1	7.2
16.	*Dekkera naardensis*	3237	cola	7.5	7.7	7.5	7.7	7.5	7.8	8.0
17.	*Eurotium amstelodami*	16902	Mouldy kernel	7.1	6.4	7.1	6.4	6.2	not tested	6.1
18.	*Eurotium chevaleri*	17181	Not known	4.8	4.4	4.9	4.6	4.8	not tested	5.1
19.	*Fusarium proliferatum*	CRA16816	Grass	7.0	7.0	7.0	7.0	6.9	7.0	7.1
20.	*Fusarium solani*	16976	Factory isolate	6.8	6.9	6.9	6.9	6.9	7.0	6.8
21.	*Geotrichum conidium*	14398	Factory isolate	8.3	8.2	8.3	8.3	8.3	8.2	8.3
22.	*Hansenisapora uvarum*	15958	Factory isolate	7.0	7.3	7.0	7.3	6.9	6.7	6.0
23.	*Hansenula anomola*	626	Sugar solution	8.1	8.1	8.1	8.1	8.1	8.3	8.3
24.	*Kloeckera apiculata*	6412	Bakery isolate	7.6	7.6	7.6	7.7	7.6	7.7	7.6
25.	*Kluyveromyces lactis var. lactis*	16455	Gassy Cheese	7.7	7.7	7.8	7.7	7.8	8.2	7.7
26.	*Kluyveromyces marxianus*	6749	Dairy isolate	7.7	7.7	7.7	7.7	not tested	7.8	7.7
27.	*Metschenikowia pulcherrina*	16167	Food spoilage	6.8	6.7	6.9	6.8	6.8	6.8	6.8
28.	*Monascus ruber*	16725	Acid product	7.0	6.9	7.0	6.9	6.9	6.9	7.4
29.	*Neosartorya fischeri*	16669	Grape juice	6.2	5.9	6.2	6.0	6.3	5.9	6.2
30.	*Paecilomyces variotii*	16670	Fruit concentrate	2.7	4.2	4.3	4.2	4.6	not tested	4.7
31.	*Penicillium aurantigriseum*	16192	Acid product	5.6	5.3	5.6	5.4	5.5	5.4	5.5
32.	*Penicillium crustosum*	16671	Lemon	5.3	5.5	5.3	5.5	5.4	5.5	5.3
33.	*Penicillium expansum*	16672	Mouldy cherry juice	5.5	6.4	5.6	5.4	5.7	5.6	5.4
34.	*Penicillium roqueforti*	16726	Acidic product	6.1	6.0	6.1	6.0	6.3	6.3	6.2
35.	*Pichia anomola*	4901	Carbonated drink	7.6	7.7	7.6	7.7	7.7	7.6	7.7

Number	Inclusivity Organism	CRA code	Source	OPYM spread - 54h (log cfu/ml)	OPYM pour - 54h (log cfu/ml)	OPYM spread - 72h (log cfu/ml)	OPYM pour - 72h (log cfu/ml)	DG18 reference method (log cfu/ml)	DRBCA - Reference method (log cfu/ml)	Non selective - MEA (log cfu/ml)
36.	*Pichia galeiformis*	16015	Spoiled tomato juice	5.6	5.5	5.7	5.5	7.4	7.3	7.0
37.	*Issatchenkia orientalis*	16164	Spoiled yogurt	7.4	7.5	7.4	7.5	7.4	7.4	7.3
38.	*Rhodotorula slooffiae*	16001	Factory environment	6.6	6.6	6.6	7.5	<4	7.7	7.6
39.	*Saccharomyces bayanus*	16434	Lager production	7.3	7.3	7.3	7.3	6.9	7.1	6.9
40.	*Saccharomyces exiguus*	16017	Spoiled mayonnaise	6.9	6.7	6.9	6.7	7.3	7.3	8.0
41.	*Saccharomyces cerevisiae*	720	Frozen fruit juice	7.3	7.1	7.3	7.1	7.2	7.4	7.3
42.	*Torulaspora delbrukeii*	16154	Spoiled yogurt	7.3	7.2	7.3	7.2	7.2	7.2	7.1
43.	*Trichosporon jirovecii*	15964	Powder	7.2	7.1	7.2	7.1	7.1	7.1	7.1
44.	*Trichosporon coremiiforme*	15962	Powder	7.0	6.9	7.0	6.9	6.9	7.0	6.9
45.	*Yarrowia lipolytica*	16146	Soft drinks factory	7.3	7.3	7.5	7.3	7.4	7.4	7.4
46.	*Wallemia sebi*	17533	Blackcurrant jelly	6.9	6.9	6.9	6.9	5.9	not tested	5.9
47.	*Willopsis saturnas*	6423	Bakery isolate	7.4	7.4	7.4	6.3	7.3	7.5	7.3
48.	*Eurotium repens*	17174	unkown	4.2	5.3	4.2	5.3	6.0	Not tested	5.4
49.	*Zygosaccharomyces baili*	16124	Nougat	6.6	6.7	6.6	6.8	6.7	6.6	7.1
50.	Zygosaccharomyces rouxii	16125	Spoiled fruit	7.7	7.7	7.8	7.8	6.9	6.9	6.9