Method Comparison Study Report for the ISO 16140-2:2016 validation of Compact Dry YMR for enumeration of yeasts and moulds in a broad range of foods with an aw >0.95

MicroVal study number: 2016 LR61

Method/Kit name: Compact Dry YMR

Report version:MCS/ILS renewal report containing includivity data 30/09/2021[v2]

MicroVal Expert Laboratory:

MCS and ILS study

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Inclusivity/Exclusivity studies

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Foreword

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

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Method/Kit name: Compact Dry YMR

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 21527-1:2008 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Colony count technique in products with water activity greater than 0.95.

Scope of validation: Broad range of foods covering

- Dairy Products
- Confectionary, bakery and eggs
- Fruits and Vegetables
- Ready to eat Foods
- Multicomponent foods

Certification orgnization: Lloyd's Register

List of abbreviations

-	AL	Acceptability Limit
-	AP	Accuracy Profile
-	Art. Cont.	Artificial contamination
-	CFU	Colony Forming Units
-	CL	confidence limit (usually 95%)
-	EL	Expert Laboratory
-	$\bar{D}$	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

Contents

1 Introduction

This proposal describes the work carried out for a MicroVal validation study, (based on ISO 16140-2:2016), protocol for validation of alternative methods) for the enumeration of yeasts and moulds in a broad range of foods. The study was carried out by Campden BRI as the MicroVal Expert Laboratory. In addition, studies on Inclusivity and Exclusivity were carried out by Q Laboratories.

The study involved the enumeration of yeasts and moulds and so the requirements of the Quantitative protocol were carried out.

The alternative method used was: Compact Dry YMR. This is a chromogenic medium for the enumeration of yeasts and moulds Characteristic yeast colonies appear blue and moulds form cottony colonies with characteristic mould colours.

The reference method used was: ISO 21527-1:2008 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Colony count technique in products with water activity greater than 0.95.

Scope of the validation study was: A broad range of foods

Categories included:

- Dairy Products
- Confectionary, bakery and eggs
- Fruits and Vegetables
- Ready to eat Foods
- Multicomponent foods

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Limits of quantification (LOQ);
- Inclusivity and exclusivity
- ILS

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

The alternative method Compact Dry YMR shows comparable performance to the reference method ISO 21527-1:2008 for the enumeration of yeasts and moulds in a broad range of foods.

Overall, the conclusions for the Method Comparison Study and ILS are:

- The alternative CD YMR enumeration method for yeasts and moulds shows satisfactory results for relative trueness.
- The alternative CD YMR enumeration method for yeasts and moulds shows satisfactory results for accuracy profile.
- The alternative CD YMR enumeration method for yeasts and moulds is selective and specific

Note: this is a revision of the original Microval report to include an inclusivity and exclusivity study. Whilst an inclusivity / exclusivity study is not strictly required by ISO16140-2:2016 for total count methods, it is a MicroVal requirement that all yeast and mould alternative methods include this an inclusivity and exclusivity study.

2 Method protocols

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

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

2.1 Reference method

The reference method was ISO 21527-1: Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Colony count technique in products with water activity greater than 0.95. This method was used as a total count for all yeasts and mould present in food products with an aw of >0.95 and with the capability to grow within 5 days at 25°C.

Sample preparations used in the reference method and the alternative method were done according to ISO 6887-series for all sample matrices in this proposal.

2.2 Alternative method

Compact Dry plates are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the centre of the self-diffusible medium. The Compact Dry YMR method contains chromogenic medium and selective agents for the detection and enumeration of yeasts and moulds. Yeasts grow as blue colonies and moulds form cottony colonies with characteristic colours.

Note: The kit insert recommends that the plates can incubated at 25±1ºC for 2 to 3 days as in some circumstances a shorter incubation period is possible. In this study only the 3-day incubation period was validated for a broad range of foods (aw>0.95).

The flow diagram is given in Annex A

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

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

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

3.1.1 Number of samples

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

Table 1 – Categories, types and number of samples analysed

Categories	Types	No of samples analysed	No of samples interpreted	ISO 6887 used
Dairy products	Cheese e.g. grated cheese, soft cheese, blue cheese	14	14	6887-5
	Yogurts with fruit	5	5	6887-5
	Fermented milk drinks	5	4	6887-5
	Total	**24**	**23**	
Confectionery, bakery and eggs	Bakery products with custard	5	5	6887-2
	Egg products without additives e.g. chilled quiches	5	5	6887-2
	Par baked egg products	5	5	6887-2
	Total	**15**	**15**	
Fruits and vegetables	Fresh fruit salad and fruit purees	6	6	6887-2
	Chilled fruit juices	5	4	6887-2

Categories	Types	No of samples analysed	No of samples interpreted	ISO 6887 used
	Fermented vegetables e.g. sauerkraut, olives	5	4	6887-2
	Total	**16**	**14**	
Ready to eat foods	Ready to eat meat and poultry e.g. turkey fillet, pate	5	5	6887-2
	Cooked and cured fish products e.g. roll herring, seafood terrine	5	5	6887-3
	Cured meats e.g. salami, ham	5	5	6887-2
	Total	**15**	**15**	
Multi component foods	Composite foods with raw ingredients e.g. sandwiches, pasta salads.	6	6	6887-2
	Mayonnaise based chilled salads	5	4	6887-2
	Ambient stable acidified foods e.g. ketchup	5	3	6887-2
	Total	**15**	**13**	
TOTAL		**85**	**80**	

Eighty five samples were analysed, leading to 80 interpretable results.

3.1.2 Test sample preparation

All of the samples tested in the relative trueness study were naturally contaminated samples. The water activity of representative food types within each category were measured to ensure they were aw >0.95. This information is shown in Annex C.

In accordance with ISO 16140-2, a minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, made up of at least three types with at least 5 interpretable results per type.

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

3.1.3 Protocols applied during the validation study.

Incubation time

The incubation time for the alternative method was 3 day (72h) at 25±1*°C.*

Confirmations if required for the alternative method

No confirmations were required

3.1.4 Test results

All raw data per category are given in Annex B. 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 1 to 5 shows the data plotted per category and Figure 6 summarises all the data for the five food categories.

Figure 1 - Scatter plot of the reference method versus alternative method for Confectionary, bakery and eggs

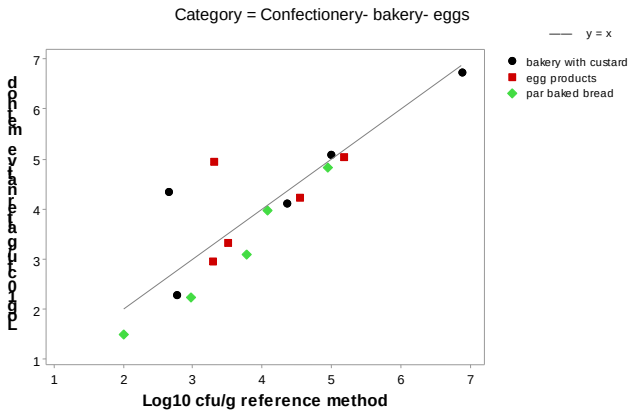


Figure 2- Scatter plot of the reference method versus alternative method results for Dairy products

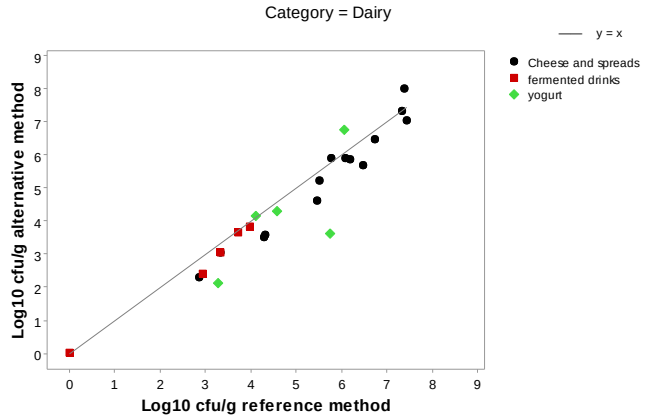


Figure 3- Scatter plot of the reference method versus alternative method results for Fruits and vegetables

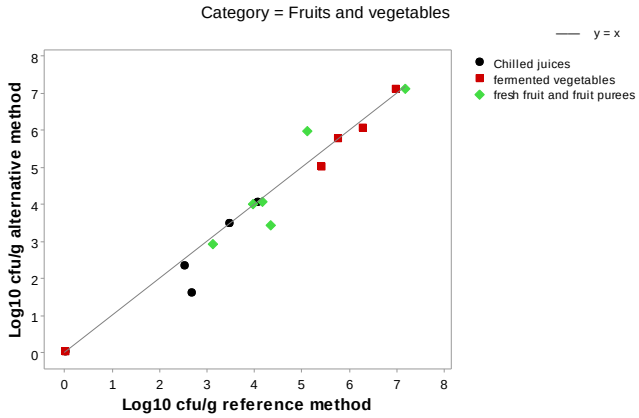


Figure 4- Scatter plot of the reference method versus alternative method results for Multicomponent foods

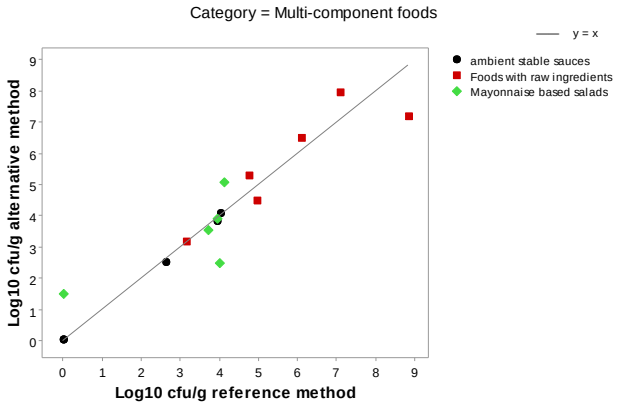


Figure 5- Scatter plot of the reference method versus alternative method results for Ready to eat Foods

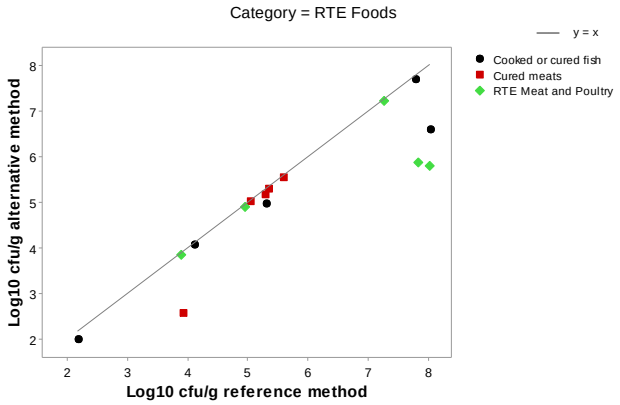
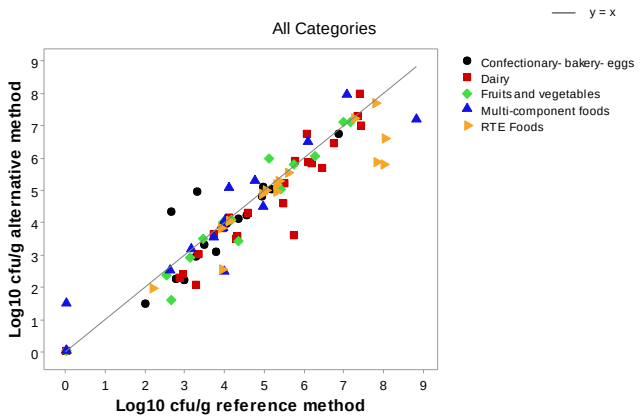


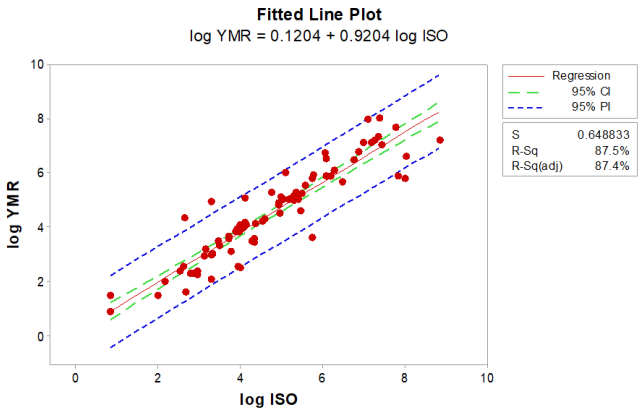
Figure 6 - Scatter plot of the reference method versus alternative method results for all the categories for L. monocytogenes plated onto 1ml OPL pour plates.



According to ISO/FDIS 16140-2:2015 section 6.1.2.3, the results of the scatter plot are interpreted based on a visual observation of the amount of bias and extreme results. The data appear generally acceptable although there was a general trend for a negative bias in the data sets for RTE foods (Fig 5) and Dairy products (Fig 2). For the confectionary category there were a couple of points with a positive bias of over 1.0.

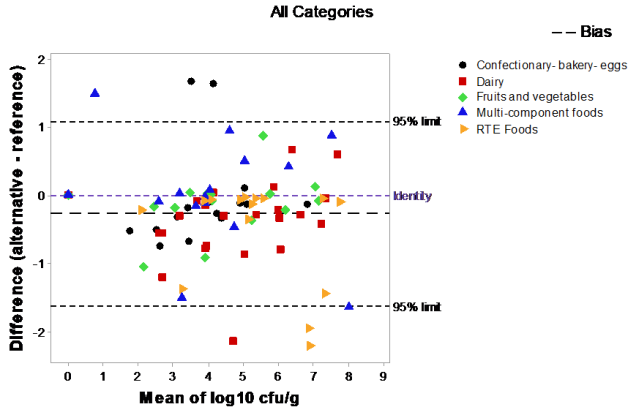
In order to help visualize which points showed an unusually high level of bias, a fitted regression line plot was created showing the 95%PI around the regression line. Five points were shown as clearly outside of the expected scatter of data as seen in Figure 7 . These were samples number 29, 75, 79, 145 and 156 which are also listed in Table 3 as samples which are outliers from the Bland Altman plot (Figure 8)

Figure 7 : fitted regression line plot



The data was analysed as described in ISO/FDIS 16140-2:2015 section 6.1.2.3 in order to produce the Bland –Altman difference plot. The average difference $\overline{D}$, the standard deviation of difference s_D and the limits of agreement were calculated per category and for all categories (Table 2).

Figure 8 Bland-Altman plot for all categories



The results of the scatter plots were interpreted according to ISO/FDIS 16140-2:2015 section 6.1.2.3 based on a visual observation on the amount of bias and extreme results. It is expected that not more than one in 20 data values will lie outside the CLs. Any disagreements with the expectation should be recorded.

For this data set there 7 in 80 data values which lie outside the CLs. These data are shown in Table 3. This is slightly outside the expectation of 1 in 20. The outliers covered 4 different food categories and showed no particular trends for food type, with 2 RTE meat samples (cooked chicken , cooked sausages), 2 yogurt samples (cherry and strawberry) and 1 multi-component food sample (chicken chili wrap) being below the lower CL and 2 confectionary/bakery/egg products (egg fried rice, egg custard tarts) being above the upper CL.

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

Table 2 - Summary of the calculated values per category L. monocytogenes 1ml OPL pour plates.

Category	n	$\bar{D}$	SD	95 % low limit	95 % upper limit
Confectionary- bakery- eggs	15	-0.045	0.728	-1.657	1.568
Dairy	23	-0.397	0.578	-1.621	0.827
Fruits and vegetables	14	-0.150	0.457	-1.171	0.871
Multi-component foods	13	-0.102	0.777	-1.858	1.655
RTE Foods	15	-0.549	0.780	-2.277	1.178
All Categories	80	-0.268	0.676	-1.622	1.086

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

Table 2 shows that there was a general negative bias in the data of -0.268 which means that on average the alternative method would slightly under recover yeasts and moulds compared to the reference method. For multi-component foods and

confectionery, the bias was low at around 0.1log or less. The largest negative bias was for the RTE food category (-0.549) and this covered samples of cooked or cured fish, RTE meat and poultry and cooked or cured fish.

Although there is an underlying negative bias, the 'all categories' Bland Altman plot show a high dispersion of the data around the line of identity showing both positive and negative deviations. The negative CL of was -1.622 and the positive CL was 1.086. Most of the samples tested contained both yeast and mould colonies although there were generally more yeasts present. The reference method states that '*enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium'* so it is perhaps not surprising to find a high level of variability based on the fact that the samples contained naturally present yeasts and moulds. In addition there are differences in the size of the plates used for the reference method and the alternate method and in the volumes analysed, 0.1ml for reference and 1ml for alternate. In addition, the alternate method relies on a chromogenic medium for detection of yeasts and moulds. Considering all these aspects, the agreement between the alternate method and the reference method is not unusual for a yeast and mould method

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 Table 3 .

Table 3 - Data which are outside of the accepted limits

Category	**Type**	**N° Sample**	**Food item**	**Difference Alt – ref**
Confectionary- bakery- eggs	With custard	156	Egg custard tarts	1.677
RTE Foods	RTE Meat and Poultry	75	Breaded chicken strips	-1.442
Multi-component foods	With raw ingredients	64	Sweet chilli chicken wrap	-1.656
Confectionary- bakery- eggs	Egg products	145	Egg Fried Rice	1.635
RTE Foods	RTE Meat and Poultry	29	Cocktail sausages	-1.970
Dairy	Yogurt	2	Strawberry Yogurt	-1.204
Dairy	Yogurt	79	Cherry Yogurt	-2.148

3.1.5 Discordant results

It is commonly recognised that a bias higher than 0.5 Log cfu/g difference between the compared methods should be explained if possible. For bacterial counts, < 0.5 log difference is typical of the natural variation you might expect between samples. For yeast and mould counts the variability may often be higher than this so the discordant results have been grouped into samples with a difference of >0.5log and a difference of >1.0 log to highlight samples with higher than expected disagreement. There are 26 discordant results, 6 with positive bias and 20 with negative bias (only 8 of these >1.0 log) (Tables 4 and 5). There was no pattern to the data in terms of the product category or type and the discordant results fell

across all five categories. The magnitude of the average bias was similar with a mean positive bias of 1.05 and a mean negative bias of -1.12.

It is likely that non-target organisms naturally present in these products are able to grow on the Reference media but not on the alternative media (Compact Dry). Previous studies in our laboratories have shown that DRBCA allows enumeration of a number of bacterial groups as well as yeasts and moulds.

The results showing a higher enumeration with the ALTERNATIVE method than with the REFERENCE method are shown below. (See Table 4)

Table 4 – Discordant results with a positive bias

Sample n°	Product category	Products	Bias log Alt - log Ref (log CFU/g)
156	Confectionary/eggs	Egg custard tarts	1.677
145	Confectionary/eggs	Egg Fried Rice	1.635
19	Fruits and Vegetables	Raspberry and Redcurrant Puree	0.866
40	Multi component foods	Hummus	0.864
77	Dairy (Yogurt)	Peach Yogurt	0.660
87	Dairy	Grated mozzarella	0.589

The results showing a lower enumeration with the ALTERNATIVE method than with the REFERENCE method are shown below (See Table 5).

Table 5 – Discordant results with a negative bias

Sample n°	Product category	Products	Bias log Alt - log Ref (log CFU/g)
Difference of >1.0 log			
75	RTE Foods	Breaded chicken strips	-2.222
79	Dairy (Yogurt)	Cherry yogurt	-2.148
29	RTE Foods	Cooked cocktail sausage	-1.970
64	Multi component foods	Sweet Chilli Chicken Wrap	-1.656
61	Multi component foods	Potato Salad	-1.523
107	RTE Foods	Hot smoked salmon	-1.450
206	RTE foods	Honey roast ham chunks	-1.377
2	Dairy (Yogurt)	Danio Strawberry Yogurt	-1.204
18	Fruits and vegetables	Mango juice	-1.061
Difference of >0.5log to <1.0 log			
203	Fruits and vegetables		-0.924

24	Dairy (Cheese)	Dorset Vinney Blue unpasteurised blue cheese	-0.873
20	Dairy (Cheese)	Reblochon de Savoie unpasteurised	-0.806
115	Dairy (Cheese)	Stilton	-0.791
143	Dairy (Cheese)	Ashmore unpasteurised cheddar	-0.760
201	Confectionary/eggs	par baked petit pains	-0.749
204	Confectionary/eggs	Par baked baguettes	-0.686
10	Dairy (fermented drinks)	Peach Probiotic Drink	-0.572
74	Dairy (Cheese)	Jarlsberg cheese	-0.566
311	Confectionary/eggs	par baked garlic bread	-0.523
4	Confectionary/eggs	frozen vanilla custard slices	-0.511

3.1.6 Conclusion (RT study)

Taking into account the overall Bland Altman analysis where are 7 outlier results which is only slightly above the expected 1 in 20, it is concluded that the relative trueness study of the ALTERNATIVE method is acceptable. Whilst there are a number of discordant results, these do not form part of the ISO16140-2:2016 analysis and therefore are informative only. For total plate count methods especially yeast and mould methods which are aimed at enumeration of a wide range of mycological groups, this level of outliers is not unreasonable, however end users should perform verification studies to show comparable results with their usual reference method

The relative trueness study of the ALTERNATIVE method Hyserve Compact Dry YMR is satisfied for a 3 day incubation period at 25±1°C.

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

Five 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 6)

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

Table 6- Categories, types, items, strains and inoculation levels for accuracy profile study

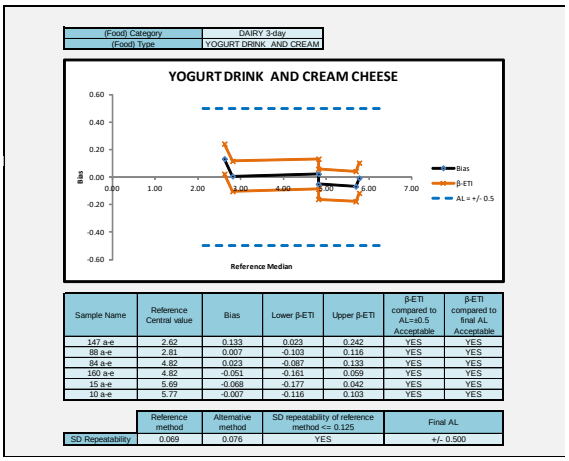
Category	Types	Strain	Item	Target Level	Test portions
Dairy products	Pasteurised dairy products	*S.cerevisiae* CRA 15968	Fermented yogurt drink	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5
			Cream cheese	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5
Fruits and vegetables	Blanched or pasteurised products	*D.hansenii* CRA 15969	Vegetable Juice	Low: 500cf/g	5
				Medium : 5000cfu/g	5
				High : 50.000cfu/g	5
			Beetroot salad	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5
Confectionary, bakery and eggs	Chilled RTE foods	*A.niger* CRA 16667	Quiche	Low: 100cf/g	5
				Medium : 1000cfu/g	5
				High : 50.000cfu/g	5
			Egg custard tarts	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5
Ready to eat foods	Fish products	*P. chrysogeum* DSM 848	Cooked prawns	Low: 100cf/g	5
				Medium : 10000cfu/g	5
				High : 100.000cfu/g	5
			Fish pate	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5
Multi component foods	Composite foods with raw ingredients	*G. candidum* CRA 14398	Sandwiches	Low 500cf/g	5
				Medium : 5000cfu/g	5
				High : 10.000cfu/g	5
			Pasta salad with protein	Low 300cf/g	5
				Medium : 5.000cfu/g	5
				High : 100.000cfu/g	5

3.2.2 Calculations and interpretation of accuracy profile study

The summary tables for the accuracy profile study are given in Annex E. The statistical results and the accuracy profiles are provided Figures 29-33.

The calculations were done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) available on http://standards.iso.org/iso/16140

Figure 8– Accuracy profile Dairy products

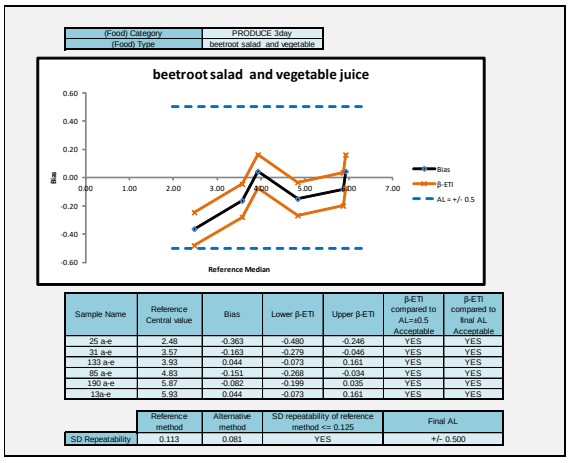


(Food) Category	DAIRY 3-day
(Food) Type	YOGURT DRINK AND CREAM

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
147 a-e	2.62	0.133	0.023	0.242	YES	YES
88 a-e	2.81	0.007	-0.103	0.116	YES	YES
84 a-e	4.82	0.023	-0.087	0.133	YES	YES
160 a-e	4.82	-0.051	-0.161	0.059	YES	YES
15 a-e	5.69	-0.068	-0.177	0.042	YES	YES
10 a-e	5.77	-0.007	-0.116	0.103	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.069	0.076	YES	+/- 0.500

Figure 9– Accuracy profile Fruit and vegetables

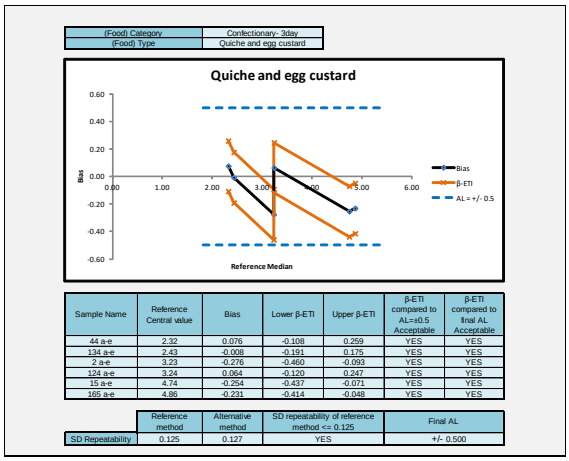


(Food) Category	PRODUCE 3day
(Food) Type	beetroot salad and vegetable

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	2.48	-0.363	-0.480	-0.246	YES	YES
31 a-e	3.57	-0.163	-0.279	-0.046	YES	YES
133 a-e	3.93	0.044	-0.073	0.161	YES	YES
85 a-e	4.83	-0.151	-0.268	-0.034	YES	YES
190 a-e	5.87	-0.082	-0.199	0.035	YES	YES
13a-e	5.93	0.044	-0.073	0.161	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.081	YES	+/- 0.500

Figure 10– Accuracy profile Confectionary bakery and eggs

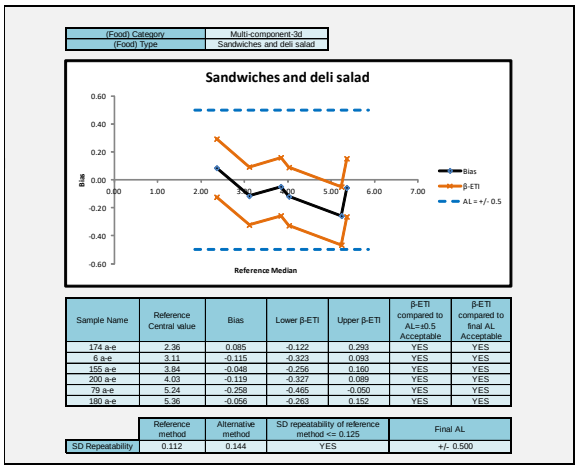


(Food) Category	Confectionary- 3day
(Food) Type	Quiche and egg custard

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
44 a-e	2.32	0.076	-0.108	0.259	YES	YES
134 a-e	2.43	-0.008	-0.191	0.175	YES	YES
2 a-e	3.23	-0.276	-0.460	-0.093	YES	YES
124 a-e	3.24	0.064	-0.120	0.247	YES	YES
15 a-e	4.74	-0.254	-0.437	-0.071	YES	YES
165 a-e	4.86	-0.231	-0.414	-0.048	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.125	0.127	YES	+/- 0.500

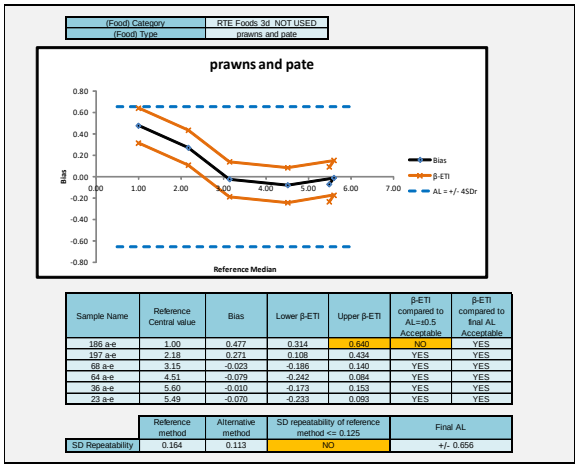
Figure 11 – Accuracy profile Multicomponent Foods



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
174 a-e	2.36	0.085	-0.122	0.293	YES	YES
6 a-e	3.11	-0.115	-0.323	0.093	YES	YES
155 a-e	3.84	-0.048	-0.256	0.160	YES	YES
200 a-e	4.03	-0.119	-0.327	0.089	YES	YES
79 a-e	5.24	-0.258	-0.465	-0.050	YES	YES
180 a-e	5.36	-0.056	-0.263	0.152	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.112	0.144	YES	+/- 0.500

Figure 12 – Accuracy profile RTE foods



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
186 a-e	1.00	0.477	0.314	0.640	NO	YES
197 a-e	2.18	0.271	0.108	0.434	YES	YES
68 a-e	3.15	-0.023	-0.186	0.140	YES	YES
64 a-e	4.51	-0.079	-0.242	0.084	YES	YES
36 a-e	5.60	-0.010	-0.173	0.153	YES	YES
23 a-e	5.49	-0.070	-0.233	0.093	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.164	0.113	NO	+/- 0.656

Conclusion accuracy profile study

The comparison of the reference method and alternative method was within the 0.5 Log Acceptability limit calculated according to ISO16140-2:2016 section 6.1.3.3 for 4 of the 5 food categories. For the 5th category, the RTE foods the AL was exceeded for the lowest level of prawns.

If any of the upper or lower values exceeded the limits for any category and the standard deviation of the reference method was >0.125, additional evaluation procedure were followed, as described in ISO 16140-2:2016 and the new acceptability limits were calculated as a function of the standard deviation $AL_s = 4 \cdot s_{ref}$.

After re-calculation of the limits according to ISO 16140, the RTE food met the re-calculated limits

All the accuracy profiles fulfil the performance criteria or the re-calculated criteria and the alternative method is accepted as being equivalent to the reference method using a 3d incubation period

3.3 Quantification limits (LOQ)

As the alternative method is based on counting visible colonies target microorganism, the LOQ was not required to be determined according to ISO/FDIS 16140-2:2015.

3.4 Inclusivity / exclusivity

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

The inclusivity of the CD YMR method was determined using 51 strains of yeasts and moulds and the exclusivity was established with 32 strains of non-target organisms. This was done as a randomized blind-coded study. All moulds were maintained in 10% glycerol solution at -80°C in Japan Food Research Laboratory (Shibuya-ku, Tokyo, Japan). Prior to use, each strain was subcultured onto a pre-poured plate of Potato Dextrose Agar (PDA) and incubated for 7-14 days at 25°C ± 1°C. The surrounding part of an isolated colony from each strain was then subcultured onto a PDA slant and incubated for 7 – 14 days at 25°C ± 1°C. The culture slant was then kept at 5 ± 3°C until required for use. For obtaining the levels for inoculations, each mould strain was cultured separately onto a PDA plate for 6 – 19 days at 25°C ± 1°C, those spores were suspended in sterilized phosphate-buffered saline (PBS) with 0.05% of Polysorbate 80. The number of cells in the suspension was enumerated with a Thoma cell counting chamber and the culture was serially diluted in PBS to the required level, 30 – 150 cfu/mL.

All yeasts were maintained on storage beads (Microbank, Iwaki & Co., Ltd.) at -80°C in Japan Food Research Laboratory. Prior to use, each strain was subcultured onto a pre-poured plate of PDA and incubated for 7-14 days at 25°C ± 1°C. An isolated colony from each strain was then subcultured onto a PDA slant and incubated for 7 – 14 days at 25°C ± 1°C. The culture slant was then kept at 5 ± 3°C until required for use. For obtaining the levels for inoculations, each yeast strain was cultured separately into Tryptone Soy Broth (TSB) for 3 days at 25°C ± 1°C. The number of cells in the suspension was enumerated with a Thoma cell counting chamber and the culture was serially diluted in PBS to the required level, 30 – 150 cfu/mL.

Each exclusivity strain was cultured in TSB for 18-24 hours at 35°C ±1°C in Table 2.

The inclusivity results are presented in Annex F. All of the 51 inclusivity strains tested showed growth on the CD YMR medium and gave the expected results. Morphology results are that yeasts and moulds form green/blue colonies. While most colonies are some shade of green/blue, any coloured colony should be counted. In addition, mould colonies may have a diffuse or cottony appearance.

The raw data for the exclusivity strains is given in Annex F. Of the 32 exclusivity strains tested, none showed growth on the CD YMR medium after 72 h at 25°C. ± 1°C

3.4.1 Conclusion
The alternative CD YMR method is selective and specific for yeasts and moulds and shows comparable performance to the reference method.

3.5 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The alternative CD YMR enumeration method for yeasts and moulds shows satisfactory results for relative trueness.
- The alternative CD YMR enumeration method for yeasts and moulds shows satisfactory results for accuracy profile.
- The alternative CD YMR enumeration method for yeasts and moulds is selective and specific

4 Interlaboratory study

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

4.1 Study organisation

4.1.1 Collaborators
There were 7 organisations used in this study representing 4 different countries. The number of collaborators from each organisation was 2 (according to ISO16140-2:2016 6.2.2) giving a maximum of 14 potential data sets. (Annex G). It was only possible to find 7 organisations that were able to participate in this collaborative trial. It was agreed at a MVTC that in order to progress the ILS we could use fewer organisations and more collaborators.

4.1.2 Matrix and strain used

Chilled salmon pâté was used for this study. The samples were inoculated with a cocktail of a yeast and a mould mixed in equal concentration. *S.cerevisae* CRA 15968 and *P.chrysogenum* DSM 848 were used.

4.1.3 Sample preparation
For each of the 14 collaborators participating in the interlaboratory study 7 x 10g samples of salmon pâté were weighed into sterile stomach bags. One sample of salmon pâté remained uninoculated. For the remaining six samples, appropriate dilutions of the yeast and mould cocktail were used to individually inoculate 2 x 10g samples at the low (~10^2 cfu/ml), middle (~10^4cfu/ml) and high (~10^6cfu/ml) contamination levels.

For each collaborator, a set of samples was prepared containing 2 samples at a low level, two samples at a medium level, two samples at a high level and a single

uninoculated blank sample. The samples were blind-coded so that the collaborators did not know the intended contamination level. A set of samples was also prepared for the EL although the data from these was not used in the data analysis

The samples were blind-coded (as shown in Table 7). Where more than 1 collaborator was used at an organization, different blind coding numbers were used for the replicate sets of samples. After weighing out, the samples were frozen for 72 hours prior to despatch. A stability test was done to establish the effect of freeze - thawing on the levels of Yeasts and moulds contained in samples and the stability of the inoculated samples during chilled 72 hours chilled transportation was tested. Additionally, a set of samples was prepared at the same time, for the Expert laboratory (Campden BRI) organising the trial to confirm the presence of the target organisms and the contamination levels. These data were not used in the analysis.

Table 7: Contamination levels

Contamination level	**Sample code set 1**	**Sample code set 2**
Uninoculated	4	8
Low (10^2 cfu/g)	1	13
Low (10^2 cfu/g)	5	14
Medium (10^4 cfu/g)	2	10
Medium (10^4 cfu/g)	6	12
High (10^6 cfu/g)	3	9
High (10^6 cfu/g)	7	11

4.1.4 Labelling and shipping

Prior to despatch, each set of samples was removed from the freezer and packed into plastic containers (Air-Sea Containers Limited, code 490). These plastic containers were then placed inside a thermal control unit (Air-Sea Containers Limited, TC-20 code 802) with cool packs (Air-Sea Containers Limited, CP-20 code 405). The samples were packaged frozen so as to allow thawing to occur during transportation. Each laboratory also received an additional vial containing water "temperature control sample" which was packed with the test samples.

This was used to enable the laboratory to take a temperature measurement, representative of the samples, upon receipt. In addition to this a continuous electronic temperature monitor (Thermochron iButton) was placed in the sample packages. The laboratories were requested to return the ibuttons to the expert laboratory upon receipt. The target storage conditions were for the temperature to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

Frozen samples for all labs were removed from the freezer on Friday 24th February 2017 and placed into the packaging. Shipping was arranged so that each laboratory would receive their samples within 24-72h dependent on location and speed of the

International courier service. The samples sent to mainland Europe were dispatched on Friday 24th February 2017 (labs 1, 2, 4, 6) . The remaining samples for the UK collaborators were placed in a chillier at <8C over the weekend already packed in their packaging, and the samples were dispatched on Monday 27th February 2017 (labs 3, 5, 7). Although this is outside of the recommended 48hr transportation time, experience has shown that samples often get held up in customs from the UK to mainland Europe and it is not possible to ensure a <48hr delivery time. It is for this reason that samples are dispatched frozen and allowed to thaw during transport. The condition of the samples was recorded by each laboratory on a receipt.

4.1.5 Analysis of Samples

The analyses were started on Tuesday 28th February 2017, although some collaborators did not start until Wednesday 1st March due to receiving the samples late.

4.2 Experimental parameters controls

4.2.1 Strain stability during transport
Stability testing was done prior to despatch of the samples. A set of samples was produced at the highest inoculation level and was tested immediately after inoculation, and 24 h, 48 h and 72h after removal from the freezer and storage at 8±°C.

Table 8 - Levels of yeasts and moulds (cfu/g) in stability samples stored at 8±1°C – preliminary trial

Time	**0h (defrost)**		**24h @ 8◦C**		**48h @ 8◦C**		**72h @ 8◦C**	
Method	YMR	Reference:	YMR	Reference:	YMR	Reference:	YMR	Reference:
Rep a	5.10E+04	5.45E+04	5.00E+04	4.60E+04	5.30E+03	4.80E+04	1.30E+05	1.30E+05
Rep b	5.45E+04	3.27E+04	5.10E+04	3.70E+04	6.20E+04	5.10E+04	1.40E+05	1.30E+05
Mean	**5.28E+04**	**4.36E+04**	**5.05E+04**	**4.15E+04**	**3.37E+04**	**4.95E+04**	**1.35E+05**	**1.30E+05**

The data after the preliminary trial showed that the levels of yeasts and moulds count were stable for the first 48 hours after placing at 8±1°C but started to show some increase in levels between 48 and 72 hours. These stability conditions are more severe than the conditions likely to be seen in the samples sent out to the laboratories as they are held at a constant 8±1°C immediately on removal from the freezer, whereas the samples dispatched to the laboratories are placed in insulated packaging with ice-blocks. As the increases were around 0.5 logs and were consistent between duplicates, it was considered acceptable to continue with study with samples prepared in this way.

4.2.2 Logistic conditions
Four collaborators (7,8,11,12) received their samples on 27/2/2017 and 8 collaborators (1,2,3,4,5,6,9,10) received their samples on 28/2/2017. Labs receiving samples either stored their samples refrigerated (<8°C) over night or tested them on

the day of receipt. Labs tested the samples on 28/2/2017 or 1/03/2017. The information is shown in Table 2 below. It was intended for collaborators to test samples on the same date but due to the logistics of the courier service, this was not possible. Despite the differences in delivery times, all laboratories received samples in a good condition, except for laboratory 1, where the water vial was measured as 13.5◦C (see Table 9)

Table 9 - Sample temperatures at receipt

Organising Laboratory (collaborators)	Date samples despatched	Date samples received	Date samples tested	Temperature of control sample upon receipt (°C)	Average storage temperature (°C) over entire transport period
1 (1, 2)	24/02/17	28/02/17	01/03/17	13.5	4.3
2 (3, 4)	24/02/17	28/02/17	01/03/17	8.4	3.75
3 (5, 6)	27/02/17	28/02/17	28/02/17	2.8	1.5
4 (7, 8)	24/02/17	27/02/17	28/02/17	9	1.8
5 (9, 10)	27/02/17	28/02/17	28/02/17	5.5	1.5
6 (11, 12)	24/02/17	27/02/17	28/02/17	5.5	3.5
7 (13, 14)	27/02/17	28/02/17	28/02/17	3.6	I-button not returned
Expert lab	27/02/17	28/02/17	28/02/17	1.8	1.0

The temperature range of the samples upon receipt by the collaborative laboratories (Table 3) was variable. It ranged from 2.8°C for lab 3 to 13.5°C for lab 1. The I-button data shows the temperature profile of the samples throughout transport and the data for lab 1 showed that the temperature was <8°C throughout. The plots are given in Annex I, and the average temperature across the whole transportation period is shown in Table 9. For laboratory number 1 which recorded a water temperature of 13.5°C, it is possible that there was some delay in analysing the temperature of the water vial on receipt as the I-button data shows good temperature control throughout distribution

4.3 Calculation and summary of data

The raw data are given in Annex H.

4.3.1 MicroVal Expert laboratory results
The results obtained by the expert laboratory are given in Table 10.

Table 10– Results obtained by the expert lab.

Level	Reference method	Alternative method
Blank	<10	<10
Low	3.80E+02	4.10E+02
Low	4.00E+02	2.40E+02
Medium	1.04E+05	9.70E+04
Medium	8.00E+04	5.40E+04
High	7.60E+05	5.40E+05
High	5.40E+05	5.70E+05

4.3.2 Results obtained by the collaborative laboratories.

The data from the collaborative trial were calculated and interpreted according to section 6.2.3 of ISO 16140-2:2016 using the freely available Excel® spreadsheet (http://standards.iso.org/iso/16140). Version 14-03-2016 was used for these calculations.

The results obtained by the collaborators are shown in Table 11.

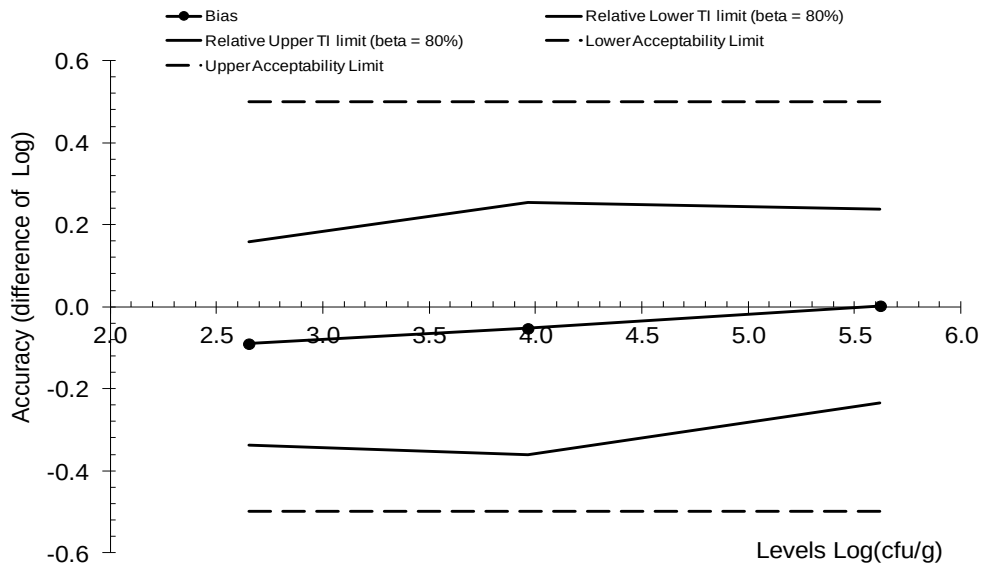
The accuracy profile plot is shown in Figure 13 and the statistical analysis of the data shown in Table 12.

Table 11: Summary of the results of the interlaboratory study per analyte level (k

Collaborator	**Level**	**Reference method (Log cfu/g)**		**Alternative method (Log cfu/g)**	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
01	low	2.61	2.81	2.36	2.82
02	low	2.89	2.69	2.85	2.65
03	low	2.69	2.61	2.41	2.45
04	low	2.68	2.51	2.34	2.44
05	low	2.54	2.45	2.44	2.04
06	low	2.59	2.26	2.37	2.42
07	low	2.68	2.73	2.69	2.72
08	low	2.88	2.80	2.81	2.80
09	low	2.76	2.68	2.65	2.63
10	low	2.74	2.72	2.69	2.61
11	low	2.72	2.64	2.56	2.53
12	low	2.88	2.80	2.69	2.65
13	low	2.61	2.41	2.55	2.42
14	low	2.49	2.38	2.53	2.61
01	medium	4.05	4.27	3.85	4.17
02	medium	4.24	4.19	4.30	4.22
03	medium	3.81	3.80	3.87	3.83
04	medium	4.04	3.93	3.97	3.71
05	medium	3.80	3.98	3.63	3.68

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
06	medium	3.93	3.58	3.59	3.76
07	medium	4.00	4.00	4.12	4.14
08	medium	4.08	4.11	4.10	4.17
09	medium	4.04	3.85	3.82	3.85
10	medium	4.13	3.70	3.94	3.82
11	medium	3.94	4.08	3.91	3.96
12	medium	4.17	4.28	4.26	4.16
13	Medium	3.80	3.79	3.68	3.57
14	medium	3.77	3.64	3.83	3.60
01	high	5.74	5.59	5.80	5.49
02	high	5.90	5.87	5.83	5.83
03	high	5.53	5.68	5.61	5.66
04	high	5.57	5.69	5.53	5.57
05	high	5.53	5.65	5.34	5.19
06	high	5.50	5.58	5.34	5.48
07	high	5.68	5.58	5.74	5.84
08	high	5.66	5.82	5.86	5.81
09	high	5.42	5.60	5.55	5.82
10	high	5.58	5.61	5.65	5.58
11	high	5.57	5.50	5.65	5.63
12	high	5.82	5.78	5.74	5.75
13	high	5.45	5.53	5.55	5.57
14	high	5.50	5.45	5.45	5.59
01	blank	<10		<10	
02	blank	<10		<10	
03	blank	<10		<10	
04	blank	<10		<10	
05	blank	<10		<10	
06	blank	<10		<10	
07	blank	<10		<10	
08	blank	<10		<10	
09	blank	<10		<10	
10	blank	<10		<10	
11	blank	<10		<10	
12	blank	<10		<10	
13	blank	<10		<10	
14	blank	<10		<10	

Figure 13. Accuracy profile of CD YMR from the ILS



The statistical analysis of the ILS data is shown in Table 6 below. It can be seen that the repeatability standard deviation (S_r) was similar for the alternative and reference method ranging from 0.095 to 0.127 for Compact Dry YMR and 0.077 to 0.132 for the reference method. The between-labs standard deviation (S_L) was also of a similar microbiological magnitude for the alternative method (0.131 to 0.197) and the reference method (0.106 to 0.138).

The mean $\log_{10}$ count from the 14 samples at each levels were very similar for the two methods with low, medium and high average counts of 2.561. 3.911 and 5.623 for the alternative method and 2.652, 3.964 and 5.621 for the reference.

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the Acceptability Limits AL (±0.5log units)then a further calculation is done to calculate the pooled average SR of the reference method. There was no requirement for this as all values met the AL's.

Looking at Figure 13, it can be seen that no values lie outside of the 0.5log ALs values and therefore the alternative method is accepted as being equivalent to the reference method.

Table 12. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile							
Study Name	Hyserve YMR						
Date	27/03/2016						
Coordinator	Campden BRI						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0.50	0.50	0.50				
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**2.652**	**3.964**	**5.621**				
Number of participants (K)	14	14	14		14	14	
Average for alternative method	2.561	3.911	5.623		2.652	3.964	
Repeatability standard deviation (sr)	0.127	0.105	0.095		0.106	0.132	
Between-labs standard deviation (sL)	0.131	0.197	0.144		0.117	0.138	
Reproducibility standard deviation (sR)	0.182	0.223	0.172		0.158	0.192	
Corrected number of dof	20.738	16.199	17.517		20.089	20.556	1
Coverage factor	1.359	1.378	1.372				
Interpolated Student t	1.324	1.336	1.332				
Tolerance interval standard deviation	0.1873	0.2302	0.1776				
Lower TI limit	2.313	3.603	5.387				
Upper TI limit	2.809	4.218	5.860				
Bias	**-0.090**	**-0.053**	**0.002**				
Relative Lower TI limit (beta = 80%)	**-0.338**	**-0.361**	**-0.234**				
Relative Upper TI limit (beta = 80%)	**0.158**	**0.254**	**0.239**				
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**				
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0.162						

FALSE

App
Step 8: If any of
the acceptability
reproducibility s

Step 9: Calcu
functior

FALSE
FALSE

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

5 Overall conclusions of the validation study

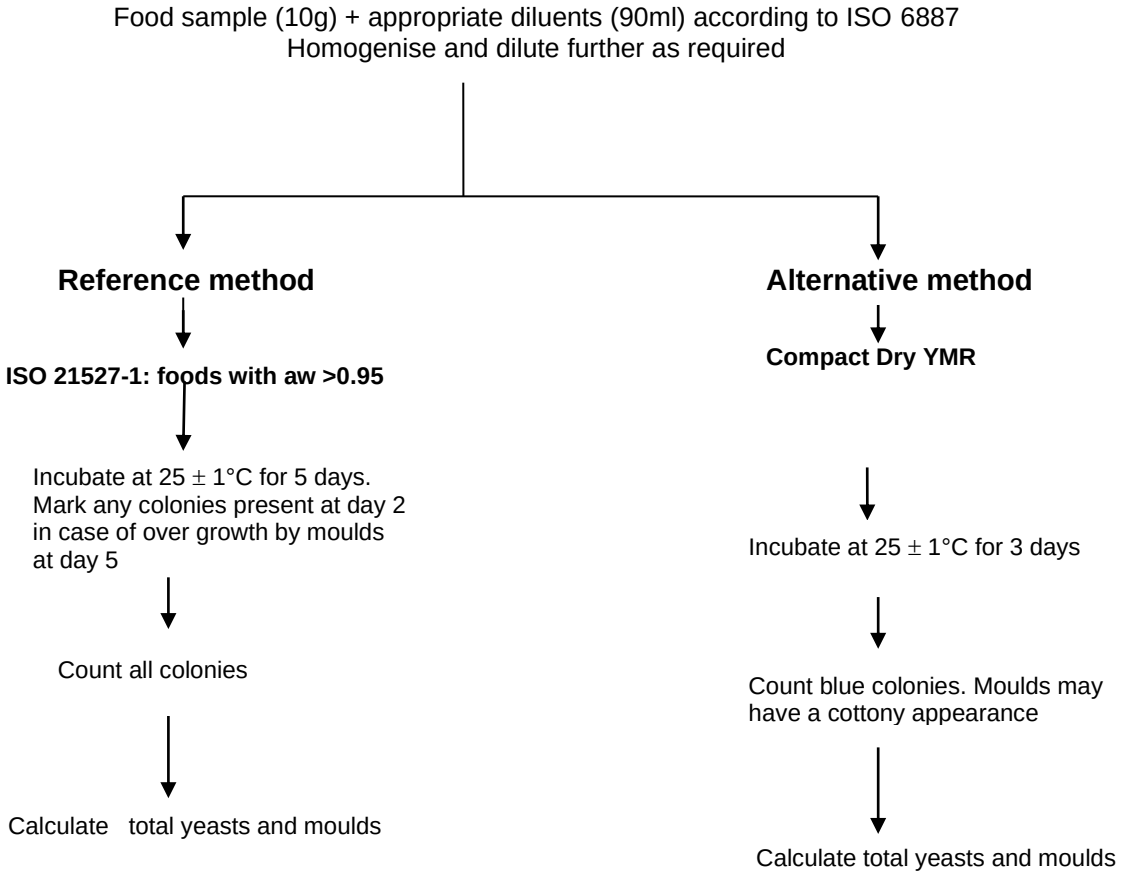
- The alternative method CD YMR for enumeration of yeasts and moulds shows satisfactory results for relative trueness.
- The alternative method CD YMR for enumeration of yeasts and moulds shows satisfactory results for accuracy profile.
- The alternative method CD YMR for enumeration of yeasts and moulds is selective and specific.
- The alternative method CD YMR for enumeration of yeasts and moulds shows satisfactory performance in the ILS.

The alternative method CD YMR for enumeration of yeasts and moulds shows comparable performance to the reference method ISO 21527-1:2008 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Colony count technique in products with water activity greater than 0.95.

Date 30/09/2021

Signature Suzanne Jordan

ANNEX A: Flow diagram of the alternative method reference method



ANNEX B: Relative Trueness raw data

Item	Sample code	Dilution	YM R	Cfu/g	Log cfu/g	Dilution	DRBCA 0.1ml	Cfu/g	Log cfu/g
Danio Strawberry Yogurt	2	-1	12	120	2.08	-1 0.5ml	98	1920	3.28
		-2	0			-1 0.5ml	94		
Frozen vanilla custard slices	4	-1	17	182	2.26	-1 0.5ml	29	590	2.77
		-2	3			-1 0.5ml	30		
Peach Probiotic Drink	10	-1	23	236	2.37	-1 0.5ml	39	880	2.94
		-2	3			-1 0.5ml	49		
Spinach & ricotta quiche	11	-3	16	16000	4.20	-2	35	34545	4.54
		-4	0			-3	3		
Half fat mayonnaise	14	-1	33	327	2.51	-1 0.5ml	30	420	2.62
		-2	3			-1 0.5ml	12		
Mango Juice	18	-1	4	40	1.60	-1 0.5ml	31	460	2.66
		-2	0			-1 0.5ml	15		
Raspberry and Redcurrant Puree	19	-3	93	927273	5.97	-2	124	126364	5.10
		-4	9			-3	12		
Reblochon de Savoie unpasteurised cheese	20	-4	49	454545	5.66	-3	28	2909091	6.46
		-5	1			-4	4		
Raspberry Probiotic drink	21	-1	80	1009	3.00	-1	20	2091	3.32
		-2	11			-2	3		
Dorset Vinney Blue unpasteurised blue cheese	24	-2	T	39000	4.59	-3	31	290909	5.46
		-3	39			-4	1		
Grapes and strawberries	25	-2	118	11455	4.06	-1	140	14091	4.15
		-3	8			-2	5		
Berkswell unpasteurised Ewes milk cheese	26	-4	74	745455	5.87	-3	125	1227273	6.09
		-5	8			-4	10		
Red Pepper hummus	28	-4	T	1250000	7.10	-3	T	9600000	6.98
		-5	125			-4	90		
Cooked cocktail sausages	29	-4	72	718182	5.86	-4	T	67000000	7.83
		-5	7			-5	67		
Pineapple and Apricot Puree	34	-2	88	9545	3.98	-1	84	9182	3.96
		-3	17			-2	15		
Microwave frozen rice	35	-3	29	30000	4.48	-2	89	90000	4.95
		-4	4			-3	10		
Ham sandwich	37	-1	146	1445	3.16	-1 0.5ml	73	1420	3.15
		-2	13			-1 0.5ml	69		
Pastrami	38	-4	36	336364	5.53	-3	37	381818	5.58
		-5	1			-4	5		
Salmon and King Prawn sandwich	39	-4	17	181818	5.26	-2	55	58182	4.76
		-5	3			-3	9		
Reduced fat hummus	40	-5	T	8909090	7.95	-4	155	12181818	7.09
		-6	85			-5	19		
Shropshire blue cheese	41	-5	T	1000000	7.00	-5	26	27272727	7.44
		-6	10			-6	4		
Gevrik Goats cheese	49	-4	82	772727	5.89	-3	61	600000	5.78
		-5	5			-2	5		
Feta and dried tomato pasta	52	-2	T	3100000	6.49	-2	T	1220000	6.09
		-3	31			-3	122		

Item	Sample code	Dilution	YM R	Cfu/g	Log cfu/g	Dilution	DRBCA 0.1ml	Cfu/g	Log cfu/g
Normandie Camembert	53	-4	16	163636	5.21	-3	31	318182	5.50
		-5	2			-4	4		
Cheese and bacon quiche	55	-1	86	882	2.95	-1 0.5ml	105	1890	3.28
		-2	11			-1 0.5ml	84		
Skyr Apple Lingonberry yogurt	57	-2	141	13909	4.14	-1	117	12882	4.11
		-3	2			-2	24		
Brussels Pate	59	-5	T	1600000	7.20	-4	T	18000000	7.26
		-6	16			-5	18		
Potato salad	61	-1	30	300	2.48	-1	101	10000	4.00
		-2	3			-2	9		
Salami	62	-3	141	140909	5.15	-3	19	190909	5.28
		-4	14			-4	2		
Cockles	63	-5	T	4700000	7.67	-4	T	60000000	7.78
		-6	42			-5	60		
Sweet Chilli Chicken Wrap	64	-5	T	1500000	7.18	-5	T	68000000	8.83
		-6	15			-6	68		
Chicken liver pate	68	-3	74	73636	4.87	-2	80	85455	4.93
		-4	7			-3	144		
Pineapple Juice	71	-2	32	3091	3.49	-1 0.5ml	137	2910	3.46
		-3	2			-1 0.5ml	154		
Tomato Ketchup 50% Less Sugars	72	-2	121	11818	4.07	-1	98	10182	4.01
		-3	9			-2	14		
Jarlsberg cheese	74	-1	19	190	2.28	-1	7	700	2.85
		-2	0			-1	0		
Breaded chicken strips	75	-4	58	600000	5.78	-4	T	10000000	8.00
		-5	8			-5	100		
JogoBella Peach Yogurt	77	-5	53	5272727	6.72	-3	114	1154545	6.06
		-6	5			-4	13		
Orange Juice	78	-2	114	11182	4.05	-1	113	11364	4.06
		-3	9			-2	12		
Cherry Yogurt	79	-2	T	4000	3.60	-3	53	563000	5.75
		-3	4			-4	6		
Grated Mozzarella	87	-5	T	9500000	7.98	-6	23	24500000	7.39
		-6	95			-7	4		
Apple and grape snack	101	-1	83	827	2.92	-1 0.5ml	60	1300	3.11
		-2	15			-1 0.5ml	70		
Ardennes Pate	102	-2	69	6455	3.81	-1	77	7727	3.89
		-3	2			-2	8		
savoury eggs	104	-3	107	106364	5.03	-2	147	146364	5.17
		-4	10			-3	14		
Grated Four Cheese	106	-4	T	1980000	7.30	-5	22	22300000	7.35
		-5	198			-6	3		
Hot smoked salmon	107	-5	33	3727273	6.57	-4	T	10500000	8.02
		-6	8			-5	105		
Salmon pate	108	-1	9	90	1.95	-1 0.5ml	6	150	2.18
		-2	0			-1 0.5ml	9		
Green and Black Olives	114	-3	T	590000	5.77	-2	T	560000	5.75
		-4	59			-3	56		

Item	Sample code	Dilution	YM R	Cfu/g	Log cfu/g	Dilution	DRBCA 0.1ml	Cfu/g	Log cfu/g
Stilton	115	-2	33	3091	3.49	-2	16	19091	4.28
		-3	1			-3	5		
Passion Fruit Yogurt	117	-3	19	19091	4.28	-2	39	38182	4.58
		-4	2			-3	3		
Chorizo	118	-4	19	190909	5.28	-3	23	218182	5.34
		-5	2			-4	1		
Unpasteurised hard cheese	122	-4	63	690909	5.84	-3	148	1527273	6.18
		-5	13			-4	20		
Frozen prawns	125	-3	96	89091	4.95	-2	T	200000	5.30
		-4	2			-3	20		
Egg yolk	130	-2	19	2000	3.30	-1	20	3091	3.49
		-3	3			-2	4		
Frozen king prawns	139	-2	105	10909	4.04	-1	127	12909	4.11
		-3	15			-2	15		
Egg fried rice	145	-3	87	86364	4.94	-1	19	2000	3.30
		-4	8			-2	3		
Orange and Raspberry Juice	148	-1	22	220	2.34	-1 0.5ml	12	330	2.52
		-2	0			-1 0.5ml	21		
Egg custard tarts	156	-3	21	20909	4.32	-1 0.5ml	25	440	2.64
		-4	2			-1 0.5ml	19		
Pizza Express light dressing	163	-2	66	6727	3.83	-1	86	8818	3.95
		-3	8			-2	11		
Fresh iced custard slices	170	-5	53	5454545	6.74	-4	74	7363636	6.87
		-6	7			-5	7		
savers white par baked baguettes	200	-3	66	64545	4.81	-2	87	85455	4.93
		-4	5			-3	7		
par baked petit pains	201	-1	16	164	2.21	-1 0.5ml	45	920	2.96
		-2	2			-1 0.5ml	47		
melon and grapes snack pack	202	-5	122	1227272	7.09	-4	151	14818182	7.17
		-6	13			-5	12		
pre-packed apple slices	203	-1	T	2600	3.41	-2	20	21818	4.34
		-2	26			-3	4		
par baked baguettes	204	-2	12	1200	3.08	-1	58	5818	3.76
		-3	1			-2	6		
vanilla creme custard pastries	205	-2	117	12273	4.09	-2	21	22727	4.36
		-3	18			-3	4		
honey roast ham chunks	206	-1	24	355	2.55	-1	85	8455	3.93
		-2	15			-2	8		
smietana cream drink	207	-2	62	6545	3.82	-1	94	9272	3.97
		-3	10			-2	8		
portugese custard tarts	208	-3	116	120000	5.08	-2	90	95455	4.98
		-4	16			-3	15		
kefir milk drink	209	-1	T	4300	3.63	-1	44	5182	3.71
		-2	43			-2	13		
bake at home crusty rolls	210	-2	88	9091	3.96	-1	110	11455	4.06
		-3	12			-2	16		
ham	211	-3	93	100000	5.00	-2	101	107273	5.03
		-4	17			-3	17		
Pimento stuffed olives with manchego	303	-3	106	103636	5.02	-2	T	250000	5.40
		-4	8			-3	25		

Item	Sample code	Dilution	YM R	Cfu/g	Log cfu/g	Dilution	DRBCA 0.1ml	Cfu/g	Log cfu/g
prawn pasta salad	305	-2	82	7818	3.89	-1	86	8363	3.92
		-3	4			-2	12		
Jalopeno coleslaw	306	-3	109	110909	5.04	-2	136	130000	5.11
		-4	13			-3	7		
mixed olives with chilli peppers	307	-4	113	1136364	6.06	-4	17	1909091	6.28
		-5	12			-5	4		
Tuna pasta salad	308	-2	37	3455	3.54	-1	47	5182	3.71
		-3	1			-2	10		
par baked garlic bread	311	-1	3	30	1.48	-1	1	100	2.00
		-1	3			-1	1		

Note: for dilutions where a low count was expected a 1ml sample was plated over 2 plates (0.5ml on each). This is a minor deviation from the ISO requirement for 1ml across 3 plates but this is a deviation covered by the laboratory ISO17025 accreditation and has been shown to give a similar performance to the ISO approach

ANNEX C: Calculation and interpretation of relative trueness

Category	Type	Aw	Sample number	Log_{10}dcfu/g		Mean	Difference
				Alternative method	Reference method		
Confectionary-bakery- eggs	bakery with custard	0.960	4	2.771	2.260	2.515	-0.511
		0.988	156	2.643	4.320	3.482	1.677
		0.990	170	6.867	6.737	6.802	-0.130
		*	205	4.357	4.089	4.223	-0.268
		*	208	4.980	5.079	5.029	0.099
	egg products	*	11	4.538	4.204	4.371	-0.334
		0.995	55	3.276	2.945	3.111	-0.331
		*	104	5.165	5.027	5.096	-0.139
		*	130	3.490	3.301	3.396	-0.189
		*	145	3.301	4.936	4.119	1.635
	par baked bread	0.966	200	4.932	4.810	4.871	-0.122
		0.960	201	2.964	2.215	2.589	-0.749
		*	204	3.765	3.079	3.422	-0.686
		0.962	210	4.059	3.959	4.009	-0.100
		*	311	2.000	1.477	1.739	-0.523
Dairy	Cheese	*	20	6.464	5.658	6.061	-0.806
		0.966	24	5.464	4.591	5.027	-0.873
		*	26	6.089	5.872	5.981	-0.217
		*	41	7.436	7.000	7.218	-0.436
		0.972	49	5.778	5.888	5.833	0.110
		*	53	5.503	5.214	5.358	-0.289
		*	74	2.845	2.279	2.562	-0.566
		0.976	87	7.389	7.978	7.683	0.589
		0.954	106	7.348	7.297	7.322	-0.052
		0.953	115	4.281	3.490	3.885	-0.791
		*	122	6.184	5.839	6.012	-0.344
		0.980	123	6.744	6.447	6.596	-0.297
		*	143	4.320	3.561	3.940	-0.760
	Fermented drinks	0.986	10	2.944	2.373	2.659	-0.572
		0.978	21	3.320	3.004	3.162	-0.316
		0.988	94	<1	<1	<1	NA
		*	207	3.967	3.816	3.892	-0.151
		*	209	3.714	3.633	3.674	-0.081
	yogurt	0.990	2	3.283	2.079	2.681	-1.204
		0.985	57	4.110	4.143	4.127	0.033
		0.971	77	6.062	6.722	6.392	0.660
		*	79	5.751	3.602	4.676	-2.148
		*	117	4.582	4.281	4.431	-0.301
Fruits and vegetables	Chilled juices	0.984	18	2.663	1.602	2.132	-1.061
		0.987	71	3.464	3.490	3.477	0.026
		0.989	78	4.056	4.049	4.052	-0.007
		0.998	148	2.519	2.342	2.430	-0.176
		0.998	301	<1	<1	<1	NA
	fermented vegetables	0.968	28	6.982	7.097	7.040	0.115
		0.987	114	5.748	5.771	5.760	0.023
		*	303	5.398	5.016	5.207	-0.382
		0.978	304	<1	<1	<1	NA
		*	307	6.281	6.056	6.168	-0.225
		0.978	19	5.102	5.967	5.534	0.866

Category	Type	Aw	Sample number	Log_{10}dcfu/g		Mean	Difference
				Alternative method	Reference method		
	Fresh fruit and fruit purees	0.988	25	4.149	4.059	4.104	-0.090
		0.978	34	3.963	3.980	3.971	0.017
		0.983	101	3.114	2.918	3.016	-0.196
		*	202	7.171	7.089	7.130	-0.082
		*	203	4.339	3.415	3.877	-0.924
Multi-component foods	ambient stable sauces	0.985	14	2.623	2.515	2.569	-0.109
		0.987	44	<1	<1	<1	NA
		0.983	72	4.008	4.073	4.040	0.065
		0.984	163	3.945	3.828	3.887	-0.118
		*	309	<1	<1	<1	NA
	Foods with raw ingredients	*	35	4.954	4.477	4.716	-0.477
		0.984	37	3.152	3.160	3.156	0.008
		0.979	39	4.765	5.260	5.012	0.495
		0.983	40	7.086	7.950	7.518	0.864
		0.981	52	6.086	6.491	6.289	0.405
		0.980	64	8.833	7.176	8.004	-1.656
	Mayonnaise based salads	*	31	<1	<1	<1	NA
		*	61	4.000	2.477	3.239	-1.523
		*	305	3.922	3.893	3.908	-0.029
		0.980	306	4.114	5.045	4.579	0.931
		*	308	3.714	3.538	3.626	-0.176
RTE Foods	Cooked or cured fish	0.973	63	7.778	7.672	7.725	-0.106
		0.969	107	8.021	6.571	7.296	-1.450
		0.993	108	2.176	1.954	2.065	-0.222
		0.984	125	5.301	4.950	5.125	-0.351
		*	139	4.111	4.038	4.074	-0.073
	Cured meats	*	38	5.582	5.527	5.554	-0.055
		0.987	62	5.281	5.149	5.215	-0.132
		0.964	118	5.339	5.281	5.310	-0.058
		*	206	3.927	2.550	3.239	-1.377
		*	211	5.030	5.000	5.015	-0.030
	RTE Meat and Poultry	*	29	7.826	5.856	6.841	-1.970
		0.971	59	7.255	7.204	7.230	-0.051
		0.977	68	4.932	4.867	4.899	-0.065
		0.966	75	8.000	5.778	6.889	-2.222
		0.957	102	3.888	3.810	3.849	-0.078

ANNEX D: Raw data accuracy profile study

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Quiche – low a	-1	22	**245**	**2.38**	-1 0.5ml	9	**270**	**2.43**
Quiche – low a	-1	27			-1 0.5ml	18		
Quiche – low b	-1	24	**250**	**2.39**	-1 0.5ml	14	**240**	**2.38**
Quiche – low b	-1	26			-1 0.5ml	10		
Quiche – low c	-1	31	**290**	**2.46**	-1 0.5ml	9	**190**	**2.28**
Quiche – low c	-1	27			-1 0.5ml	10		
Quiche – low d	-1	24	**255**	**2.40**	-1 0.5ml	8	**200**	**2.30**
Quiche – low d	-1	27			-1 0.5ml	12		
Quiche – low e	-1	11	**210**	**2.32**	-1 0.5ml	10	**210**	**2.32**
Quiche – low e	-1	31			-1 0.5ml	11		
Quiche –med a	-1	T	**800**	**2.90**	-1	15	**1500**	**3.18**
Quiche –med a	-2	8			-2	0		
Quiche –med b	-1	T	**900**	**2.95**	-1	17	**1700**	**3.23**
Quiche –med b	-2	9			-2	0		
Quiche –med c	-1	T	**700**	**2.84**	-1	13	**1300**	**3.11**
Quiche –med c	-2	7			-2	0		
Quiche –med d	-1	T	**1000**	**3**	-1	19	**1900**	**3.28**
Quiche –med d	-2	10			-2	0		
Quiche –med e	-1	T	**1300**	**3.11**	-1	28	**2727**	**3.44**
Quiche –med e	-2	13			-2	2		
Quiche –med e	-3	2						
Quiche – high a	-3	36	**36364**	**4.56**	-2	73	**77273**	**4.89**
Quiche – high a	-4	4			-3	12		
Quiche – high b	-3	26	**27273**	**4.43**	-2	57	**55455**	**4.74**
Quiche – high b	-4	4			-3	4		
Quiche – high c	-3	25	**25000**	**4.39**	-2	24	**22727**	**4.36**
Quiche – high c	-4	0			-3	1		
Quiche – high d	-3	39	**46364**	**4.66**	-2	55	**56364**	**4.75**
Quiche – high d	-4	12			-3	7		

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Quiche – high e	-3	29	**30909**	**4.49**	-2	39	**40000**	**4.60**
Quiche – high e	-4	5			-3	5		
Egg custard –low	-1	30	**305**	**2.48**	-1 0.5ml	15	**300**	**2.48**
Egg custard –low	-1	31			-1 0.5ml	15		
Egg custard –low	-1	24	**265**	**2.42**	-1 0.5ml	5	**130**	**2.11**
Egg custard –low	-1	29			-1 0.5ml	8		
Egg custard –low	-1	29	**235**	**2.37**	-1 0.5ml	14	**240**	**2.38**
Egg custard –low	-1	18			-1 0.5ml	10		
Egg custard –low	-1	25	**235**	**2.37**	-1 0.5ml	10	**270**	**2.43**
Egg custard –low	-1	22			-1 0.5ml	17		
Egg custard –low	-1	21	**280**	**2.44**	-1 0.5ml	14	**290**	**2.46**
Egg custard –low	-1	35			-1 0.5ml	15		
Egg custard –low	-2				-1			
Egg custard –med	-1	T	**1400**	**3.14**	-1	16	**1727**	**3.24**
Egg custard –med	-2	14			-2	2		
Egg custard –med	-1	T	**2091**	**3.32**	-1	16	**1727**	**3.24**
Egg custard –med	-2	21			-2	3		
Egg custard –med	-3	2						
Egg custard –med	-1	Spread	**3000**	**3.47**	-1	19	**1818**	**3.26**
Egg custard –med	-2	Spread			-2	1		
Egg custard –med	-3	3						
Egg custard –	-1	Spread	**2000**	**3.30**	-1	18	**1800**	**3.26**
Egg custard –	-2	Spread			-2	1		
Egg custard –	-3	3						
Egg custard –med	-1	T	**700**	**2.84**	-1	22	**2273**	**3.36**
Egg custard –	-2	7			-2	3		
Egg custard –high	-3	52	**51818**	**4.71**	-2	74	**76364**	**4.88**
Egg custard –high	-4	5			-3	10		
Egg custard –	-3	35	**34545**	**4.53**	-2	57	**56364**	**4.75**
Egg custard –	-4	3			-3	4		
Egg custard –	-3	36	**34545**	**4.53**	-2	76	**79091**	**4.90**
Egg custard –high	-4	2			-3	11		

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Egg custard –	-3	42	**42723**	**4.63**	-2	73	**72727**	**4.86**
Egg custard –	-4	5			-3	7		
Egg custard –	-3	43	**63636**	**4.80**	-2	60	**61818**	**4.79**
Egg custard –	-4	7			-3	8		
Prawns – low a	-1	3	**20**	**1.30**	-1 0.5ml	1	**10**	**1.00**
Prawns – low a	-1	1			-1 0.5ml	0		
Prawns – low b	-1	3	**25**	**1.39**	-1 0.5ml	2	**30**	**1.48**
Prawns – low b	-1	2			-1 0.5ml	1		
Prawns – low c	-1	7	**40**	**1.60**	-1 0.5ml	1	**10**	**1.00**
Prawns – low c	-1	1			-1 0.5ml	0		
Prawns – low d	-1	5	**35**	**1.54**	-1 0.5ml	1	**30**	**1.48**
Prawns – low d	-1	2			-1 0.5ml	1		
Prawns – low e	-1	5	**30**	**1.47**	-1 0.5ml	1	**10**	**1.00**
Prawns – low e	-1	1			-1 0.5ml	0		
Prawns – med a	-3	18	**17273**	**4.23**	-2	30	**30000**	**4.48**
Prawns – med a	-4	1			-3	0		
Prawns – med b	-3	32	**34545**	**4.53**	-2	40	**39091**	**4.59**
Prawns – med b	-4	3			-3	3		
Prawns – med c	-3	28	**27273**	**4.43**	-2	43	**41818**	**4.62**
Prawns – med c	-4	2			-3	3		
Prawns – med d	-3	31	**31000**	**4.49**	-2	35	**32727**	**4.51**
Prawns – med d	-4	0			-3	1		
Prawns – med e	-3	17	**17273**	**4.23**	-2	23	**24545**	**4.39**
Prawns – med e	-4	2			-3	4		
Prawns – med e	-5				-5			
Prawns – high a	-4	39	**409091**	**5.61**	-3	41	**400000**	**5.60**
Prawns – high a	-5	6			-4	3		
Prawns – high b	-4	32	**318182**	**5.50**	-3	35	**327273**	**5.51**
Prawns – high b	-5				-7			
Prawns – high c	-4	42	**427273**	**5.63**	-3	63	**600000**	**5.78**

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Prawns – high c	-5	5			-4	6		
Prawns – high d	-4	38	**390909**	**5.59**	-3	40	**427273**	**5.63**
Prawns – high d	-5	5			-4	7		
Prawns – high e	-4	20	**227273**	**5.35**	-3	32	**309091**	**5.49**
Prawns – high e	-5	5			-4	2		
Tuna pate– low a	-1	22	**255**	**2.40**	-1 0.5ml	3	**150**	**2.18**
Tuna pate– low a	-1	29			-1 0.5ml	12		
Tuna pate– low b	-1	31	**295**	**2.46**	-1 0.5ml	10	**200**	**2.30**
Tuna pate– low b	-1	28			-1 0.5ml	10		
Tuna pate– low c	-1	30	**280**	**2.44**	-1 0.5ml	4	**140**	**2.15**
Tuna pate– low c	-1	26			-1 0.5ml	10		
Tuna pate– low d	-1	34	**325**	**2.51**	-1 0.5ml	11	**140**	**2.15**
Tuna pate– low d	-1	31			-1 0.5ml	3		
Tuna pate– low e	-1	25	**270**	**2.43**	-1 0.5ml	20	**400**	**2.60**
Tuna pate– low e	-1	29			-1 0.5ml	20		
Tuna pate – med a	-1	T	**1818**	**3.25**	-1	22	**2091**	**3.32**
Tuna pate – med a	-2	19			-2	1		
Tuna pate – med a	-3	1						
Tuna pate – med b	-1	76	**1000**	**3.00**	-1	14	**1400**	**3.15**
Tuna pate – med b	-2	10			-2	0		
Tuna pate – med c	-1	T	**1909**	**3.280**	-1	14	**1400**	**3.15**
Tuna pate – med c	-2	20			-2	0		
Tuna pate – med c	-3	1			-3	0		
Tuna pate – med d	-1	125	**1327**	**3.12**	-1	27	**3182**	**3.50**
Tuna pate – med d	-2	21			-2	8		
Tuna pate – med d	-3	1						
Tuna pate – med e	-1	95	**927**	**2.96**	-1	10	**1000**	**3.00**
Tuna pate – med e	-2	7			-2	1		
Tuna pate – high a	-4	22	**236364**	**5.37**	-3	40	**372727**	**5.57**
Tuna pate – high a	-5	4			-4	1		
Tuna pate – high b	-4	28	**290909**	**5.46**	-3	24	**236363**	**5.37**

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Tuna pate – high b	-5	4			-4	2		
Tuna pate – high c	-4	27	**263636**	**5.42**	-3	26	**254545**	**5.41**
Tuna pate – high c	-5	2			-4	2		
Tuna pate – high d	-4	28	**281818**	**5.44**	-3	37	**381818**	**5.58**
Tuna pate – high d	-5	3			-4	5		
Tuna pate – high e	-4	19	**181818**	**5.25**	-3	31	**310000**	**5.49**
Tuna pate – high e	-5	1			-4	0		
Pasta salad –low a	-1	86	**905**	**2.95**	-1 0.5ml	59	**1160**	**3.06**
Pasta salad –low a	-1	95			-1 0.5ml	57		
Pasta salad –low b	-1	100	**1005**	**3.00**	-1 0.5ml	72	**1290**	**3.11**
Pasta salad –low b	-1	101			-1 0.5ml	57		
Pasta salad –low c	-1	103	**990**	**2.99**	-1 0.5ml	64	**1310**	**3.12**
Pasta salad –low c	-1	95			-1 0.5ml	68		
Pasta salad –low d	-1	118	**1205**	**3.08**	-1 0.5ml	79	**1690**	**3.23**
Pasta salad –low d	-1	123			-1 0.5ml	90		
Pasta salad –low e	-1	83	**835**	**2.92**	-1 0.5ml	92	**1870**	**3.27**
Pasta salad –low e	-1	84			-1 0.5ml	95		
Pasta salad –med a	-2	64	**6182**	**3.79**	-1	75	**7454**	**3.87**
Pasta salad –med a	-3	4			-2	7		
Pasta salad –med b	-2	56	**6000**	**3.77**	-1	71	**6909**	**3.84**
Pasta salad –med b	-3	10			-2	5		
Pasta salad –med c	-2	75	**7727**	**3.88**	-1	46	**4727**	**3.67**
Pasta salad –med c	-3	10			-2	6		
Pasta salad –med d	-2	85	**8273**	**3.91**	-1	97	**9182**	**3.96**
Pasta salad –med d	-3	6			-2	9		
Pasta salad –med e	-2	57	**6182**	**3.79**	-1	50	**5000**	**3.70**
Pasta salad –med e	-5				-5			
Pasta salad –high a	-3	63	**70000**	**4.84**	-3	18	**181818**	**5.26**
Pasta salad –high a	-4	14			-4	2		
Pasta salad –high b	-3	91	**95455**	**4.97**	-3	15	**145455**	**5.16**

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Pasta salad –high b	-4	14			-4	1		
Pasta salad –high c	-3	87	**89091**	**4.94**	-3	17	**172727**	**5.24**
Pasta salad –high c	-4	11			-4	2		
Pasta salad –high d	-3	T	**181818**	**5.25**	-3	18	**200000**	**5.30**
Pasta salad –high d	-4	19			-4	4		
Pasta salad –high d	-5	1			-7			
Pasta salad –high e	-3	T	**163636**	**5.21**	-3	16	**160000**	**5.20**
Pasta salad –high e	-4	16			-4	0		
Pasta salad –high e	-5	2						
Sandwich low a	-1	15	**163**	**2.21**	-1 0.5ml	7	**200**	**2.30**
Sandwich low a	-2	3			-1 0.5ml	13		
Sandwich low b	-1	28	**270**	**2.43**	-1 0.5ml	6	**230**	**2.36**
Sandwich low b	-1	27			-1 0.5ml	10		
Sandwich low c	-1	46	**370**	**2.56**	-1 0.5ml	11	**350**	**2.54**
Sandwich low c	-1	28			-1 0.5ml	9		
Sandwich low c	-2	2			-1	5		
Sandwich low d	-1	38	**280**	**2.44**	-1 0.5ml	14	**230**	**2.36**
Sandwich low d	-1	34			-1 0.5ml	9		
Sandwich low d	-2	2			-3			
Sandwich low e	-1	42	**430**	**2.63**	-1 0.5ml	19	**410**	**2.61**
Sandwich low e	-1	44			-1 0.5ml	22		
Sandwich low e	-2	4			-3			
Sandwich med a	-2	52	**5363**	**3.72**	-1	45	**5128**	**3.71**
Sandwich med a	-3	7			-2	12		
Sandwich med b	-2	T	**20000**	**4.30**	-1	T	**11000**	**4.04**
Sandwich med b	-3	19			-2	11		
Sandwich med b	-4	3			-4			
Sandwich med c	-2	77	**8091**	**3.90**	-1	108	**11000**	**4.04**
Sandwich med c	-3	12			-2	13		
Sandwich med d	-2	42	**5545**	**3.74**	-1	73	**7000**	**3.85**
Sandwich med d	-3	9			-2	4		
Sandwich med e	-2	91	**9273**	**3.96**	-1	110	**10636**	**4.03**

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Sandwich med e	-3	11			-2	7		
Sandwich high a	-3	T	**200000**	**5.30**	-2	T	**272727**	**5.44**
Sandwich high a	-4	19			-3	28		
Sandwich high a	-5	3			-4	2		
Sandwich high b	-3	T	**209091**	**5.32**	-2	T	**227273**	**5.36**
Sandwich high b	-4	22			-3	21		
Sandwich high b	-5	1			-4	1		
Sandwich high c	-3	T	**181818**	**5.25**	-2	T	**330000**	**5.52**
Sandwich high c	-4	19			-3	33		
Sandwich high c	-5	1			-4	0		
Sandwich high d	-3	T	**190909**	**5.28**	-2	T	**210000**	**5.32**
Sandwich high d	-4	18			-3	21		
Sandwich high d	-5	3			-4	0		
Sandwich high e	-3	T	**281818**	**5.44**	-2	T	**190909**	**5.28**
Sandwich high e	-4	29			-3	20		
Sandwich high e	-5	2			-4	1		
Beetroot salad –	-1	10	**130**	**2.11**	-1	4	**400**	**2.60**
Beetroot salad –	-1	13			-1	0		
Beetroot salad –	-1	20	**195**	**2.29**	-1	2	**200**	**2.30**
Beetroot salad –	-1	19			-1	0		
Beetroot salad –	-1	10	**130**	**2.11**	-1	26	**490**	**2.69**
Beetroot salad –	-1	16			-1	23		
Beetroot salad –	-1	13	**130**	**2.11**	-1	4	**400**	**2.60**
Beetroot salad –	-1	13			-1	0		
Beetroot salad –	-1	15	**165**	**2.21**	-1	3	**300**	**2.48**
Beetroot salad –	-1	18			-1	0		
Beetroot salad –	-2	107	**10455**	**4.01**	-1	91	**9273**	**3.97**
Beetroot salad –	-3	8			-2	11		
Beetroot salad –	-2	76	**7273**	**3.86**	-1	89	**8455**	**3.93**
Beetroot salad –	-3	4			-2	4		
Beetroot salad –	-2	110	**10636**	**4.02**	-1	97	**10091**	**4.00**
Beetroot salad –	-3	7			-2	14		

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Beetroot salad –	-2	87	**9091**	**3.95**	-1	81	**8364**	**3.92**
Beetroot salad –	-3	13			-2	11		
Beetroot salad –	-1	93	**9364**	**3.97**	-1	72	**7091**	**3.85**
Beetroot salad –	-1	10			-2	6		
Beetroot salad –	-4	100	**1036364**	**6.01**	-3	84	**809091**	**5.91**
Beetroot salad –	-5	14			-4	5		
Beetroot salad –	-4	88	**872727**	**5.94**	-3	87	**872727**	**5.94**
Beetroot salad –	-5	8			-4	9		
Beetroot salad –	-4	99	**981818**	**5.99**	-3	103	**1018182**	**6.01**
Beetroot salad –	-5	9			-4	9		
Beetroot salad –	-4	92	**936364**	**5.97**	-3	86	**8545455**	**6.93**
Beetroot salad –	-5	11			-4	7		
Beetroot salad –	-4	93	**909091**	**5.95**	-3	71	**672727**	**5.83**
Beetroot salad –	-5	7			-4	3		
Veg juice –low a	-2	34	**3364**	**3.52**	-1	41	**4100**	**3.61**
Veg juice –low a	-3	3			-2	0		
Veg juice –low b	-2	25	**2636**	**3.42**	-1	37	**3700**	**3.57**
Veg juice –low b	-3	4			-2	0		
Veg juice –low c	-2	27	**2545**	**3.40**	-1	35	**3500**	**3.54**
Veg juice –low c	-3	1			-2	0		
Veg juice –low d	-2	26	**2455**	**3.39**	-1	47	**4700**	**3.67**
Veg juice –low d	-3	1			-2	0		
Veg juice –low e	-2	25	**2455**	**3.39**	-1	12	**2200**	**3.34**
Veg juice –low e	-3	2			-2	15		
Veg juice –med a	-3	68	**74545**	**4.87**	-2	87	**85455**	**4.93**
Veg juice –med a	-4	14			-3	7		
Veg juice –med b	-3	62	**63636**	**4.80**	-2	91	**93636**	**4.97**
Veg juice –med b	-4	8			-3	2		
Veg juice –med c	-3	49	**48182**	**4.68**	-2	67	**67273**	**4.83**
Veg juice –med c	-4	4			-3	7		

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Veg juice –med d	-3	40	**41818**	**4.62**	-2	50	**46364**	**4.67**
Veg juice –med d	-4	6			-3	1		
Veg juice –med e	-3	42	**40909**	**4.61**	-2	72	**68182**	**4.83**
Veg juice –med e	-4	3			-3	3		
Veg juice -high a	-4	62	**718182**	**5.85**	-3	81	**854545**	**5.93**
Veg juice -high a	-5	17			-4	13		
Veg juice -high b	-4	41	**390909**	**5.59**	-3	52	**527273**	**5.72**
Veg juice -high b	-5	2			-4	6		
Veg juice -high c	-4	58	**609091**	**5.78**	-3	72	**736364**	**5.87**
Veg juice -high c	-5	9			-4	4		
Veg juice -high d	-4	55	**527273**	**5.72**	-3	67	**645455**	**5.81**
Veg juice -high d	-5	3			-4	4		
Veg juice -high e	-4	66	**681818**	**5.83**	-3	115	**1172727**	**6.07**
Veg juice -high e	-5	9			-4	14		
Yogurt drink – low	-1	72	**695**	**2.84**	-1 0.5ml	34	**670**	**2.83**
Yogurt drink – low	-1	67			-1 0.5ml	33		
Yogurt drink – low	-2	3						
Yogurt drink – low	-1	69	**605**	**2.78**	-1 0.5ml	29	**650**	**2.81**
Yogurt drink – low	-1	52			-1 0.5ml	36		
Yogurt drink – low	-2	8						
Yogurt drink – low	-1	Not plated	**800**	**2.903**	-1 0.5ml	40	**620**	**2.79**
Yogurt drink – low	-2	8			-1 0.5ml	22		
Yogurt drink – low	-1	69	**660**	**2.81**	-1 0.5ml	38	**780**	**2.89**
Yogurt drink – low	-1	63			-1 0.5ml	40		
Yogurt drink – low	-2	8						
Yogurt drink – low	-1	67	**620**	**2.79**	-1 0.5ml	30	**590**	**2.77**
Yogurt drink – low	-1	57			-1 0.5ml	29		
Yogurt drink – low	-2	10						
Yogurt drink –	-3	71	**70000**	**4.84**	-4	70	**66364**	**4.82**
Yogurt drink –	-4	6			-5	3		
Yogurt drink –	-3	63	**64545**	**4.80**	-2	65	**62727**	**4.80**
Yogurt drink –	-4	8			-3	4		

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Yogurt drink –	-3	73	**70000**	**4.84**	-2	68	**66364**	**4.82**
Yogurt drink –	-4	4			-3	5		
Yogurt drink –	-3	79	**772773**	**5.88**	-2	65	**62727**	**4.80**
Yogurt drink –	-4	6			-3	4		
Yogurt drink –	-3	71	**73636**	**4.86**	-2	71	**70709**	**4.85**
Yogurt drink –	-4	10			-3	7		
Yogurt drink –	-4	56	**581818**	**5.76**	-3	72	**754545**	**5.88**
Yogurt drink –	-5	8			-4	11		
Yogurt drink –	-4	50	**472727**	**5.67**	-3	60	**590909**	**5.77**
Yogurt drink –	-5	2			-4	5		
Yogurt drink –	-4	58	**663636**	**5.82**	-3	47	**481818**	**5.68**
Yogurt drink –	-5	5			-4	6		
Yogurt drink –	-4	75	**736364**	**5.86**	-3	75	**736364**	**5.87**
Yogurt drink –	-5	6			-4	6		
Yogurt drink –	-4	51	**518182**	**5.71**	-3	50	**481818**	**5.68**
Yogurt drink –	-5	6			-4	3		
Cream cheese –	-1	60	**575**	**2.75**	-1 0.5ml	23	**510**	**2.71**
Cream cheese –	-1	65			-1 0.5ml	28		
	-2	5						
Cream cheese –	-1	48	**510**	**2.70**	-1 0.5ml	19	**420**	**2.62**
Cream cheese –	-1	54			-1 0.5ml	23		
Cream cheese –	-2	3						
Cream cheese –	-1	26	**570**	**2.75**	-1 0.5ml	12	**360**	**2.56**
Cream cheese –	-1	37			-1 0.5ml	24		
Cream cheese –	-2	3						
Cream cheese –	-1	43	**882**	**2.94**	-1 0.5ml	21	**450**	**2.65**
Cream cheese –	-1	54			-1 0.5ml	24		
Cream cheese –	-2	7						
Cream cheese –	-1	49	**465**	**2.67**	-1 0.5ml	21	**400**	**2.60**
Cream cheese –	-1	44			-1 0.5ml	19		
Cream cheese –	-2	5						

Item - Inoculum level	Alternative Compact Dry YM – 3 days				Reference method			
	Dilution (1ml)	No of colonies	Count (cfu/g)	log cfu/g	Dilution (0.1ml)	No of colonies	Count (cfu/g)	log cfu/g
Cream cheese –	-3	58	**55455**	**4.74**	-2	58	**59091**	**4.77**
Cream cheese –	-4	3			-3	7		
Cream cheese –	-3	62	**58182**	**4.76**	-2	66	**65455**	**4.82**
Cream cheese –	-4	2			-3	6		
Cream cheese –	-3	62	**60909**	**4.78**	-2	72	**72727**	**4.86**
Cream cheese –	-4	5			-3	8		
Cream cheese –	-3	53	**52727**	**4.72**	-2	67	**65455**	**4.82**
Cream cheese –	-4	5			-3	5		
Cream cheese –	-3	78	**74545**	**4.87**	-2	85	**87277**	**4.94**
Cream cheese –	-4	4			-3	11		
Cream cheese -	-4	67	**636364**	**5.80**	-3	68	**681818**	**5.83**
Cream cheese -	-5	3			-4	7		
Cream cheese -	-4	39	**381818**	**5.58**	-3	51	**490909**	**5.69**
Cream cheese -	-5	3			-4	3		
Cream cheese -	-4	39	**363636**	**5.56**	-3	45	**472727**	**5.67**
Cream cheese -	-5	1			-4	7		
Cream cheese -	-4	55	**536364**	**5.72**	-3	63	**618182**	**5.79**
Cream cheese -	-5	4			-4	5		
Cream cheese -	-4	42	**420000**	**5.62**	-3	37	**390909**	**5.59**
Cream cheese -	-5	0			-4	6		

ANNEX E: Summary data accuracy profile study

(Food) Category 2	Confectionary
(Food) Type 2	Quiche and egg custard

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
44 a-e	Quiche	Low	270	240	190	200	210	245	250	290	255	210
134 a-e	Egg custard	Low	300	125	240	270	290	305	265	235	235	280
2 a-e	Quiche	Med	1500	1700	1300	1900	2727	800	900	700	1000	1300
124 a-e	Egg custard	Med	1636	1727	1818	1400	2273	1400	2091	3000	2000	700
15 a-e	Quiche	High	77273	55455	22727	56364	40000	36364	27273	25000	46364	30909
165 a-e	Egg custard	High	76364	56364	79091	72727	61818	51818	34545	34545	42723	63636

(Food) Category 2	Dairy
(Food) Type 2	Yogurt drink and cream cheee

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
88 a-e	Yogurt drink	Low	670	650	620	780	590	695	605	800	660	620
147 a-e	Cream cheese	Low	510	420	360	450	400	575	510	570	882	465
84 a-e	Yogurt drink	Med	66364	62727	66364	62727	70709	70000	64545	70000	77273	73636
160 a-e	Cream cheese	Med	59091	65455	72727	65455	87277	55455	58182	60909	52727	74545
10 a-e	Yogurt drink	High	754545	590909	481818	736364	481818	581818	472727	663636	736364	518182
15 a-e	Cream cheese	High	681818	490909	472727	618182	390909	636364	381818	363636	536364	420000

(Food) Category 2	Multi-component
(Food) Type 2	Sandwiches and deli-salad

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
174 a-e	Sandwiches	Low	200	230	350	230	410	163	270	370	280	430
6 a-e	Salad	Low	1160	1290	1310	1090	1870	905	1005	990	1205	835
200 a-e	Sandwiches	Med	5128	11000	11000	7000	10636	5363	20000	8091	5545	9273
155 a-e	Salad	Med	7454	6909	4727	9182	5000	6182	6000	7727	8273	6182
180 a-e	Sandwiches	High	272727	227273	330000	210000	190909	200000	209091	181818	190909	281818
79 a-e	Salad	High	181818	145455	172727	200000	160000	70000	95455	89091	181818	163636

(Food) Category 2	PRODUCE 3day
(Food) Type 2	beetroot salad and vegetable juice

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
25 a-e	beetroot salad	Low	400	200	200	400	300	130	195	130	130	165
31 a-e	vegetable juice	Low	4100	3700	3500	4700	2200	3364	2636	2545	2455	2455
133 a-e	beetroot salad	Med	9273	8455	10091	8364	7091	10455	7273	10636	9091	9364
85 a-e	vegetable juice	Med	85455	93636	67273	46364	68182	74545	63636	48182	41818	40909
190 a-e	vegetable juice	High	854545	527273	736364	645455	1172727	718182	390909	609091	527273	681818
13a-e	beetroot salad	High	809091	872727	1018182	845455	672727	1036364	872727	981818	936364	909091

(Food) Category 4	RTE Foods
(Food) Type 4	prawns and pate

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
186 a-e	prawns	Low	10	30	10	20	10	20	25	40	35	30
197 a-e	pate	Low	150	200	140	140	400	255	295	280	325	270
68 a-e	pate	Med	1100	2091	1400	3182	1000	1818	1000	1909	1327	927
64 a-e	prawns	Med	30000	39091	41818	32727	24545	17273	34545	27273	31000	17273
36 a-e	prawns	High	400000	327273	600000	427273	309091	409091	318182	427273	390909	227273
23 a-e	pate	High	372727	236363	254545	381818	310000	236364	290909	263636	281818	181818

ANNEX F: Raw data inclusivity and exclusivity study

Inclusivity strains

No.	Species	Type	Source[a]	Origin	CFU reference (PDA)	CFU CD YMR	Morphology on CD YMR at 72 h
1	*Alternaria alternata*	Mold	IFO 31188	living leaf, *Stevia rebaudiana*	38	10	blue green - dark green,
2	*Aspergillus brasiliensis*	Mold	NBRC 9455	Blueberry, *Vaccinium* sp.	31	35	white - blue green
3	*Aspergillus flavus*	Mold	NBRC 6343	Shoe sole	38	TNTC	pale blue green
4	*Aspergillus fumigatus*	Mold	NBRC 33022	unknown	65	55	pale blue green
5	*Aspergillus niger*	Mold	NBRC 105649	Leather	95	82	deep blue green; black
6	*Aspergillus oryzae*	Mold	NBRC 5375	unknown	38	36	blue green; pale brown
7	*Aspergillus terreus*	Mold	NBRC 6346	Haversack	39	31	White; not clearly formed
8	*Aspergillus versicolor*	Mold	NBRC 4098	tobacco	53	41	pale blue green; not
9	*Aureobasidium pullulans*	Yeast	NBRC 6353	unknown	45	51	blue - dark green
10	*Candida albicans*	Yeast	NBRC 1594	Clinical bronchomycosis	58	54	white - pale green
11	*Candida apicola*	Yeast	NBRC 10261	intestine of bee	98	54	blue green
12	*Candida lactis-condensi*	Yeast	NBRC 1286	fermenting condensed milk	54	79	blue green; blue green
13	*Chaetomium globosum*	Mold	NBRC 6347	Stored cotton	41	43	blue green
14	*Cladosporium cladosporioides*	Mold	NBRC 6348	unknown	63	29	pale blue green - blue
15	*Cladosporium halotolerans*	Mold	NBRC 4460	Air	52	8	white - pale blue green; not
16	*Debaryomyces hansenii*	Yeast	IFO 0026	beef-and-pork sausage	31	30	white
17	*Debaryomyces maramus*	Yeast	NBRC 0668	Air	41	4	white
18	*Fusarium oxysporum*	Mold	NBRC 7155	unknown	53	53	pale blue green; deep
19	*Fusarium solani*	Mold	NBRC 5232	unknown	45	42	blue green; cottony center
20	*Geotrichum candidum*	Yeast	NBRC 4598	unknown	58	41	pale greenish white; cottony
21	*Hormoconis resinae*	Mold	NBRC 100535	unknown	76	23	very pale blue green; atypical
22	*Monascus purpureus*	Mold	NBRC 32316	red rice	38	18	blue green; pale brown
23	*Moniliella acetoabutans*	Yeast	NBRC 9482	sweet fruit sauce	59	45	blue green
24	*Myrothecium verrucaria*	Mold	NBRC 6113	Deteriorated baled cotton	64	59	blue green; pale brown
25	*Neosartorya fischeri*	Mold	IFO 8789	rubber tire scrap	31	29	pale blue green - blue
26	*Paecilomyces variotii*	Mold	NBRC 33284	unknown	66	64	pale blue green - blue
27	*Penicillium aurantiogriseum*	Mold	NBRC 7733	Rotting grain of *Zea mays*	46	40	blue green

No.	Species	Type	Source[a]	Origin	CFU reference (PDA)	CFU CD YMR	Morphology on CD YMR at 72 h
28	*Penicillium brevicompactum*	Mold	NBRC 5727	soil	58	42	blue green; white - pale
29	*Penicillium chrysogenum*	Mold	IFO 32030	cheese	57	46	blue green; white cottony
30	*Penicillium citrinum*	Mold	NBRC 6352	unknown	71	65	yellow green; white cottony
31	*Penicillium funiculosum*	Mold	NBRC 100958	Mercury-treated fabric	57	27	pale white; not clearly formed
32	*Penicillium martensii*	Mold	NBRC 8142	unknown	58	55	Green; not clearly formed
33	*Penicillium ochrochloron*	Mold	NBRC 4612	unknown	48	37	blue green; not clearly formed
34	*Penicillium pinophilum*	Mold	NBRC 33285	unknown	79	47	white - very pale blue
35	*Phialophora fastigiata*	Mold	IFO 6850	unknown	52	42	blue green; not clearly formed
36	*Phoma herbarum*	Mold	NBRC 107643	Polyester straw on drinking pot	49	21	white - very pale blue
37	*Pichia anomala*	Yeast	IFO 10213	unknown	31	31	blue green (nearly green)
38	*Pseudocochliobolus lunatus*	Mold	NBRC 30883	leaf of sudangrass 'Greenleaf'	61	52	pale blue green; not
39	*Rhizopus oryzae*	Mold	NBRC 31005	Radio set	33	TNTC	partly pale yellow brown;
40	*Rhodotorula acuta*	Yeast	IFO 1912	grape must	81	75	blue green; pale blue
41	*Rhodotorula glutinis*	Yeast	NBRC 1125	Air	33	29	blue green; pale blue
42	*Rhodotorula mucilaginosa*	Yeast	NBRC 0889	unknown	70	49	pale blue green; very
43	*Saccharomyces cerevisiae*	Yeast	NBRC 101557	Fermenting sake mash	37	31	white - pale green
44	*Scopulariopsis brevicaulis*	Mold	NBRC 100536	Chrysalis of silkworm	56	61	pale blue green - blue
45	*Torulaspora delbrueckii*	Yeast	IFO 1180	grape must	54	43	blue green (nearly green);
46	*Trichoderma citrinoviride*	Mold	IFO 31137	Soil in organic layer	65	68	pale blue green; not
47	*Trichoderma virens*	Mold	NBRC 6355	Soil	37	22	blue green - yellow green;
48	*Trichophyton mentagrophytes*	Mold	IFO 6202	unknown	64	62	pale blue green
49	*Trichosporon asahii*	Yeast	NBRC 103889	case of trichosporia cutis	76	62	blue green
50	*Zygosaccharomyces bailii*	Yeast	NBRC 1098	unknown	94	72	pale blue green - blue
51	*Zygosaccharomyces rouxii*	Yeast	NBRC 1960	cane sugar	72	83	white - pale blue green;

[a]IFO = Campden Culture Collection (Campden BRI, Chipping Campden, UK); NBRC = National Institute of Technology and Evaluation Biological Resource Center (Tokyo, Japan)
[b]"+" indicates growth occurred.

Exclusivity strains

No.	Species	Source[a]	Origin	Growth[b]
1	*Acinetobactor baumannii*	JCM 6841	Urine	-
2	*Acinetobacter calcoaceticus*	ATCC 19606	unknown	-
3	*Alcaligenes faecalis*	IFO 13111	unknown	-
4	*Bacillus cereus*	IFO 13494	unknown	-
5	*Bacillus licheniformis*	NBRC 12200	unknown	-
6	*Bacillus subtilis*	NBRC 3134	unknown	-
7	*Burkholderia cepacia*	NBRC 15124	10% benzalkonium chloride solution	-
8	*Citrobacter freundii*	IFO 12681	unknown	-
9	*Enterobacter aerogens*	ATCC 13048	Sputum, South Carolina Dept. of Health and Environmental Control	-
10	*Enterobacter cloacae* subsp. *Cloacae*	NBRC 13535	Spinal fluid	-
11	*Enterococcus faecalis*	NBRC 12965	Citrus juice	-
12	*Enterococcus faecium*	ATCC 19434	unknown	-
13	*Escherichia coli*	NBRC 3301	Human feces	-
14	*Escherichia coli*	NBRC 3972	Feces	-
15	*Flavobacterium odoratum*	ATCC 4651	unknown	-
16	*Kocuria rhizophila*	ATCC 9341	Soil	-
17	*Lactobacillus casei*	NBRC 15883	Cheese	-
18	*Lactococcus lactis*	NS 6938	human	-
19	*Listeria monocytogenes*	VTU 206	unknown	-
20	*Micrococcus luteus*	NBRC 3333	unknown	-
21	*Morganella morganiii*	ATCC 25830	Patient with summer diarrhea	-
22	*Paenibacillus polymyxa*	NBRC 15309	unknown	-
23	*Proteus mirabilis*	IFO 3849	unknown	-
24	*Pseudomonas aeruginosa*	NBRC 13275	Outer ear infection	-
25	*Pseudomonas stutzeri*	ATCC 17587	Bile	-
26	*Rhodococcus equi*	IFO 14956	Lung abscess of foal	-
27	*Salmonella* Typhimurium	ATCC 14028	Tissue, animal - pools of heart and liver from 4-week-old chickens	-
28	*Serratia marcescens* subsp. *Marcescens*	NBRC 102204	Pond water	-

No.	Species	Source[a]	Origin	Growth[b]
29	*Shigella flexneri*	ATCC 12022	unknown	-
30	*Staphylococcus aureus* subsp. *Aureus*	NBRC 12732	unknown	-
31	*Staphylococcus epidermidis*	ATCC 35984	Catheter sepsis, Tennessee	-
32	*Streptococcus pyogenes*	JCM 5674	Scarlet fever	-

[a]JCM = Japan Collection of Microorganisms (RIKEN BioResource Center, Ibaraki, Japan); ATCC = American Type Culture Collection (Manassas, VA, USA); IFO = Campden Culture Collection (Campden BRI, Chipping Campden, UK); NBRC = National Institute of Technology and Evaluation Biological Resource Center (Tokyo, Japan); VTU = Visvesvaraya Technological University (Bangalore, India)
[b]"-" indicates growth did not occur.

ANNEX G: List of collaborative laboratories

Laboratories	Country	Adresse	Number of collaborators
Jars	Poland	ŁAJSKI, 2a Kościelna Str. 05-119 Legionowo Poland	2
Micro-Smedt	Belgium	Vennen 4 Bus 1 Herentals B 2200 BELGIUM	2
Coca Cola	Belgium	Bergensesteenweg 1424, 1070 Anderlecht, BELGIUM	2
GNT International B.V.	Netherlands	GNT International B.V. Industrieweg 26 5731 HR Mierlo Netherlands	2
Muller	UK	Donnington Wood Business Park, Granville Road, Donnington Wood, Telford, Shropshire, TF2 7GJ	2
Britvic	UK	Breakspear Park, Hemel Hempstead HP2 4TZ	2
Campden BRI Nutfield site	UK	Nutfield, Coopers Hill Road, Nutfield, Surrey, RH1 4HY, UK	2

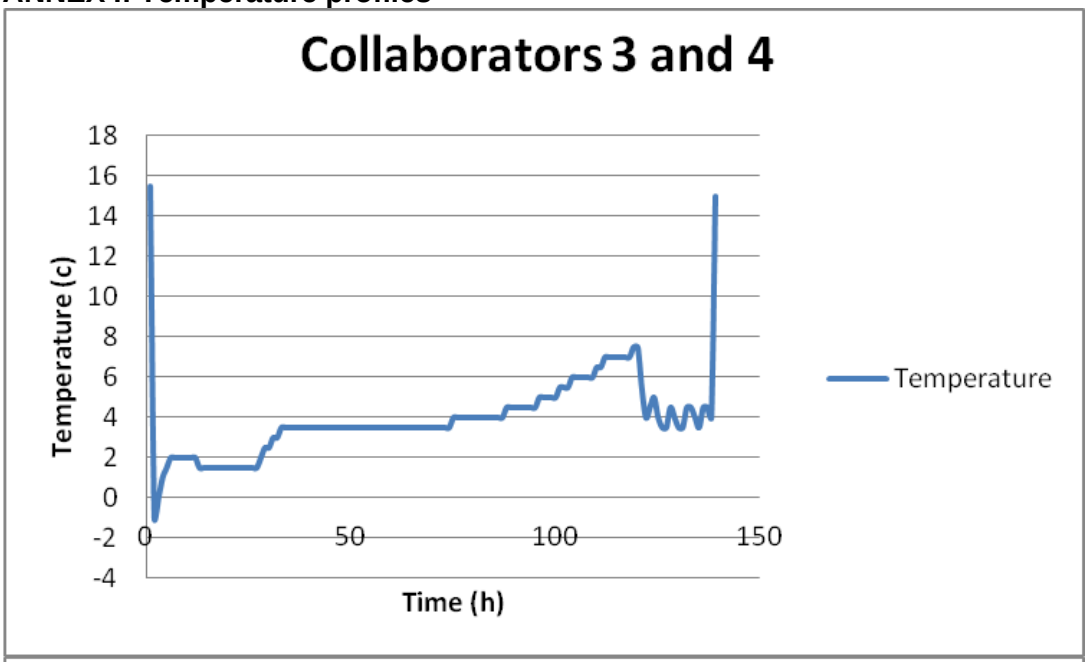
ANNEX H: Raw data from the ILS

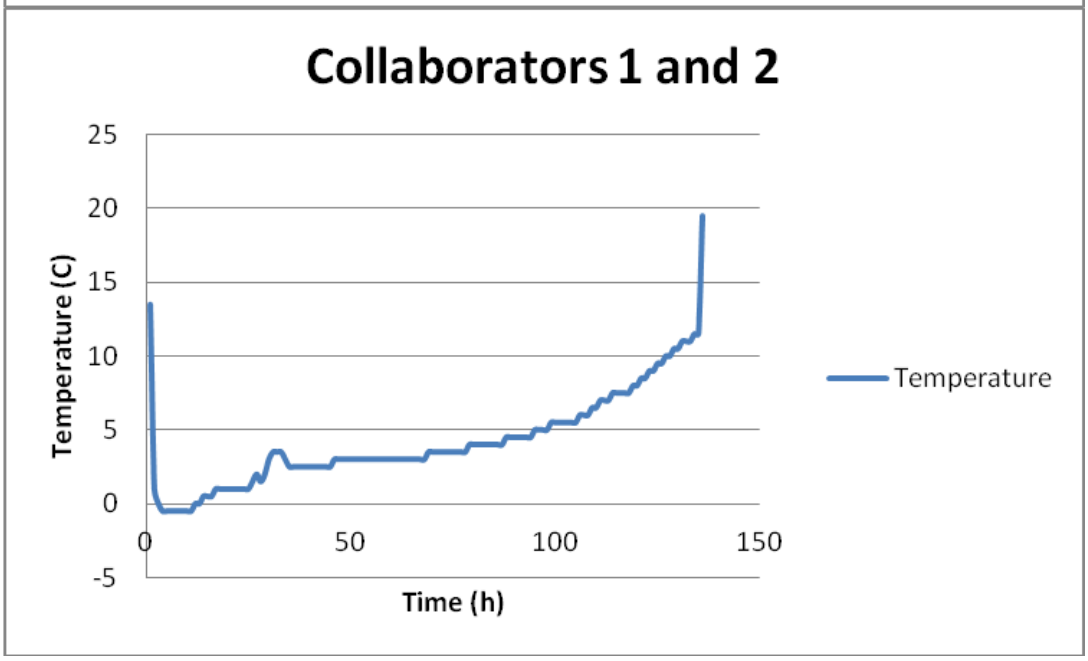
Laboratory	Collaborator	Sample code	Level	Reference method	Alternative Method	Date samples tested
1	1	4	Blank	<10	<10	01/03/2017
	1	1	Low	410	227	01/03/2017
	1	5	Low	650	655	01/03/2017
	1	2	Medium	11182	7091	01/03/2017
	1	6	Medium	18545	14818	01/03/2017
	1	3	High	554545	636364	01/03/2017
	1	7	High	390909	309091	01/03/2017
1	2	8	Blank	<10	<10	01/03/2017
	2	13	Low	770	700	01/03/2017
	2	14	Low	490	445	01/03/2017
	2	10	Medium	17455	20000	01/03/2017
	2	12	Medium	15455	16455	01/03/2017
	2	9	High	790909	672727	01/03/2017
	2	11	High	745455	672727	01/03/2017
2	3	4	Blank	<10	<10	01/03/2017
	3	1	Low	490	255	01/03/2017
	3	5	Low	410	282	01/03/2017
	3	2	Medium	6455	7364	01/03/2017
	3	6	Medium	6364	6727	01/03/2017
	3	3	High	336364	410000	01/03/2017
	3	7	High	481818	460000	01/03/2017
2	4	8	Blank	<10	<10	01/03/2017
	4	13	Low	480	218	01/03/2017
	4	14	Low	320	273	01/03/2017
	4	10	Medium	11091	9364	01/03/2017
	4	12	Medium	8545	5091	01/03/2017
	4	9	High	372727	336364	01/03/2017
	4	11	High	490909	372727	01/03/2017
3	5	4	Blank	<10	<10	28/02/2017
	5	1	Low	350	273	28/02/2017
	5	5	Low	280	110	28/02/2017
	5	2	Medium	6272	4273	28/02/2017
	5	6	Medium	9455	4727	28/02/2017
	5	3	High	336364	218182	28/02/2017
	5	7	High	445455	156364	28/02/2017
3	6	8	Blank	<10	<10	28/02/2017
	6	13	Low	390	236	28/02/2017
	6	14	Low	180	264	28/02/2017
	6	10	Medium	8455	3909	28/02/2017
	6	12	Medium	3818	5727	28/02/2017
	6	9	High	318182	218182	28/02/2017
	6	11	High	381818	300000	28/02/2017
4	7	4	Blank	<10	<10	28/02/2017
	7	1	Low	480	491	28/02/2017

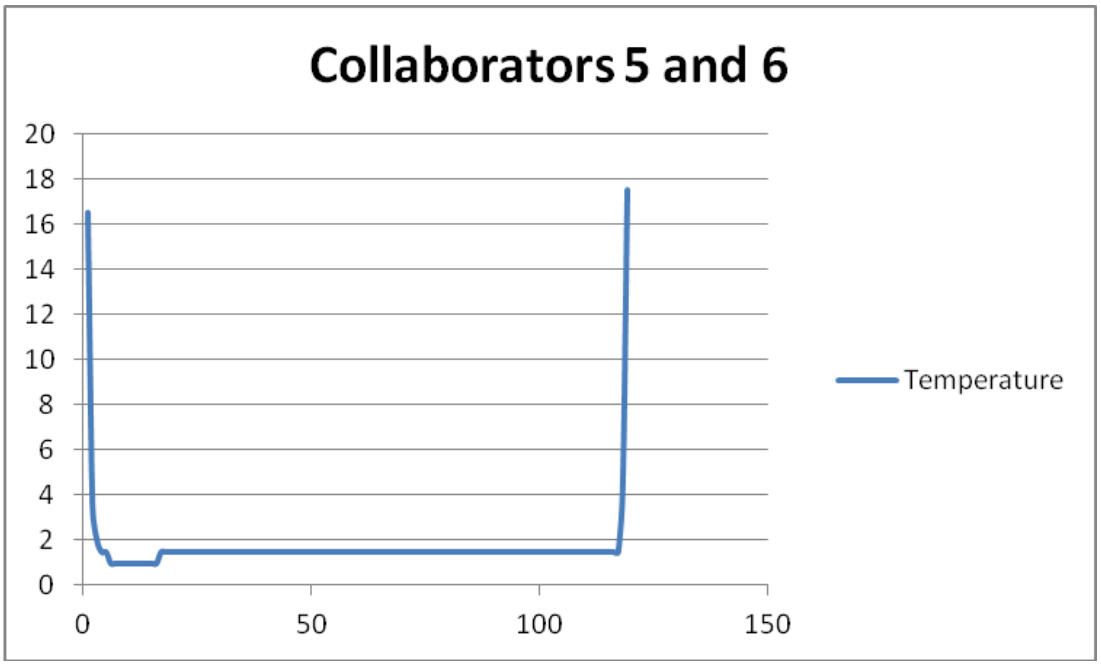
Laboratory	Collaborator	Sample code	Level	Reference method	Alternative Method	Date samples tested
	7	5	Low	540	527	28/02/2017
	7	2	Medium	10000	13182	28/02/2017
	7	6	Medium	9909	13909	28/02/2017
	7	3	High	481818	554545	28/02/2017
	7	7	High	654545	690909	28/02/2017
	8	8	Blank	<10	<10	28/02/2017
	8	13	Low	750	645	28/02/2017
	8	14	Low	630	636	28/02/2017
4	8	10	Medium	12000	12545	28/02/2017
	8	12	Medium	12909	14636	28/02/2017
	8	9	High	454545	727273	28/02/2017
	8	11	High	654545	645455	28/02/2017
	9	4	Blank	<10	<10	28/02/2017
	9	1	Low	570	445	28/02/2017
	9	5	Low	480	427	28/02/2017
5	9	2	Medium	10909	6545	28/02/2017
	9	6	Medium	7000	7000	28/02/2017
	9	3	High	263636	354545	28/02/2017
	9	7	High	400000	654545	28/02/2017
	10	8	Blank	<10	<10	28/02/2017
	10	13	Low	550	491	28/02/2017
	10	14	Low	520	409	28/02/2017
5	10	10	Medium	13636	8727	28/02/2017
	10	12	Medium	5000	6636	28/02/2017
	10	9	High	381818	445455	28/02/2017
	10	11	High	409091	381818	28/02/2017
	11	4	Blank	<10	<10	28/02/2017
	11	1	Low	520	364	28/02/2017
	11	5	Low	440	336	28/02/2017
6	11	2	Medium	8636	8182	28/02/2017
	11	6	Medium	11909	9091	28/02/2017
	11	3	High	372727	445455	28/02/2017
	11	7	High	318182	430000	28/02/2017
	12	8	Blank	<10	<10	28/02/2017
	12	13	Low	750	491	28/02/2017
	12	14	Low	630	445	28/02/2017
6	12	10	Medium	14909	18182	28/02/2017
	12	12	Medium	19000	14545	28/02/2017
	12	9	High	654545	545455	28/02/2017
	12	11	High	609091	563636	28/02/2017
	13	4	Blank	<10	<10	28/02/2017
	13	1	Low	410	355	28/02/2017
7	13	5	Low	260	264	28/02/2017
	13	2	Medium	6273	4818	28/02/2017
	13	6	Medium	6182	3727	28/02/2017
	13	3	High	281818	354545	28/02/2017

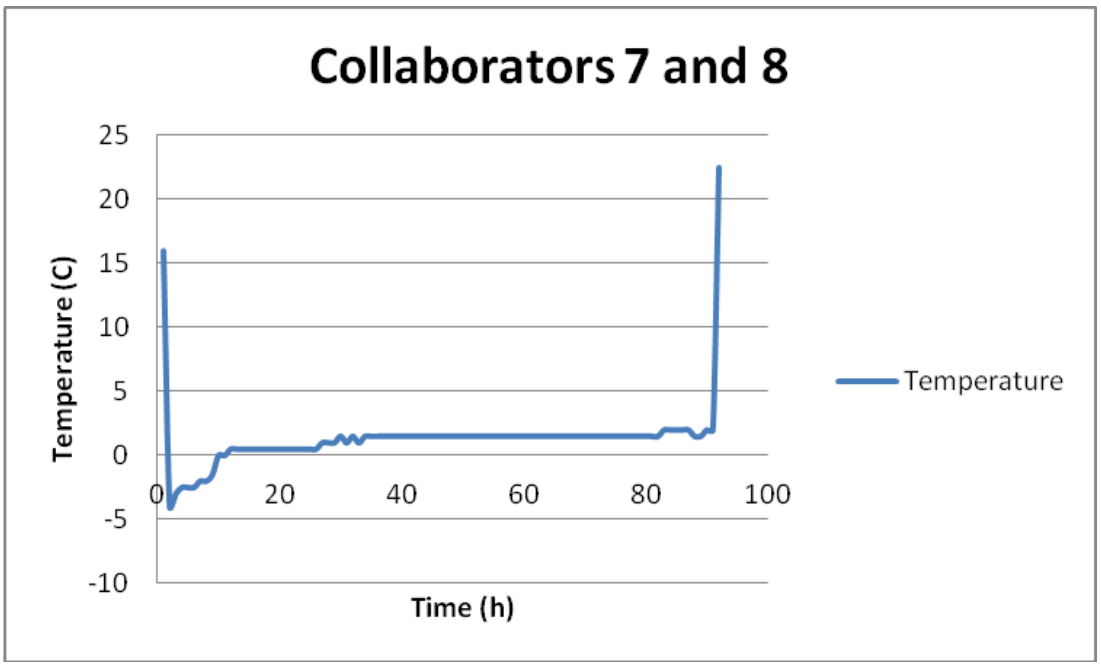
Laboratory	Collaborator	Sample code	Level	Reference method	Alternative Method	Date samples tested
	13	7	High	336364	372727	28/02/2017
7	14	8	Blank	<10	<10	28/02/2017
	14	13	Low	310	336	28/02/2017
	14	14	Low	240	409	28/02/2017
	14	10	Medium	5909	6818	28/02/2017
	14	12	Medium	4364	4000	28/02/2017
	14	9	High	318181	281818	28/02/2017
	14	11	High	281818	390909	28/02/2017
8 (Expert Lab)	Expert Lab	4	Blank	<10	<10	28/02/2017
	Expert Lab	1	Low	260	409	28/02/2017
	Expert Lab	5	Low	360	300	28/02/2017
	Expert Lab	2	Medium	7182	6636	28/02/2017
	Expert Lab	6	Medium	6818	6455	28/02/2017
	Expert Lab	3	High	427273	281818	28/02/2017
	Expert Lab	7	High	363636	318182	28/02/2017

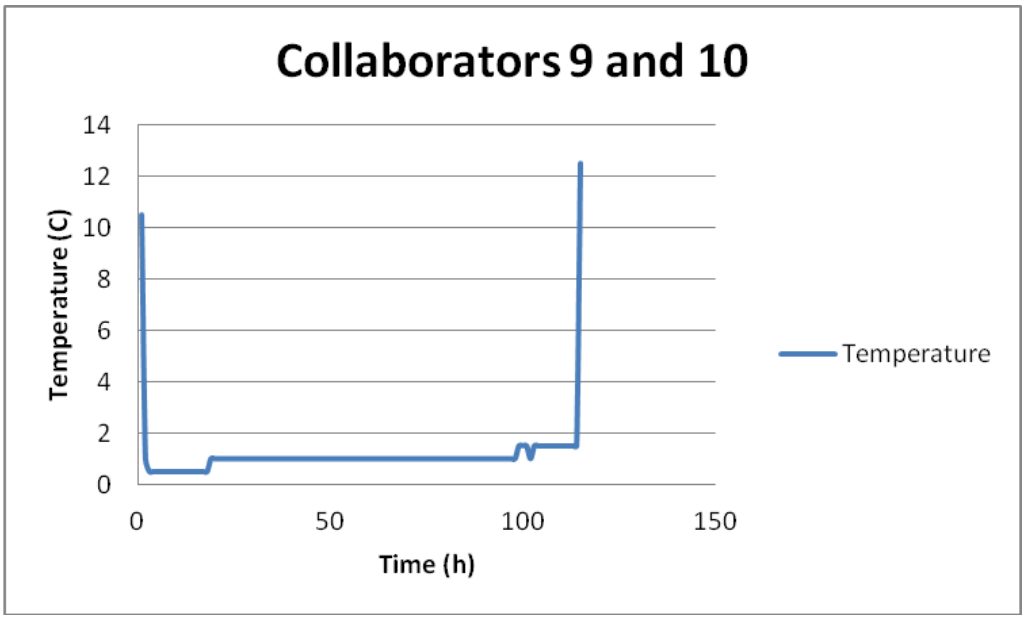
ANNEX I: Temperature profiles

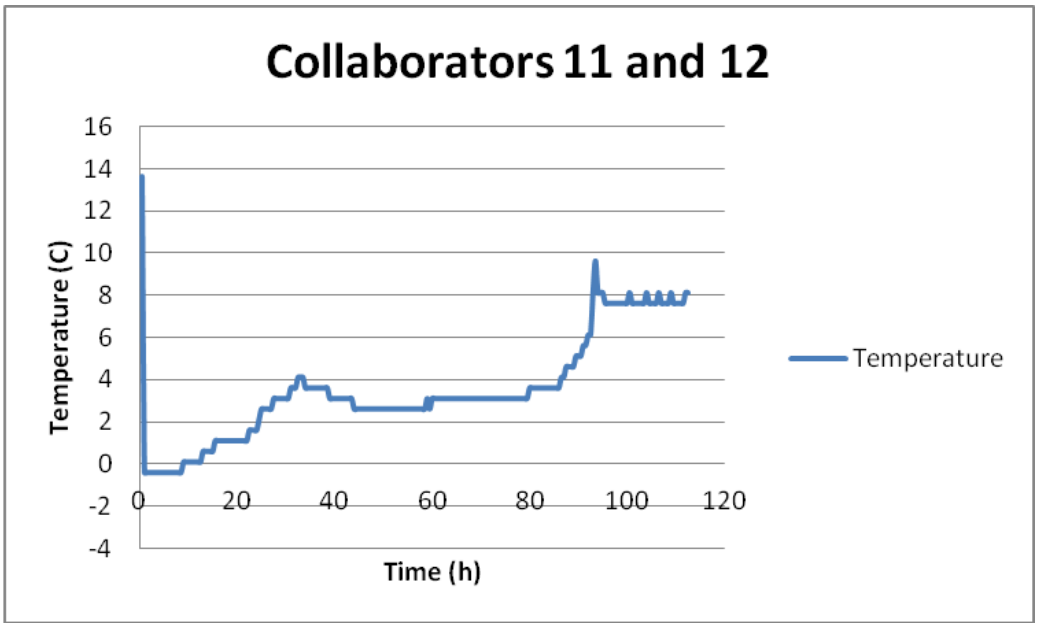





Collaborators 5 and 6
20
18
16
14
12
10
8
6
4
2
0
0
50
100
150
Temperature


Collaborators 7 and 8
25
20
15
10
5
0
-5
-10
Temperature (C)
0
20
40
60
80
100
Time (h)
Temperature


Collaborators 9 and 10
Temperature (C)
14
12
10
8
6
4
2
0
0
50
100
150
Time (h)
Temperature


Collaborators 11 and 12
Temperature (C)
16
14
12
10
8
6
4
2
0
-2
-4
0
20
40
60
80
100
120
Time (h)
Temperature