Quantitative study protocol for the ISO 16140-2:2016 validation of Compact Dry CFR, for the enumeration of coliforms in 2 selected categories: raw milk and dairy products and pasteurised milk and dairy products

MicroVal study number: 2023LR123

Method/Kit name: CompactDry CFR

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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.2.5

Company: **Shimadzu Diagnostics Coroporation**

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Method/Kit name: CompactDry CFR

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 methods: ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms**.**

Scope of validation: 2 categories (raw milk and dairy products, heat processed milk and dairy products).

Certification organization: Lloyd's Register

List of abbreviations

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

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of an alternative method for the enumeration of coliforms in 2 categories (Raw dairy products and Heat processed dairy products) was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

Compact Dry CFR (Shimadzu Diagnostics Corporation) 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. This is a ready to use, chromogenic plate for the enumeration of Coliforms. Following incubation at 35±1°C for 16-18h Coliforms grow to give blue/blue-green colonies due to chromogens contained in the medium.

The validation has been carried out at 2 incubation time points and the data generated during the validation will be used to determine which incubation window will be selected for use in the kit insert. The first time point was 16h incubation, of which the incubation window is 16-18h. The second time point was18 hours with an incubation window if 18-20h.

The reference method used was:

ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

An incubation temperature of 37°C±1°C was used for the reference method for dairy products.

Scope of the validation study is: 2 named categories

Categories included:

- Raw milk and dairy products
- Heat processed milk and dairy products

Criteria evaluated during the study have been:

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

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

The alternative method CompactDry CFR shows comparable performance to the reference method (ISO 4832:2006). The full conclusions can be found in Section 3.3.3.

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 the same sample.

2.1 Reference method

The reference method use was:

ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

An incubation temperature of 37°C±1°C was used for the reference method for dairy products.

See the flow diagram in Annex A.

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

2.2 Alternative method

See the flow diagram in Annex A.

Compact Dry CFR (Shimadzu Diagnostics Corportation) 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. This is a ready to use, chromogenic plate for the enumeration of Coliforms. Following incubation at 35±1°C for 16-18h Coliforms grow to give blue/blue-green colonies due to chromogens contained in the medium.

The validation has been carried out at 2 incubation time points, the data generated during the validation will be used to determine which incubation window will be selected for use in the kit insert. The first time point is 16h incubation, of which the incubation window is 16-18h. The second time point is 18 hours with an incubation window if 18-20h.

2.3 Study design

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

The reference method and alternative are performed with the same sample.

3 Method comparison study

3.1 Relative trueness study

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

A total of 2 categories were included in this validation study. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category. Each category was made up of 3 types, with at least 5 items representative for each type.

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

Table 1 – Categories, types and number of samples analyzed

Category	Types	Items	No of samples	ISO 6887	Diluent used
Raw milk and dairy products	Raw milk	Raw milk	5	6887-5	MRD
	Raw dairy products	raw milk hard cheese e.g. Comte	5	6887-5	Sodium citrate diluent
	Raw dairy products	raw milk soft cheese	5	6887-5	Sodium citrate diluent
Heat processed milk and dairy products	Pasteurised milk	pasteurised skim milk (non-fat milk), milk based drinks	5	6887-5	MRD
	Pasteurised milk based products	Processed cheese, mlk based deserts, creams, ice cream	5	6887-5	MRD Sodium citrate diluent for cheese
	Dry milk products	Milk powders and powder for milk based desserts	5	6887-5	MRD

30 samples were analyzed, leading to 30 exploitable results.

3.1.2 Test sample preparation

Naturally contaminated samples were preferentially analyzed. 20 samples were screened for the presence of the target organism. From these samples 25 % were positive for the target organism and these samples were used in the data analysis. The remaining 75% were negative for the target organism. It was therefore necessary to use artificial contamination procedures

Samples were inoculated with coliform strains before storage of the inoculated samples, e.g. frozen foods were stored for at least 2 weeks at -20 °C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature.

In addition, 5 pasteurised milk samples were spiked with a heat-treated coliform isolate. The injury level achieved for the isolate was at least 0.5 log.

Six colifom islates were used for the artificial contamination in a mixture of seeding and spiking protocols. Each isolate was used to contaminate no more than 5 items during the study.

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

16 % of the samples used for the relative trueness study were naturally contaminated.

Artificially contaminated samples are indicated in bold characters in the results tables.

3.1.3 Protocols applied during the validation study

Incubation time

An incubation time of 16 hours and 18 hours was used for the CompactDry CFR.

3.1.4 Test results

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

3.1.5 Calculation and interpretation of relative trueness study

The calculations are provided in Annex B.

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

16h incubation

Figures 1-3 show the scatter plots for 16h incubation of CompactDry CFR.

Figure 1 - Scatter plot of the reference method versus alternative method results for the raw milk and dairy products – 16h incubation

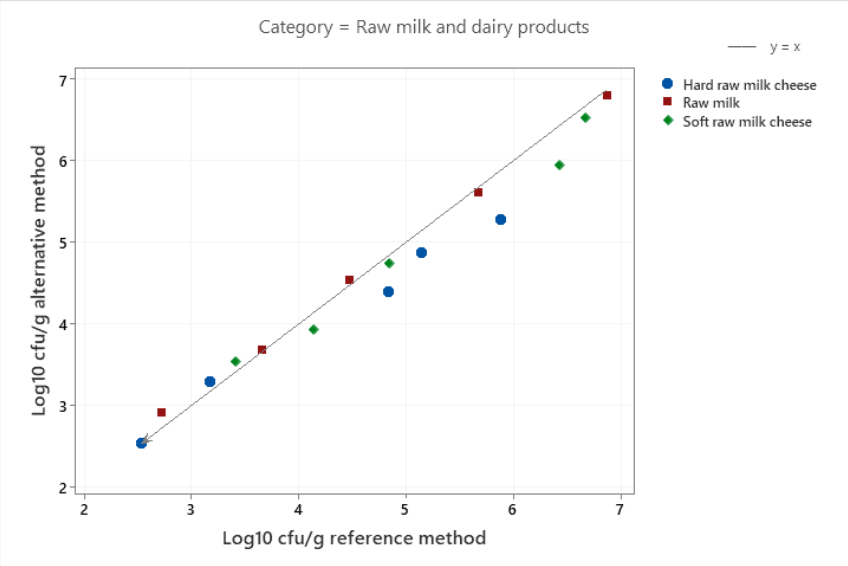


Figure 2- Scatter plot of the reference method versus alternative method results for the heat processed milk and dairy products – 16h incubation

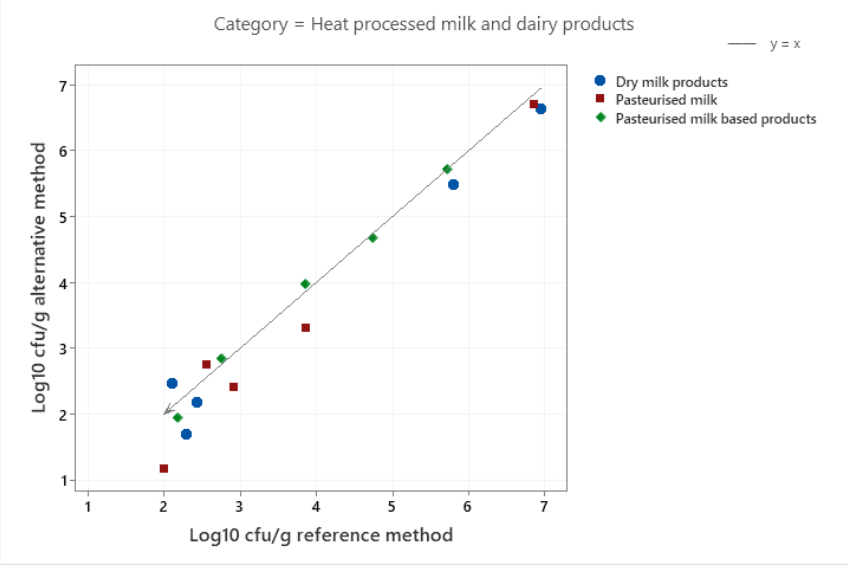
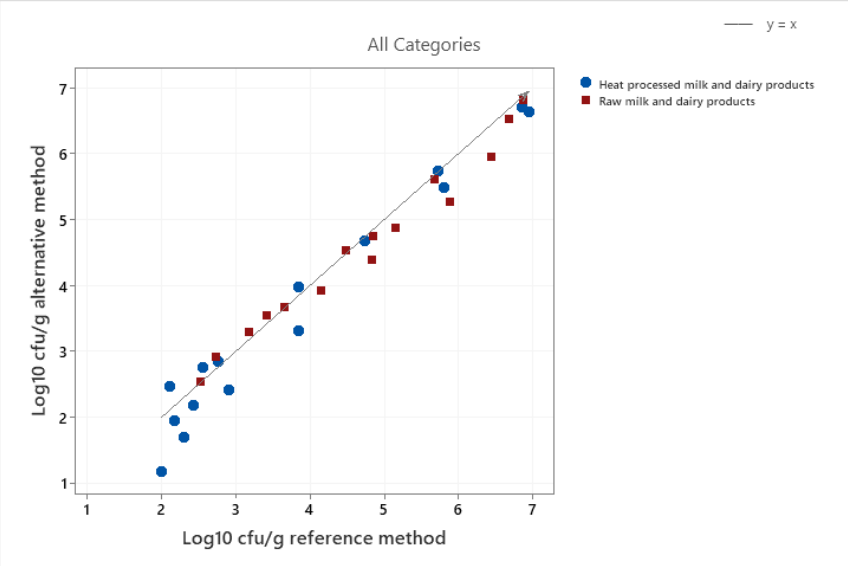


Figure 3 - Scatter plot of the reference method versus alternative method results for all the categories – 16h incubation



16h incubation

According to ISO16140-2:2016 6.1.2.3, the results of the scatter plot are interpreted on the visual observation of the amount of bias and extreme results. The data in the scatter plots show no obvious disagreement. There is a trend for slight negative bias in heat processed milk and dairy products.

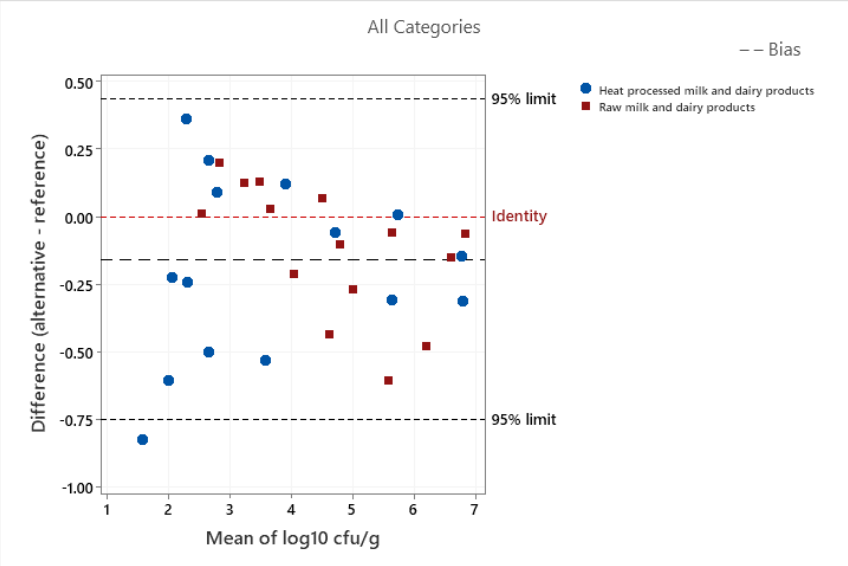
Table 2 - Summary of the calculated values per category 16h incubation

Category	**n**	$\overline{D}$	**SD**	**95 % low limit**	**95 % upper limit**
Heat processed milk and dairy products	15	-0.197	0.329	-0.924	0.531
Raw milk and dairy products	15	-0.120	0.238	-0.648	0.408
All Categories	30	-0.158	0.285	-0.750	0.433

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

The Bland-Altman difference plot for all the samples at a 16-hour incubation is given Figure 4.

Figure 4 – Bland-Altman difference plot for all the samples – 16-hour incubation



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

Table 3 - Data which are outside of the accepted limits – 16h incubation

Category	**Type**	**Inoculation**	**N° Sample**	**Reference method** **Log cfu/g**	**Alternative method** **Log cfu/g**	**Lower limit**	**Difference** **Alternative – reference)**	**Incubation time**
Heat processed milk and dairy products	Pasteurised milk	*Klebsiella ozaene* 4273 heat stress	M19	1.18	2.00	-0.750	-0.82	16 hours

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

The datapoint that is outside the limits is a pasteurized milk sample, inoculated with heat stressed *Klebsiella ozaene*. As highlighted in the scatterplots and the calculated values displayed in Table 2, there is indication of a slight negative bias in the heat processed milk and dairy product category. The sample types which are affected appear to be low level milk powder and pasteurised milk. These samples types were inoculated with lyophilized

cells and heat stressed cells respectively. There is a potential trend towards the effect of stressed cells on slowing growth of coliforms on CD CFR. A root cause analysis has been carried out and is detailed in the next section.

Root cause analysis for 16h incubation – stressed cells

Due to the identification of a negative bias in powdered and pasteurised milk samples, additonal samples were tested, as detailed below:

- Skimmed milk powder samples, inoculated with a different strain to the relative trueness study, *Enterobacter aerogenes* CRA 4232
- Pasteurised milk samples, inoculated with a different strain to the relative trueness study, *Escherichia coli* CRA 11017

The same stress protocols were applied as in the relative trueness study, as detailed in Tables 4 and 5, to determine whether the method has a negative bias towards stressed cells. The results with the log differences at each incubation time are shown in Tables 4 and 5.

Table 4 – Additional testing results for dried powder samples

Sample code	Sample	Inoculation	Stress	CD CFR 16h	CD CFR 18h	Reference	Log differences	
							16h - VB	18h - VB
M31	Skim milk powder 1	*Enterobacter aerogenes* CRA 4232	Lyophilised culture - storage for 2 weeks	5.6	5.6	5.8	-0.27	-0.27
M32	Skim milk powder 2			4.7	4.7	4.9	-0.25	-0.25
M33	Skim milk powder 3			3.9	3.9	4.1	-0.22	-0.22
M34	Skim milk powder 4			3.0	3.1	3.0	0.00	0.04
M35	Skim milk powder 5			2.2	2.2	2.2	0.03	0.06

Table 5 – Additional testing results for dried powder samples

Sample code	Sample	Inoculation	Stress	CD CFR 16h	CD CFR 18h	Reference	Log differences	
							16h - VB	18h - VB
M36	Pasteurised milk 1	*E.coli* CRA 11017	Heat treatment 55°C for 10 minutes, 0.5 log injury	6.5	6.5	6.1	0.41	0.41
M37	Pasteurised milk 2			5.7	5.7	5.4	0.22	0.22
M38	Pasteurised milk 3			4.4	4.4	4.3	0.07	0.07
M39	Pasteurised milk 4			3.6	3.6	3.4	0.21	0.21
M40	Pasteurised milk 5			2.6	2.6	2.4	0.17	0.17

Data from the repeat samples do not indicate a consistent negative bias in dried and pasteurised milk with stressed cells. The slight negative bias observed with selected samples in the relative trueness study is more likely to be a strain-sample combination rather than an indication of slower growth in stressed cells.

In addition, there was little change in the log differences between the reference and alternative methods with increased incubation time. These additional results have been added to the exisisting relative trueness results and displayed in Figures 5 and 6.

Figure 5. Scatter plot of the reference method versus alternative method results for the heat processed milk and dairy product category – 16h incubation, including additonal troubleshooting samples

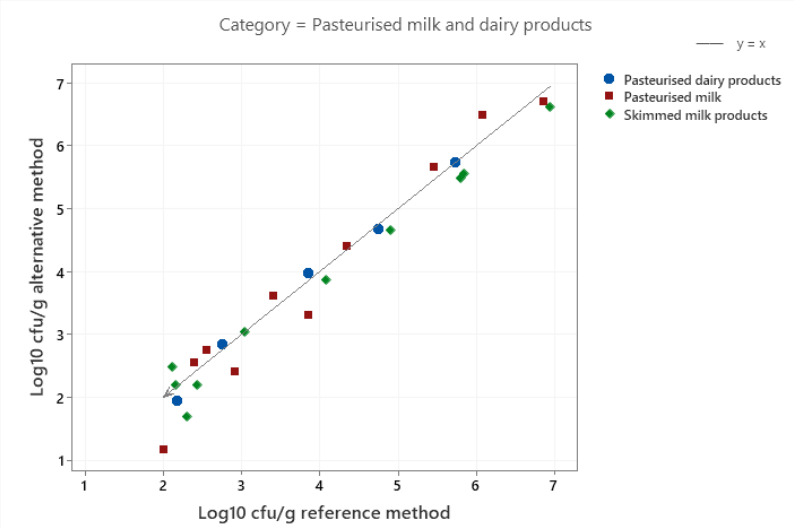
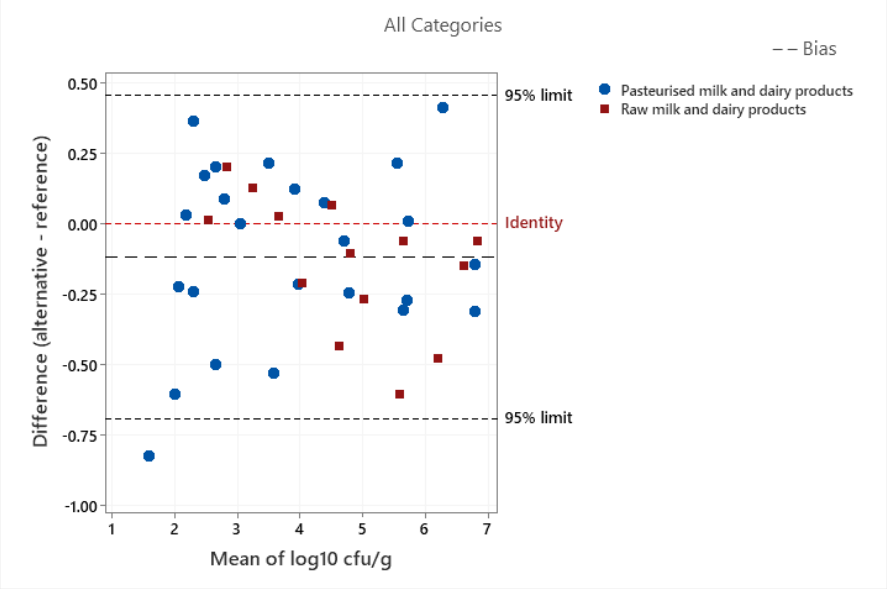
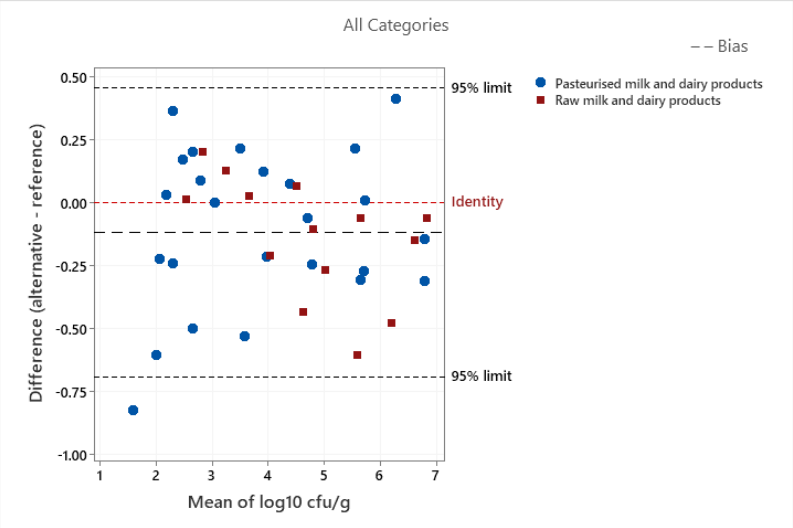


Figure 6. Bland-altman plot of the reference method versus alternative method results for both categories – 16h incubation, including additonal troubleshooting samples





Data from the additional powdered and pasteurised milk samples do not suggest that stressed cells slow the growth of coliforms on CD CFR. The conclusion for the root cause analysis was that there is no systematic bias of the CD CFR method with stressed cells. The slight negative bias observed with selected samples in the relative trueness study is more likely to be a strain-sample combination rather than an indication of slower growth in stressed cells.

18h incubation

Figures 7-9 show the scatter plots for 18h incubation of CompactDry CFR.

Figure 7 - Scatter plot of the reference method versus alternative method results for the raw milk and dairy products – 18h incubation

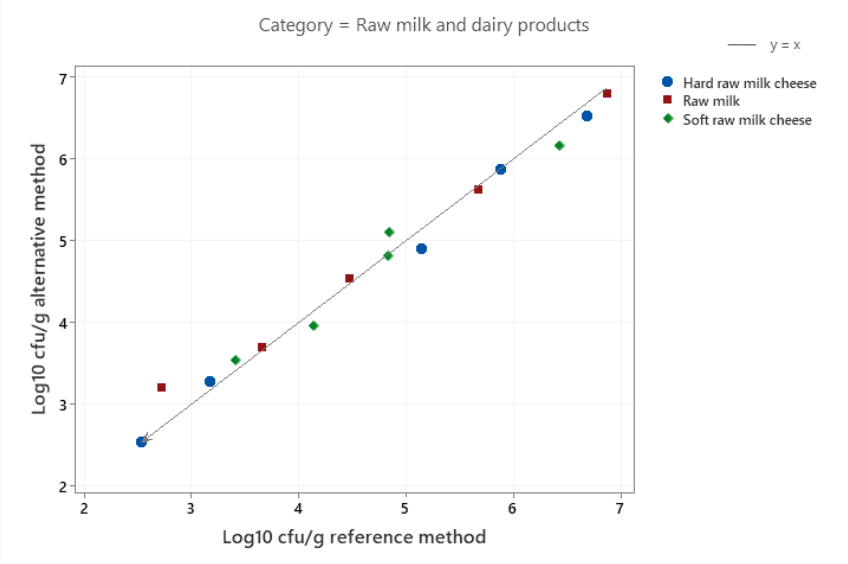


Figure 8- Scatter plot of the reference method versus alternative method results for the heat processed milk and dairy products – 18h incubation

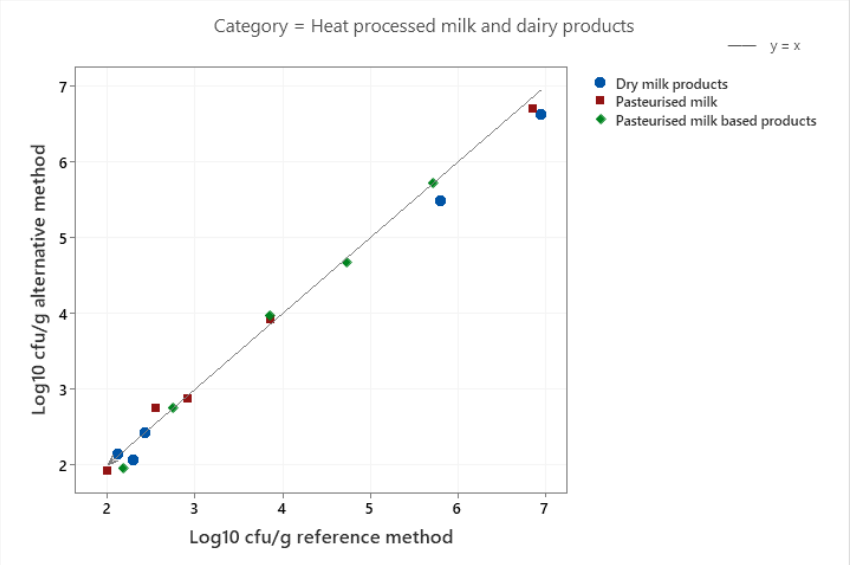
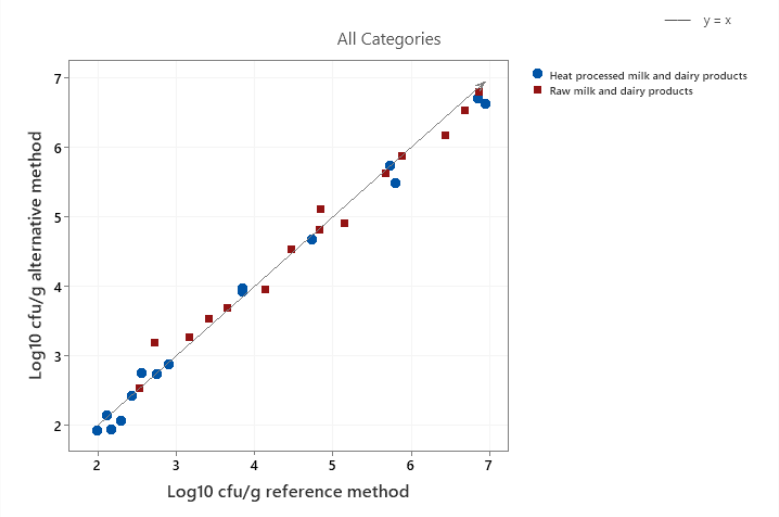


Figure 9 - Scatter plot of the reference method versus alternative method results for all the categories – 18h incubation



<u>18h incubation</u>

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

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

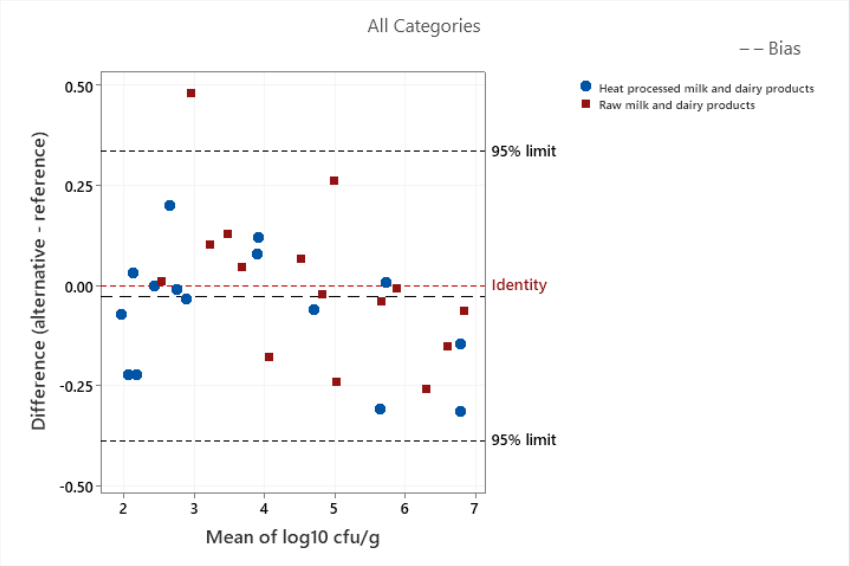
The Bland-Altman difference plot for all the samples at an 18-hour incubation is given in Figure 10.

Table 6 - Summary of the calculated values per category 18h incubation

Category	**n**	$\overline{D}$	**SD**	**95 % low limit**	**95 % upper limit**
Heat processed milk and dairy products	15	-0.062	0.152	-0.399	0.274
Raw milk and dairy products	15	0.010	0.191	-0.413	0.434
All Categories	30	-0.026	0.173	-0.387	0.335

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

Figure 10 – Bland-Altman difference plot for all the samples – 18-hour incubation



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in the Table 7.

Table 7 - Data which are outside of the accepted limits

Category	Type	Inoculation	N° Sample	Reference method Log cfu/g	Alternative method Log cfu/g	Difference Alternative – reference)	Incubation time
Raw milk and dairy products	Raw milk	*Siccibacter turicensis* 17681 chilled stress	M5	2.72	3.20	0.48	18 hours

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

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method is satisfied for both incubation times as the expectation of not more than 1 in 20 data points outside of the acceptability limits is met.

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

One type per category will be tested with 2 items per type as shown in Table 8.

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 8):

Table 8 - Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Type	Strain	Item
Raw milk and raw dairy products	Raw Milk and milk based products	*E. coli* CRA 1476 isolated from dry milk	Raw milk
			Raw milk cheese
Heat processed Milk and dairy products	Pasteurised and milk dried milk based products	*Franconibacter helvecticus* 17678 isolated from powder	Pasteurised milk
			Milk powder

Preparation of samples was done as a bulk inoculation. A 100g sample was inoculated with 1ml of appropriate dilution of inoculating strain and homogenised by hand massaging or stomaching to evenly distribute the inoculum. For all matrices excluding milk powder, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis. Milk powder was inoculated with a lyophilised culture and stored at ambient for 2 weeks prior to analysis.

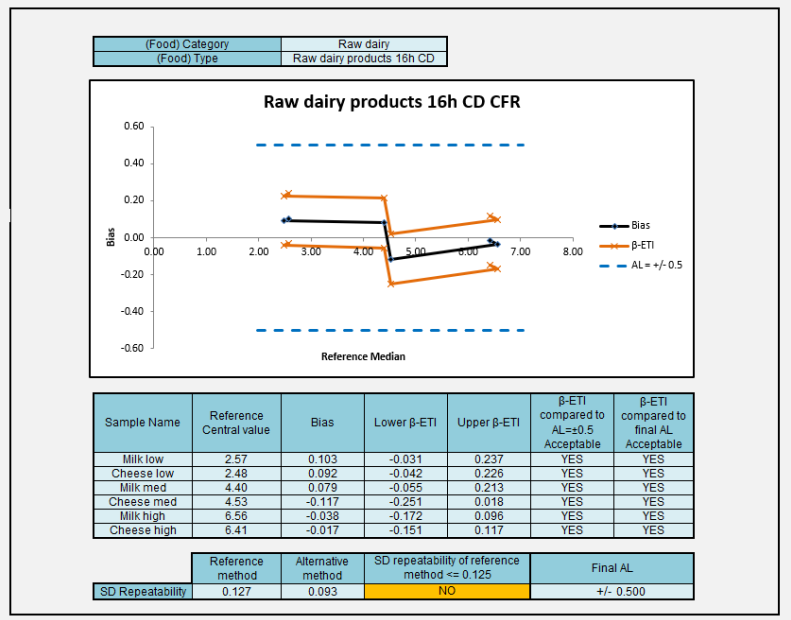
Five separate 10g test portions were removed from the bulk sample and mixed with 90ml peptone salt diluent (PSD) or appropriate diluent and enumerated on both methods.

All results have been tabulated, calculated and interpreted according to ISO 16140-2

3.2.2 Calculations and interpretation of accuracy profile study
The raw data are provided Annex C and the summary tables (in CFU/g) in Annex D. The statistical results and the accuracy profiles are provided Figures 11-14.

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 11 – Accuracy profile for raw milk and dairy products – 16h incubation

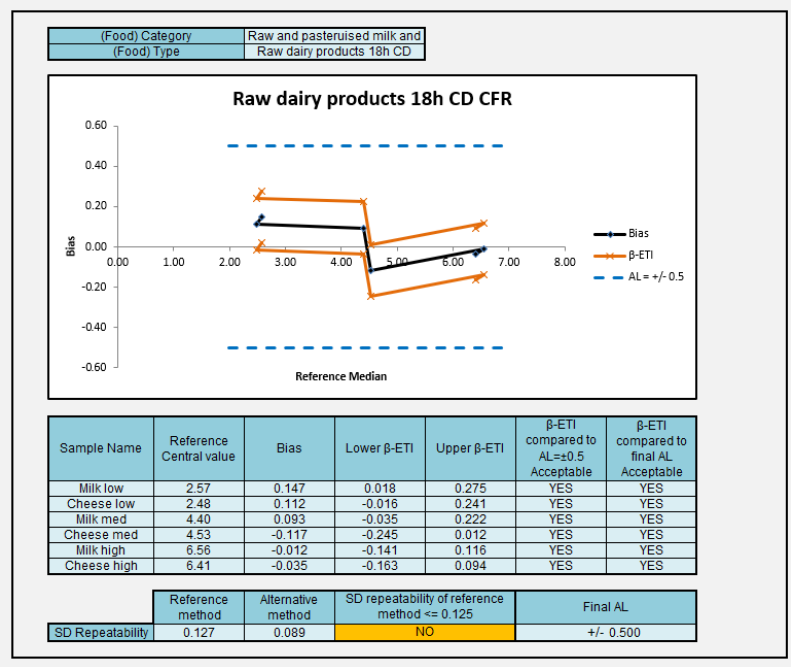


(Food) Category	Raw dairy
(Food) Type	Raw dairy products 16h CD

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Milk low	2.57	0.103	-0.031	0.237	YES	YES
Cheese low	2.48	0.092	-0.042	0.226	YES	YES
Milk med	4.40	0.079	-0.055	0.213	YES	YES
Cheese med	4.53	-0.117	-0.251	0.018	YES	YES
Milk high	6.56	-0.038	-0.172	0.096	YES	YES
Cheese high	6.41	-0.017	-0.151	0.117	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.127	0.093	NO	+/- 0.500

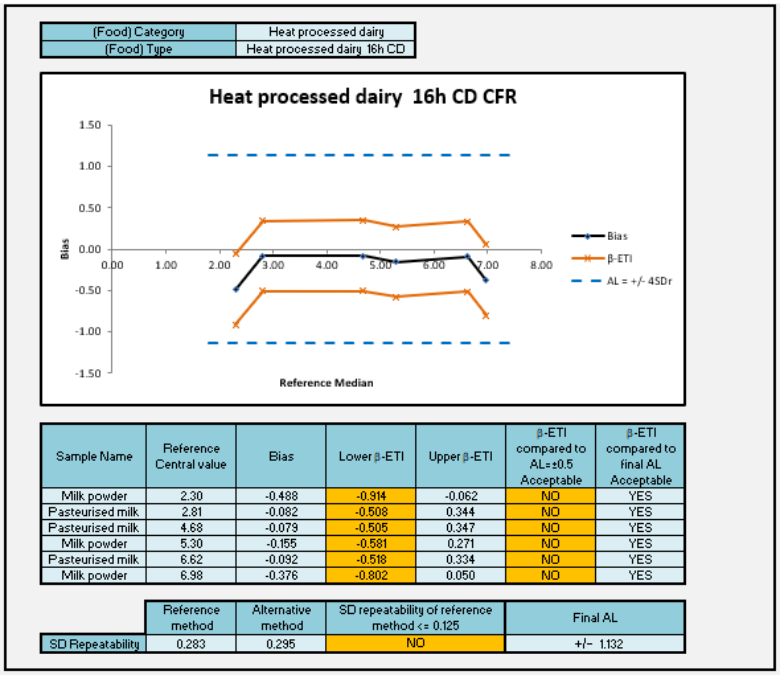
Figure 12 - Accuracy Profile for raw milk and dairy products – 18h incubation



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Milk low	2.57	0.147	0.018	0.275	YES	YES
Cheese low	2.48	0.112	-0.016	0.241	YES	YES
Milk med	4.40	0.093	-0.035	0.222	YES	YES
Cheese med	4.53	-0.117	-0.245	0.012	YES	YES
Milk high	6.56	-0.012	-0.141	0.116	YES	YES
Cheese high	6.41	-0.035	-0.163	0.094	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.127	0.089	NO	+/- 0.500

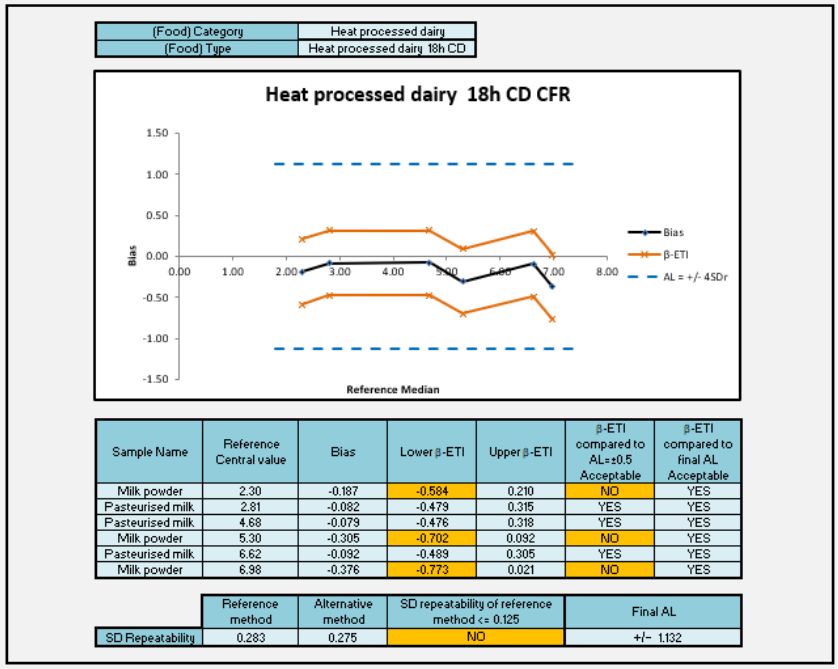
Figure 13 – Accuracy Profile for heat processed milk and dairy products – 16h incubation



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Milk powder	2.30	-0.488	-0.914	-0.062	NO	YES
Pasteurised milk	2.81	-0.082	-0.508	0.344	NO	YES
Pasteurised milk	4.68	-0.079	-0.505	0.347	NO	YES
Milk powder	5.30	-0.155	-0.581	0.271	NO	YES
Pasteurised milk	6.62	-0.092	-0.518	0.334	NO	YES
Milk powder	6.98	-0.376	-0.802	0.050	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.283	0.295	NO	+/- 1.132

Figure 14 – Accuracy Profile for heat processed milk and dairy products – 18h incubation



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
Milk powder	2.30	-0.187	-0.584	0.210	NO	YES
Pasteurised milk	2.81	-0.082	-0.479	0.315	YES	YES
Pasteurised milk	4.68	-0.079	-0.476	0.318	YES	YES
Milk powder	5.30	-0.305	-0.702	0.092	NO	YES
Pasteurised milk	6.62	-0.092	-0.489	0.305	YES	YES
Milk powder	6.98	-0.376	-0.773	0.021	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.283	0.275	NO	+/- 1.132

Comments

In this study the following categories met the AL of 0.5 log: raw milk and dairy products for the 16 and 18h incubation times.

In this study, the following categories required the new AL to be calculated: pasteurised milk and dairy products, for the 16 and 18h incubation times. Both incubation times met the new AL value of 1.132 log.

There was a high repeatability observed in the reference and alternative methods for milk powder samples. One possible reason for this could be a variation in die off occurring in the milk powder between the replicate samples at each level.

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

3.3 Inclusivity / exclusivity

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

3.3.1 Protocols
Inclusivity

50 coliform strains were grown in NB medium at 37°C overnight. Each strain was tested once with the alternative method, the reference method and a non-selective agar.

Exclusivity

30 non coliform *(*predominantly Gram negative) strains were included in the exclusivity study. The isolates were grown in an appropriate non-selective medium at 37°C overnight. Each strain was tested once with the alternative method, the reference method and a non-selective agar. Results

All raw data are given in Annex E.

Inclusivity

A total of 50 strains were tested for inclusivity. All 50 of these strains tested showed a positive result on the reference and alternative methods.

Exclusivity

A total of 30 isolates were tested for exclusivity and all 30 of these strains tested showed a negative result on the reference and alternative methods.

3.3.2 Conclusion
The alternative CompactDry CFR detection method is selective and specific.

3.3.3 Conclusion (MCS)
Overall, the conclusions for the Method Comparison are:

- The alternative method, CompactDry CFR, for enumeration of coliforms shows satisfactory results for relative trueness at both incubation times, 16h and 18h;
- The alternative method, CompactDry CFR, for enumeration of coliforms shows satisfactory results for accuracy profile at both incubation times 16h and 18h;
- The alternative method, CompactDry CFR, for enumeration of coliforms selective and specific at both incubation times 16h and 18h.

4 Interlaboratory study

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

4.1 Study organisation

4.1.1 Collaborators
Samples were sent to 10 participants in 8 laboratories. Three countries particpated in the study: England, Scotland and France.

4.1.2 Matrix and strain used
Roquefort cheese was inoculated with *Escherichia coli* CRA 11017 (NCTC 12241).

4.1.3 Sample preparation
Samples were prepared and inoculated on 20th March 2024 as described below:

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

The target levels and codes are shown in Table 9 below.

Table 9. Contamination levels

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

4.1.4 Labelling and shipping
Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

Samples were shipped in 24 h to 48 h to the involved laboratories. The temperature conditions were required to stay lower or equal to 8°C during transport and during storage in the laboratories.

4.1.5 Analysis of Samples
Collaborative study laboratories and the expert laboratory carried out the analyses on 25th March 2024 with the alternative and reference methods. The analyses by the reference method and the alternative method were performed on the same day.

4.2 Experimental parameters controls

4.2.1 Detection of Coliforms in the matrix before inoculation
In order to detect the presence of coliforms, the reference method was performed on five portions (10 g) before the inoculation. All the results were negative.

4.2.2 Strain stability during transport
Duplicate samples inoculated at four levels (10^2, 10^3, 10^5, 10^6 cfu/g) were tested for the enumeration of coliforms after 7 days of storage at 2-8°C (Table 10). Frozen samples were thawed under controlled conditions prior to analysis.

Table 10 – Coliform stability in the matrix

Day	Storage conditions	Alternative method (log cfu/g) – 16 hour count								Reference method (log cfu/g)							
		Level 1		Level 2		Level 3		Level 4		Level 1		Level 2		Level 3		Level 4	
		A	b	a	b	a	B	a	b	A	B	a	b	a	b	a	b
Day 0	N/A	2.6	2.5	3.7	3.8	5.8	5.6	5.8	5.6	2.9	2.7	3.5	3.8	5.9	5.6	6.8	6.6
Day 7	Storage at 2-8°C	2.4	1.8	3.4	2.9	5.3	5.0	6.2	6.1	2.3	1.9	3.2	3.0	5.4	4.6	6.2	5.0

No growth was observed during storage at 5°C ± 3°C. A small amount of die off was observed at all levels. This was considered when inoculating the samples.

4.2.3 Logistic conditions
The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 11.

Table 11 - Sample temperatures at receipt

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis Date
1	4.9	1.5	21/3/24 15:58	25/3/24
2	3.5	0	21/3/24 16:01	25/3/24
3	3.2	0	21/3/24 13:30	25/3/24
4	3.7	0.7	22/3/24 15:20	25/3/24
5	3.7	0.7	22/3/24 15:20	25/3/24
6	4.2	0	22/3/24 14:31	25/3/24
7	5.1	0.7	21/3/24 12:45	25/3/24
8	1.3	0	22/03/24 14:00	25/3/24
9	2.5	-0.3	22/03/24 13:30	25/3/24
10	6.5	12.7	27/3/24 12:30	27/3/2024

No issues were encountered during the transport or at receipt for 9 collaborators. 1 parcel was held in customs, the temperature on receipt was 12.9°C. This dataset was excluded from analysis. For the remaining 9 participants, the samples were delivered on time and in appropriate conditions. Temperatures during shipment and at receipt were all correct.

4.3 Calculation and summary of data

4.3.1 MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 12.

Table 12 – Results obtained by the expert lab.

Level	Reference method	Alternative method
Blank	<1	<1
Low	3.1	3.3
Low	3.0	3.0
Medium	4.1	4.2
Medium	4.1	4.1
High	6.9	6.7
High	6.2	6.2

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 13.

The accuracy profile plot is shown in Figures 14 and 15 and the statistical analysis of the data shown in Tables 14 and 15.

Table 13: Summary of the results of the interlaboratory study per analyte level

Collaborator	**Level**	**Reference method (Log cfu/g)**		**Alternative method (Log cfu/g) – 16h incubation**		**Alternative method (Log cfu/g) – 18h incubation**	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
1	Low	2.3	2.3	2.8	2.8	2.8	2.8
2	Low	2.3	2.3	2.6	2.4	2.6	2.4
3	Low	3.0	3.4	2.9	3.2	2.9	3.2
4	Low	3.0	3.0	3.0	3.1	3.0	3.1
5	Low	2.8	3.0	2.8	3.0	2.8	3.0
6	Low	2.5	3.2	2.6	3.0	2.6	3.0
7	Low	3.1	3.1	3.3	3.0	3.3	3.0
8	Low	3.5	3.3	3.3	3.0	3.3	3.0

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g) – 16h incubation		Alternative method (Log cfu/g) – 18h incubation	
		Duplicate 1	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*	*Duplicate 1*	*Duplicate 2*
9	Low	3.0	3.0	3.0	3.2	3.0	3.2
10*	Low	3.2	2.7	3.4	2.7	3.4	2.7
1	Med	3.6	3.8	3.7	4.1	3.7	4.1
2	Med	3.4	4.0	3.5	4.1	3.5	4.1
3	Med	4.6	4.6	4.7	4.8	4.7	4.8
4	Med	4.9	4.1	4.0	4.1	4.0	4.1
5	Med	4.0	4.1	3.9	4.0	3.9	4.0
6	Med	4.2	4.4	4.1	4.3	4.1	4.3
7	Med	4.7	4.6	4.7	4.7	4.7	4.7
8	Med	4.6	4.5	4.2	4.1	4.2	4.1
9	Med	4.8	3.9	4.4	3.9	4.4	3.9
10*	Med	3.7	4.2	3.7	4.0	3.7	4.0
1	High	4.9	5.4	5.2	5.8	5.2	5.8
2	High	4.2	5.8	4.5	5.7	4.5	5.7
3	High	6.7	6.4	6.7	6.6	6.7	6.6
4	High	6.4	6.0	6.4	6.0	6.4	6.0
5	High	6.0	5.9	6.0	5.9	6.0	5.9
6	High	6.2	6.2	6.1	6.2	6.1	6.2
7	High	6.5	6.5	6.5	6.6	6.5	6.6
8	High	5.6	6.4	5.1	6.4	5.1	6.4
9	High	6.5	6.8	6.4	6.5	6.4	6.5
10*	High	6.6	6.4	6.6	6.5	6.6	6.5
1	Blank	<1		<1		<1	
2	Blank	<1		<1		<1	
3	Blank	<1		<1		<1	
4	Blank	<1		<1		<1	
5	Blank	<1		<1		<1	
6	Blank	<1		<1		<1	
7	Blank	<1		<1		<1	
8	Blank	<1		1.3**		1.3**	
9	Blank	<1		<1		<1	
10	Blank	<1		<1		<1	

* Data excluded due to high temperature on receipt

**estimated count, less than 4 colonies on plate

Figure 14. Accuracy profile of CompactDry CFR from the ILS – 16h incubation

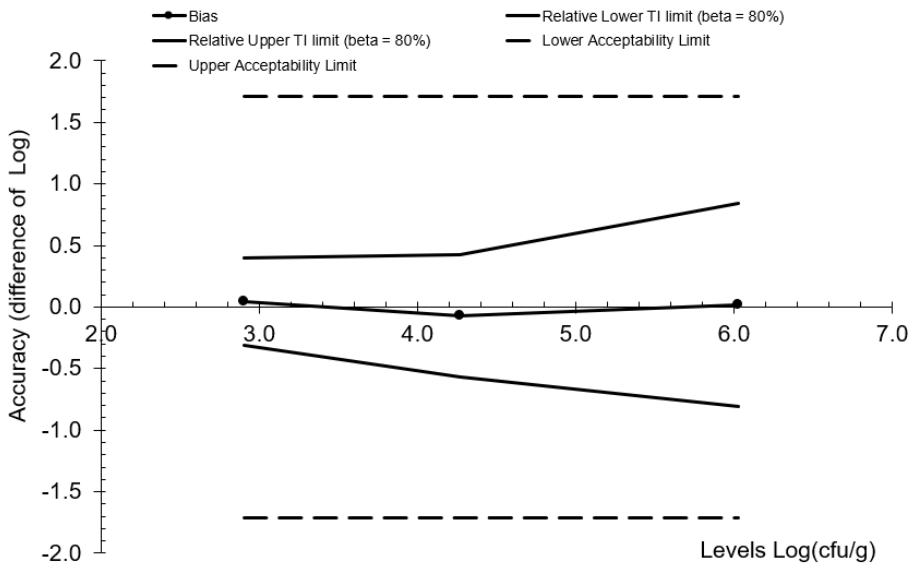


Figure 15. Accuracy profile of CompactDry CFR from the ILS – 18h incubation

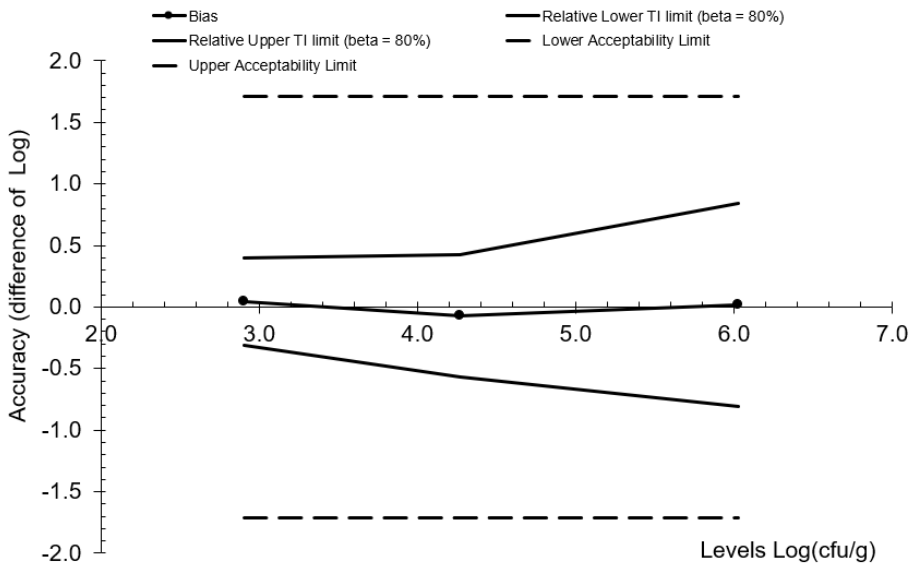


Table 14. Statistical analysis of the ILS data according to the ISO spreadsheet – 16h incubation

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	2.905	4.272	6.027			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	2.948	4.200	6.042	2.905	4.272	6.027
Repeatability standard deviation (sr)	0.176	0.222	0.449	0.194	0.332	0.451
Between-labs standard deviation (sL)	0.182	0.275	0.382	0.349	0.297	0.497
Reproducibility standard deviation (sR)	0.254	0.353	0.589	0.399	0.446	0.672
Corrected number of dof	12.764	11.790	13.817	10.124	13.554	12.409
Coverage factor	1.407	1.417	1.398			
Interpolated Student t	1.352	1.358	1.346			
Tolerance interval standard deviation	0.2642	0.3686	0.6121			
Lower TI limit	2.591	3.700	5.218			
Upper TI limit	3.305	4.701	6.866			
Bias	**0.043**	**-0.072**	**0.015**			
Relative Lower TI limit (beta = 80%)	**-0.314**	**-0.573**	**-0.809**	TRUE		
Relative Upper TI limit (beta = 80%)	**0.400**	**0.428**	**0.839**	TRUE		
Lower Acceptability Limit	**-1.71**	**-1.71**	**-1.71**			
Upper Acceptability Limit	**1.71**	**1.71**	**1.71**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.519					

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

Table 15. Statistical analysis of the ILS data according to the ISO spreadsheet – 18h incubation

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	2.905	4.272	6.027			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	2.948	4.200	6.042	2.905	4.272	6.027
Repeatability standard deviation (sr)	0.176	0.222	0.449	0.194	0.332	0.451
Between-labs standard deviation (sL)	0.182	0.275	0.382	0.349	0.297	0.497
Reproducibility standard deviation (sR)	0.254	0.353	0.589	0.399	0.446	0.672
Corrected number of dof	12.764	11.790	13.817	10.124	13.554	12.409
Coverage factor	1.407	1.417	1.398			
Interpolated Student t	1.352	1.358	1.346			
Tolerance interval standard deviation	0.2642	0.3686	0.6121			
Lower TI limit	2.591	3.700	5.218			
Upper TI limit	3.305	4.701	6.866			
Bias	**0.043**	**-0.072**	**0.015**			
Relative Lower TI limit (beta = 80%)	**-0.314**	**-0.573**	**-0.809**	TRUE		
Relative Upper TI limit (beta = 80%)	**0.400**	**0.428**	**0.839**	TRUE		
Lower Acceptability Limit	**-1.71**	**-1.71**	**-1.71**			
Upper Acceptability Limit	**1.71**	**1.71**	**1.71**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.519					

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

Minimal bias was observed at all levels at 16h and 18h incubation (0.043, -0.072, 0.015 respectively).

A review of the accuracy profile and statistical analysis revealed that there was a high acceptability limit of 1.71 and -1.71 observed in the ILS. To investigate possible reasons for the high AL seen in the ILS, a root cause analysis was carried out.

4.3.3 Root cause analysis

The same batches of media were used by all participants and the incubation time and temperature of analysis were correct. Participants prepared their own VRBA, which could have introduced variation between labs. As part of the investigation, the repeatability of both methods as well as the standard deviation between labs were analysed. Table 16 shows the repeatability of the reference and alternative methods, and Table 17 shows the standard deviation between labs.

Table 16. Repeatability of the reference and alternative methods

Method	**Low**	**Medium**	**High**
Reference	0.194	0.332	0.451
16h alternative	0.176	0.222	0.449
18h alternative	0.176	0.222	0.449

Data revealed that the repeatability of samples within methods is consistent between reference and alternative methods. The repeatability was increased for high level samples for the reference and alternative methods.

Table 17. Standard deviation between labs of the reference and alternative methods

Method	**Low**	**Medium**	**High**
Reference	0.349	0.297	0.497
16h alternative	0.182	0.275	0.382
18h alternative	0.182	0.275	0.382

The results show that the standard deviation between labs is higher for the reference method than for the alternative method. There could be several possible reasons for the higher standard deviation seen between labs for the reference method:

- Differences in stability of the strain in the matrix
- Variation in the levels of mould between samples which is affecting the recovery of coliforms
- Differences in inoculation between samples

<u>Stability of the strain</u>

Analysis of stability results showed that there was a slight die off in all levels observed over the sample storage. Although the die off was accounted for in sample inoculation, it is possible that die off occurred at different rates between labs which could explain for large variation observed. The impact of strain stability on the results is likely to be minimal as this is a paired study.

Variation in levels of mould

The matrix, Roquefort cheese, contains high levels of the mould *Penicillium roqueforti*. It is possible that the mould in the sample could have affected the recovery of coliforms from the sample.

Roquefort samples were purchased from a supermarket in 100g amounts. The samples were purchased at the same time and are from the same batch. Samples were not homogenised before individual 10g test portion were taken. This could have caused variation in the levels background microflora, including moulds, between samples. This could have impacted the level of coliforms recovered from each sample.

Performance of the method

Table 18 shows the log differences between the reference and alternative method.

Table 18. Log differences between methods (reference - alternative 16h incubation)

Participant number	Low		Medium		High	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	0.5	0.5	0.2	0.3	0.3	0.4
2	0.3	0.1	0.2	0.2	0.3	0.0
3	-0.1	-0.1	0.1	0.2	0.0	0.2
4	0.0	0.1	-0.9	0.0	-0.1	0.0
5	0.0	0.0	-0.2	0.0	0.0	0.0
6	0.1	-0.1	-0.1	-0.1	0.0	0.0
7	0.1	0.0	0.0	0.1	0.0	0.1
8	-0.2	-0.3	-0.4	-0.3	-0.5	0.0
9	0.0	0.1	-0.4	0.0	0.0	-0.4
10	0.2	0.0	0.0	-0.2	0.0	0.1

4.3.4 Interlaboratory conclusions

The conclusion of the root cause analysis is that the high acceptability limits are due to high standard deviation between labs.

The conclusion of the interlaboratory study is that the accuracy profile data meets the requirements of ISO 16140-2 and that there is good agreement between reference and alternative methods.

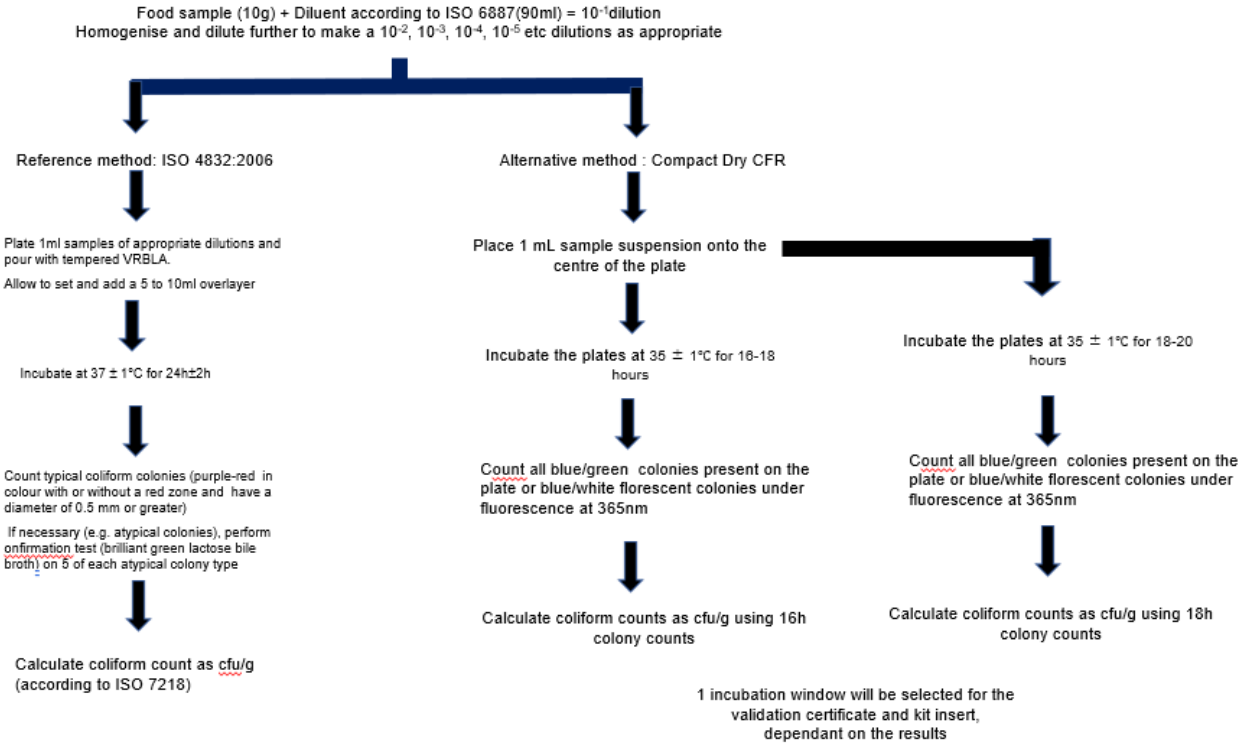
5 Overall conclusions of the validation study

- The alternative method, CompactDry CFR, for enumeration of coliforms in raw and pasteurized dairy products shows satisfactory results for relative trueness;
- The alternative method, CompactDry CFR, for enumeration of coliforms in raw and pasteurized dairy products shows satisfactory results for accuracy profile;
- The alternative method, CompactDry CFR, for enumeration of coliforms is selective and specific.
- The alternative method, CompactDry CFR, for enumeration of coliforms in raw and pasteurized dairy products shows satisfactory performance in the ILS
- The alternative method, CompactDry CFR for enumeration of coliforms in raw and pasteurized dairy products shows comparable performance to the reference method ISO 4832:2006, with the reference method incubated at 37°C±1°C
- A minimum incubation time of 16h can be used for CompactDry CFR

10 June 2024

Alice Foxall
Project Manager – Molecular Microbiology and Methods

ANNEX A: Flow diagram of the reference and alternative methods



ANNEX B: Calculation and interpretation of relative trueness

Key: Sample codes in bold were artificially contaminated

Calculation and interpretation of relative trueness – 16h incubation

Type	Item	Sample code	Log Ref	Log Alt	Mean	Difference
Raw milk and dairy products						
Raw milk	Raw milk 1	**M1**	6.87	6.81	6.84	-0.06
Raw milk	Raw milk 2	**M2**	5.67	5.61	5.64	-0.06
Raw milk	Raw milk 3	**M3**	4.48	4.54	4.51	0.07
Raw milk	Raw milk 4	**M4**	3.65	3.68	3.67	0.03
Raw milk	Raw milk 5	**M5**	2.72	2.92	2.82	0.20
Hard raw milk cheese	Roquefort	**M6**	5.15	4.88	5.01	-0.27
Hard raw milk cheese	Framgerie la tournette	M7	3.18	3.30	3.24	0.12
Hard raw milk cheese	Semi curado	**M8**	2.53	2.54	2.54	0.01
Hard raw milk cheese	Comte	**M9**	5.88	5.28	5.58	-0.60
Hard raw milk cheese	Wiamore sheep milk cheese	**M10**	4.83	4.40	4.62	-0.43
Soft raw milk cheese	Saint Marcellin	**M11**	6.43	5.95	6.19	-0.48
Soft raw milk cheese	Golden Cenarth	M12	4.85	4.75	4.80	-0.10
Soft raw milk cheese	Rouzaire bride de meaux	M13	6.68	6.53	6.61	-0.15
Soft raw milk cheese	Brillat saviarin	**M14**	4.15	3.93	4.04	-0.21
Soft raw milk cheese	Neuf Chatel	**M15**	3.41	3.54	3.48	0.13
Heat processed milk and dairy products						
Pasteurised milk	British skimmed milk 1	**M16**	6.85	6.71	6.78	-0.14
Pasteurised milk	Semi-skimmed milk 1	**M17**	2.55	2.76	2.65	0.21
Pasteurised milk	Pasteurised whole milk	**M18**	2.91	2.41	2.66	-0.50
Pasteurised milk	Pasteurised semi skimmed milk 2	**M19**	2.00	1.18	1.59	-0.82
Pasteurised milk	Whole milk 2	M20	3.85	3.32	3.59	-0.53
Pasteurised milk based products	Pasteurised mozzarella	**M21**	2.76	2.85	2.80	0.09
Pasteurised milk based products	Strawberry milkshake	**M22**	3.85	3.97	3.91	0.12
Pasteurised milk based products	Cream	**M23**	4.74	4.68	4.71	-0.06
Pasteurised milk based products	Ice cream	**M24**	5.72	5.73	5.73	0.01
Pasteurised milk based products	Grated cheddar	M25	2.18	1.95	2.07	-0.22
Dry milk products	Skim milk powder 1	**M26**	6.94	6.63	6.79	-0.31
Dry milk products	Skim milk powder 2	**M27**	2.43	2.19	2.31	-0.24

Type	Item	Sample code	Log Ref	Log Alt	Mean	Difference
Dry milk products	Skim milk powder 3	**M28**	5.80	5.49	5.65	-0.31
Dry milk products	Skim milk powder 4	**M29**	2.11	2.48	2.30	0.36
Dry milk products	Skim milk powder 5	**M30**	2.30	1.70	2.00	-0.60

Calculation and interpretation of relative trueness – 18h incubation

Type	Item	Sample code	Log Ref	Log Alt	Mean	Difference
Raw milk and dairy products						
Raw milk	Raw milk 1	**M1**	6.87	6.81	6.84	-0.06
Raw milk	Raw milk 2	**M2**	5.67	5.63	5.65	-0.04
Raw milk	Raw milk 3	**M3**	4.48	4.54	4.51	0.07
Raw milk	Raw milk 4	**M4**	3.65	3.70	3.68	0.05
Raw milk	Raw milk 5	**M5**	2.72	3.20	2.96	0.48
Hard raw milk cheese	Roquefort	**M6**	5.15	4.91	5.03	-0.24
Hard raw milk cheese	Framgerie la tournette	M7	3.18	3.28	3.23	0.10
Hard raw milk cheese	Semi curado	**M8**	2.53	2.54	2.54	0.01
Hard raw milk cheese	Comte	**M9**	6.68	6.53	6.61	-0.15
Hard raw milk cheese	Wiamore sheep milk cheese	**M10**	5.88	5.88	5.88	-0.01
Soft raw milk cheese	Saint Marcellin	**M11**	6.43	6.18	6.30	-0.26
Soft raw milk cheese	Golden Cenarth	M12	4.85	5.11	4.98	0.26
Soft raw milk cheese	Rouzaire bride de meaux	M13	4.83	4.81	4.82	-0.02
Soft raw milk cheese	Brillat saviarin	**M14**	4.15	3.97	4.06	-0.18
Soft raw milk cheese	Neuf Chatel	**M15**	3.41	3.54	3.48	0.13
Heat processed milk and dairy products						
Pasteurised milk	British skimmed milk 1	**M16**	6.85	6.71	6.78	-0.14
Pasteurised milk	Semi-skimmed milk 1	**M17**	2.56	2.76	2.66	0.20
Pasteurised milk	Pasteurised whole milk	**M18**	2.91	2.88	2.90	-0.03
Pasteurised milk	Pasteurised semi skimmed milk 2	**M19**	2.00	1.93	1.96	-0.07
Pasteurised milk	Whole milk 2	M20	3.85	3.93	3.89	0.08
Pasteurised milk based products	Pasteurised mozzarella	**M21**	2.76	2.75	2.75	-0.01
Pasteurised milk based products	Strawberry milkshake	**M22**	3.85	3.97	3.91	0.12
Pasteurised milk based products	Cream	**M23**	4.74	4.68	4.71	-0.06

Pasteurised milk based products	Ice cream	**M24**	5.72	5.73	5.73	0.01
Pasteurised milk based products	Grated cheddar	M25	2.18	1.95	2.07	-0.22
Dry milk products	Skim milk powder 1	**M26**	6.94	6.63	6.79	-0.31
Dry milk products	Skim milk powder 2	**M27**	2.43	2.43	2.43	0.00
Dry milk products	Skim milk powder 3	**M28**	5.80	5.49	5.65	-0.31
Dry milk products	Skim milk powder 4	**M29**	2.11	2.15	2.13	0.03
Dry milk products	Skim milk powder 5	**M30**	2.30	2.08	2.19	-0.22

ANNEX C: Raw data accuracy profile study

Raw data Accuracy Profile study - 16h incubation

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
Category: Raw milk and raw dairy products																					
1A	Raw milk	58	48	5	1					5.3E+02	2.7	24	42	6	1					3.6E+02	2.6
1B	Raw milk	49	43	7	0					4.8E+02	2.7	38	44	3	0					4.0E+02	2.6
1C	Raw milk	47	37	7	0					4.5E+02	2.6	38	42	1	2					3.7E+02	2.6
1D	Raw milk	44	54	3	1					4.7E+02	2.7	38	37	2	0					3.6E+02	2.6
1E	Raw milk	40	45	7	0					4.5E+02	2.7	37	37	4	0					3.7E+02	2.6
2A	Raw milk		T	T	29	4				3.0E+04	4.5		T	T	26	2				2.5E+04	4.4
2B	Raw milk		T	T	26	3				2.6E+04	4.4		T	T	20	1				1.9E+04	4.3
2C	Raw milk		T	T	31	4				3.2E+04	4.5		T	T	39	4				3.9E+04	4.6
2D	Raw milk		T	T	37	5				3.8E+04	4.6		T	T	20	2				2.0E+04	4.3
2E	Raw milk		T	T	29	1				2.7E+04	4.4		T	T	26	0				2.6E+04	4.4
3A	Raw milk				T	T	17	4		1.9E+06	6.3				T	T	21	0		2.1E+06	6.3
3B	Raw milk				T	T	35	4		3.5E+06	6.5				T	T	30	2		2.9E+06	6.5
3C	Raw milk				T	T	34	2		3.3E+06	6.5				T	T	39	1		3.6E+06	6.6
3D	Raw milk				T	T	46	6		4.7E+06	6.7				T	T	46	3		4.5E+06	6.7
3E	Raw milk				T	T	33	1		3.1E+06	6.5				T	T	45	2		4.3E+06	6.6
4A	Raw milk cheese	36	41	3	0					3.8E+02	2.6	29	28	5	0					3.1E+02	2.5
4B	Raw milk cheese	43	49	5	0					4.6E+02	2.7	39	34	4	0					3.7E+02	2.6
4C	Raw milk cheese	49	38	5	0					4.4E+02	2.6	33	26	2	3					2.9E+02	2.5

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
4D	Raw milk cheese	38	35	4	0					3.7E+02	2.6	31	28	2	0					2.9E+02	2.5
4E	Raw milk cheese	36	34	3	1					3.5E+02	2.5	67	43	2	1					5.2E+02	2.7
5A	Raw milk cheese		T	T	16	3				1.7E+04	4.2		T	T	33	4				3.4E+04	4.5
5B	Raw milk cheese		T	T	22	2				2.2E+04	4.3		T	T	17	2				1.7E+04	4.2
5C	Raw milk cheese		T	T	26	0				2.6E+04	4.4		T	T	40	3				3.9E+04	4.6
5D	Raw milk cheese		T	T	37	3				3.6E+04	4.6		T	T	39	5				4.0E+04	4.6
5E	Raw milk cheese		T	T	30	3				3.0E+04	4.5		T	T	26	2				2.5E+04	4.4
6A	Raw milk cheese				T	T	22	2		2.2E+06	6.3				T	T	20	4		2.2E+06	6.3
6B	Raw milk cheese				T	T	20	3		2.1E+06	6.3				T	T	33	0		3.3E+06	6.5
6C	Raw milk cheese				T	T	32	4		3.3E+06	6.5				T	T	42	1		3.9E+06	6.6
6D	Raw milk cheese				T	T	32	3		3.2E+06	6.5				T	T	26	0		2.6E+06	6.4
6E	Raw milk cheese				T	T	22	1		2.2E+06	6.3				T	T	15	1		1.5E+06	6.2
Category: pasteurised milk and dairy products																					
7A	Pasteurised milk	49	42	4	0					4.5E+02	2.7	51	57	3	0					5.1E+02	2.7
7B	Pasteurised milk	55	45	3	0					4.8E+02	2.7	75	59	5	0					6.4E+02	2.8
7C	Pasteurised milk	62	58	6	1					6.0E+02	2.8	84	59	7	0					7.1E+02	2.9
7D	Pasteurised milk	57	50	5	0					5.3E+02	2.7	62	72	9	2					6.9E+02	2.8
7E	Pasteurised milk	58	53	3	0					5.3E+02	2.7	63	42	5	0					5.2E+02	2.7
8A	Pasteurised milk			T	40	4	3			4.0E+04	4.6			T	48	4	0			4.7E+04	4.7
8B	Pasteurised milk			T	31	4	1			3.2E+04	4.5			T	50	4	0			4.9E+04	4.7
8C	Pasteurised milk			T	37	2	0			3.5E+04	4.5			T	34	2	0			3.3E+04	4.5
8D	Pasteurised milk			T	41	8	0			4.5E+04	4.7			T	52	4	0			5.1E+04	4.7
8E	Pasteurised milk			T	42	5	0			4.3E+04	4.6			T	41	3	0			4.8E+04	4.7

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
9A	Pasteurised milk					T	39	4	0	3.9E+06	6.6					T	42	0	0	3.8E+06	6.6
9B	Pasteurised milk					T	24	2	0	2.4E+06	6.4					T	40	6	0	4.2E+06	6.6
9C	Pasteurised milk					T	48	2	0	4.5E+06	6.7					T	46	1	0	4.3E+06	6.6
9D	Pasteurised milk					T	26	0	0	2.4E+06	6.4					T	33	2	0	3.2E+06	6.5
9E	Pasteurised milk					T	35	2	0	3.4E+06	6.5					T	51	8	0	5.4E+06	6.7
10A	Milk powder	8	5	3	0					65	1.8	29	16	1	0					2.1E+02	2.3
10B	Milk powder	5	4	1	0					45	1.7	27	17	1	0					2.1E+02	2.3
10C	Milk powder	6	8	0	0					70	1.8	16	26	1	0					2.0E+02	2.3
10D	Milk powder	5	7	2	0					60	1.8	21	16	0	0					1.7E+02	2.2
10E	Milk powder	10	15	1	0					75	1.9	19	22	1	0					2.0E+02	2.3
11A	Milk powder			T	T	60	3	1		5.7E+04	4.8			T	T	145	12	0		1.4E+06	6.1
11B	Milk powder			T	T	64	11	1		6.8E+05	5.8				T	T	93	6		9.0E+05	6.0
11C	Milk powder			T	131	21	0	0		1.4E+05	5.1				T	T	19	3	0	2.0E+05	5.3
11D	Milk powder			T	95	14	0	0		9.9E+04	5.0				T	T	21	1	0	2.0E+05	5.3
11E	Milk powder			T	22	4	0	0		2.4E+04	4.4				T	42	3	0	0	4.1E+04	4.6
12A	Milk powder					T	89	5	3	8.5E+06	6.9					T	121	14	0	1.2E+07	7.1
12B	Milk powder					T	33	1	0	3.1E+06	6.5					T	47	5	0	4.7E+06	6.7
12C	Milk powder					T	T	25	1	2.4E+07	7.4					T	T	32	1	3.0E+07	7.5
12D	Milk powder					T	33	7	0	3.6E+06	6.6					T	74	6	0	7.3E+06	6.9
12E	Milk powder					T	39	5	0	4.0E+06	6.6					T	94	11	2	9.5E+06	7.0

Raw data Accuracy Profile study - 18h incubation

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
Category: raw milk and dairy products																					
1A	Raw milk	50	66	5	1					5.7E+02	2.8	24	42	6	1					3.6E+02	2.6
1B	Raw milk	53	47	7	2					5.2E+02	2.7	38	44	3	0					4.0E+02	2.6
1C	Raw milk	55	44	8	0					5.2E+02	2.7	38	42	1	2					3.7E+02	2.6
1D	Raw milk	62	50	3	1					5.4E+02	2.7	38	37	2	0					3.6E+02	2.6
1E	Raw milk	51	49	7	1					5.2E+02	2.7	37	37	4	0					3.7E+02	2.6
2A	Raw milk		T	T	29	4				3.0E+04	4.5		T	T	26	2				2.5E+04	4.4
2B	Raw milk		T	T	27	3				2.7E+04	4.4		T	T	20	1				1.9E+04	4.3
2C	Raw milk		T	T	33	4				3.4E+04	4.5		T	T	39	4				3.9E+04	4.6
2D	Raw milk		T	T	40	5				4.1E+04	4.6		T	T	20	2				2.0E+04	4.3
2E	Raw milk		T	T	33	1				3.1E+04	4.5		T	T	26	0				2.6E+04	4.4
3A	Raw milk				T	T	18	4		2.0E+06	6.3				T	T	21	0		2.1E+06	6.3
3B	Raw milk				T	T	35	4		3.5E+06	6.5				T	T	30	2		2.9E+06	6.5
3C	Raw milk				T	T	41	2		3.9E+06	6.6				T	T	39	1		3.6E+06	6.6
3D	Raw milk				T	T	50	6		5.1E+06	6.7				T	T	46	3		4.5E+06	6.7
3E	Raw milk				T	T	38	1		3.5E+06	6.5				T	T	45	2		4.3E+06	6.6

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
4A	Raw milk cheese	37	41	3	0					3.8E+02	2.6	29	28	5	0					3.1E+02	2.5
4B	Raw milk cheese	43	49	5	0					4.6E+02	2.7	39	34	4	0					3.7E+02	2.6
4C	Raw milk cheese	50	38	5	0					4.5E+02	2.6	33	26	2	3					2.9E+02	2.5
4D	Raw milk cheese	38	35	4	0					3.7E+02	2.6	31	28	2	0					2.9E+02	2.5
4E	Raw milk cheese	36	32	3	1					3.4E+02	2.5	67	43	2	1					5.2E+02	2.7
5A	Raw milk cheese		T	210	16	3				2.1E+04	4.3		T	T	33	4				3.4E+04	4.5
5B	Raw milk cheese		T	T	22	2				2.2E+04	4.3		T	T	17	2				1.7E+04	4.2
5C	Raw milk cheese		T	T	26	0				2.6E+04	4.4		T	T	40	3				3.9E+04	4.6
5D	Raw milk cheese		T	T	37	3				3.6E+04	4.6		T	T	39	5				4.0E+04	4.6
5E	Raw milk cheese		T	T	30	3				3.0E+04	4.5		T	T	26	2				2.5E+04	4.4
6A	Raw milk cheese				T	T	22	2		2.2E+06	6.3				T	T	20	4		2.2E+06	6.3
6B	Raw milk cheese				T	247	20	3		2.4E+06	6.4				T	T	33	0		3.3E+06	6.5
6C	Raw milk cheese				T	T	32	4		3.3E+06	6.5				T	T	42	1		3.9E+06	6.6
6D	Raw milk cheese				T	T	33	3		3.3E+06	6.5				T	T	26	0		2.6E+06	6.4
6E	Raw milk cheese				T	227	22	1		2.3E+06	6.4				T	T	15	1		1.5E+06	6.2
Category: pasteurised milk and dairy products																					
7A	Pasteurised milk	49	42	4	0					4.5E+02	2.7	51	57	3	0					5.1E+02	2.7
7B	Pasteurised milk	55	45	3	0					4.8E+02	2.7	75	59	5	0					6.4E+02	2.8
7C	Pasteurised milk	62	58	6	1					6.0E+02	2.8	84	59	7	0					7.1E+02	2.9
7D	Pasteurised milk	57	50	5	0					5.3E+02	2.7	62	72	9	2					6.9E+02	2.8
7E	Pasteurised milk	58	53	3	0					5.3E+02	2.7	63	42	5	0					5.2E+02	2.7
8A	Pasteurised milk			T	40	4	3			4.0E+04	4.6			T	48	4	0			4.7E+04	4.7
8B	Pasteurised milk			T	31	4	1			3.2E+04	4.5			T	50	4	0			4.9E+04	4.7

Sample code	Item	Alternative method CD CFR (1ml plate)										VB ISO reference method (ISO 4832:2006)									
		-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g	-1	-1	-2	-3	-4	-5	-6	-7	cfu/g	log cfu/g
8C	Pasteurised milk			T	37	2	0			3.5E+04	4.5			T	34	2	0			3.3E+04	4.5
8D	Pasteurised milk			T	41	8	0			4.5E+04	4.7			T	52	4	0			5.1E+04	4.7
8E	Pasteurised milk			T	42	5	0			4.3E+04	4.6			T	41	3	0			4.8E+04	4.7
9A	Pasteurised milk					T	39	4	0	3.9E+06	6.6					T	42	0	0	3.8E+06	6.6
9B	Pasteurised milk					T	24	2	0	2.4E+06	6.4					T	40	6	0	4.2E+06	6.6
9C	Pasteurised milk					T	48	2	0	4.5E+06	6.7					T	46	1	0	4.3E+06	6.6
9D	Pasteurised milk					T	26	0	0	2.4E+06	6.4					T	33	2	0	3.2E+06	6.5
9E	Pasteurised milk					T	35	2	0	3.4E+06	6.5					T	51	8	0	5.4E+06	6.7
10A	Milk powder	10	13	3	0					1.3E+02	2.1	29	16	1	0					2.1E+02	2.3
10B	Milk powder	13	12	1	0					1.2E+02	2.1	27	17	1	0					2.1E+02	2.3
10C	Milk powder	11	8	0	0					9.5E+01	2.0	16	26	1	0					2.0E+02	2.3
10D	Milk powder	11	17	2	0					1.5E+02	2.2	21	16	0	0					1.7E+02	2.2
10E	Milk powder	10	14	2	0					1.3E+02	2.1	19	22	1	0					2.0E+02	2.3
11A	Milk powder			T	T	60	3	1		5.7E+04	4.8			T	T	145	12	0		1.4E+06	6.1
11B	Milk powder			T	T	64	11	1		6.8E+05	5.8				T	T	93	6		9.0E+05	6.0
11C	Milk powder			T	131	21	0	0		1.4E+05	5.1				T	T	19	3	0	2.0E+05	5.3
11D	Milk powder			T	95	14	0	0		9.9E+04	5.0				T	T	21	1	0	2.0E+05	5.3
11E	Milk powder			T	22	4	0	0		2.4E+04	4.4				T	42	3	0	0	4.1E+04	4.6
12A	Milk powder					T	89	5	3	8.5E+06	6.9					T	121	14	0	1.2E+07	7.1
12B	Milk powder					T	33	1	0	3.1E+06	6.5					T	47	5	0	4.7E+06	6.7
12C	Milk powder					T	T	25	1	2.4E+07	7.4					T	T	32	1	3.0E+07	7.5
12D	Milk powder					T	33	7	0	3.6E+06	6.6					T	74	6	0	7.3E+06	6.9
12E	Milk powder					T	39	5	0	4.0E+06	6.6					T	94	11	2	9.5E+06	7.0

ANNEX D: Summary tables accuracy profile study.

(Food) Category 2	Raw dairy										
(Food) Type 2	Raw 16h CD CFR										
			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Milk low	Milk	low	355	400	373	359	373	527	482	445	473	450
Cheese low	Cheese	low	305	368	286	286	518	377	464	441	368	345
Milk med	Milk	interm ediate	25000	19000	39000	20000	26000	30000	26000	32000	38000	27000
Cheese med	Cheese	interm ediate	34000	17000	39000	40000	25000	17000	22000	26000	36000	30000
Milk high	Milk	high	2100000	2900000	3600000	4500000	4300000	1900000	3500000	3300000	4700000	3100000
Cheese high	Cheese	high	2200000	3300000	3900000	2600000	1500000	2200000	2100000	3300000	3200000	2500000

(Food) Category 3	Raw and										
(Food) Type 3	Raw 18h CD CFR										
			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Milk low	Milk	low	355	400	373	359	373	573	518	523	536	518
Cheese low	Cheese	low	305	368	286	286	518	395	477	455	377	345
Milk med	Milk	interm ediate	25000	19000	39000	20000	26000	30000	27000	34000	41000	31000
Cheese med	Cheese	interm ediate	34000	17000	39000	40000	25000	21000	22000	26000	36000	30000
Milk high	Milk	high	2100000	2900000	3600000	4500000	4300000	2000000	3500000	3900000	5100000	3500000
Cheese high	Cheese	high	2200000	3300000	3900000	2600000	1500000	2200000	2400000	3300000	3300000	2300000

(Food) Category 5	Heat processed										
(Food) Type 5	16h Heat										
			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Milk powder	10	low	210	210	200	170	200	65	45	70	60	75
Pasteurised milk	7	low	510	640	710	690	520	450	480	600	530	530
Pasteurised milk	8	interme diate	47000	49000	33000	51000	48000	40000	32000	35000	45000	43000
Milk powder	11	interme diate	1400000	900000	200000	200000	41000	570000	680000	140000	99000	24000
Pasteurised milk	9	high	3800000	4200000	4300000	3200000	5400000	3900000	2400000	4500000	2400000	3400000
Milk powder	12	high	12000000	4700000	30000000	7300000	9500000	8500000	3100000	24000000	3600000	4000000

(Food) Category 6	Heat processed										
(Food) Type 6	18h heat										
			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
Milk powder	10	low	210	210	200	170	200	130	120	95	150	130
Pasteurised milk	7	low	510	640	710	690	520	450	480	600	530	530
Pasteurised milk	8	interme diate	47000	49000	33000	51000	48000	40000	32000	35000	45000	43000
Milk powder	11	interme diate	1400000	900000	200000	200000	41000	57000	680000	140000	99000	24000
Pasteurised milk	9	high	3800000	4200000	4300000	3200000	5400000	3900000	2400000	4500000	2400000	3400000
Milk powder	12	high	12000000	4700000	30000000	7300000	9500000	8500000	3100000	24000000	3600000	4000000

ANNEX E: Raw data inclusivity and exclusivity study

Inclusivity data for 16h incubation of CD CFR

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 16h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Citrobacter amalonaticus	6784	T	101	9	1.00E+09	9.0	T	77	8	7.70E+08	8.9	T	77	16	8.50E+08	8.9
Citrobacter amalonaticus	7458	T	104	8	1.00E+09	9.0	T	89	6	8.60E+08	8.9	T	41	5	4.20E+08	8.6
Citrobacter amalonaticus	7467	T	130	13	1.30E+09	9.1	T	88	13	9.20E+08	9.0	T	53	7	5.50E+08	8.7
Citrobacter amnigenus	7426	125	5	0	1.20E+08	8.1	107	7	0	1.10E+08	8.0	91	10	0	9.20E+07	8.0
Siccibacter turicensis	17681	120	8	0	9.40E+07	8.0	93	10	0	9.40E+07	8.0	82	5	2	7.90E+07	7.9
Citrobacter diversus	7119	T	90	10	9.10E+08	9.0	T	75	10	7.70E+08	8.9	T	56	8	5.80E+08	8.8
Citrobacter species	16262	T	121	11	1.20E+09	9.1	T	107	13	1.00E+09	9.0	T	67	10	7.00E+08	8.8
Cronobacter sazakii	17682	T	31	1	1.70E+08	8.2	T	18	1	1.70E+08	8.2	T	13	2	1.40E+08	8.1
Citrobacter freundii	6759	T	32	6	3.50E+08	8.5	T	30	3	3.00E+08	8.5	T	33	2	3.20E+08	8.5
Citrobacter youngae	NA	300	40	5	4.10E+08	8.6	T	33	3	3.30E+08	8.5	131	12	0	1.30E+08	8.1
Enterobacter aerogenes	4232	T	29	4	3.00E+08	8.5	T	52	0	5.20E+08	8.7	T	33	3	3.30E+08	8.5
Enterobacter agglomerans	1488	T	38	6	4.00E+08	8.6	T	46	6	4.70E+08	8.7	T	42	4	4.20E+08	8.6

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 16h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Enterobacter amingenus	16908	T	54	4	5.30E+08	8.7	T	46	6	4.70E+08	8.7	T	54	3	5.20E+08	8.7
Franconibacter helvectius	17678	T	22	4	3.20E+08	8.5	T	29	6	3.20E+08	8.5	T	15	2	1.50E+08	8.2
Enterobacter asburiae	17021	T	29	7	3.30E+08	8.5	T	43	4	4.30E+08	8.6	153	18	1	1.60E+08	8.2
Enterobacter cloacae	7547	T	92	8	9.10E+08	9.0	T	99	7	9.60E+08	9.0	T	87	10	8.80E+08	8.9
Enterobacter dispar	NA	T	50	1	4.60E+08	8.7	T	31	4	3.20E+08	8.5	93	15	1	1.50E+08	8.2
Enterobacter gergoviae	NA	T	60	7	6.10E+08	8.8	T	56	6	5.60E+08	8.7	T	37	5	3.80E+08	8.6
Enterobacter intermedius	NA	53	3	0	5.10E+07	7.7	77	9	3	7.80E+07	7.9	54	4	0	5.30E+07	7.7
Enterobacter intermedius	17023	44	7	3	4.60E+07	7.7	50	4	0	4.90E+07	7.7	52	6	0	5.30E+07	7.7
Enterobacter taylorae	7530	T	104	13	1.10E+09	9.0	T	80	10	8.20E+08	8.9	T	89	9	8.90E+08	8.9
Enterobacter xiangfangensis	NA	T	59	5	5.80E+08	8.8	T	60	5	5.90E+08	8.8	T	66	6	6.50E+08	8.8
Escherichia adecarboxylata	5501	T	47	9	5.10E+08	8.7	T	41	4	4.10E+08	8.6	T	41	1	3.80E+08	8.6
Escherichia vulneris	16260	209	27	2	2.60E+08	8.4	T= 175	22	0	2.20E+08	8.3	58	10	0	6.20E+07	7.8

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 16h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Citrobacter freundii	40	T	63	8	6.50E+08	8.8	T	70	7	7.00E+08	8.8	218	44	0	4.40E+08	8.6
Cronobacter sakazakii	16909	T	84	9	8.50E+08	8.9	T	67	4	6.50E+08	8.8	T	66	10	6.90E+08	8.8
Cronobacter turicensis lausannensis	17535	T	44	3	4.30E+08	8.6	T	37	3	3.60E+08	8.6	T	31	2	3.00E+08	8.5
Cronobacter universalis	17541	T	26	6	2.90E+08	8.5	T	35	2	3.40E+08	8.5	T	25	4	2.60E+08	8.4
Escherichia coli	1476	T	48	2	4.50E+08	8.7	T	18	3	1.90E+08	8.3	T	41	1	3.80E+08	8.6
Escherichia coli	2003	T	95	5	9.10E+08	9.0	T	114	13	1.20E+09	9.1	T	98	4	9.30E+08	9.0
Escherichia coli	2091	T	54	7	5.50E+08	8.7	T	62	3	5.90E+08	8.8	T	28	0	2.80E+08	8.4
Escherichia coli	2092	T	45	4	4.50E+08	8.7	T	35	2	3.40E+08	8.5	T	40	1	3.70E+08	8.6
Escherichia coli	11017	T	84	4	8.00E+08	8.9	T	93	12	9.50E+08	9.0	T	72	3	6.80E+08	8.8
Escherichia coli	15943	T	59	2	5.50E+08	8.7	T	56	3	5.40E+08	8.7	T	57	5	5.60E+08	8.7
Escherichia coli	16041	T	49	6	5.00E+08	8.7	T	50	2	4.70E+08	8.7	T	54	2	5.10E+08	8.7
Escherichia coli	1593	T	45	3	4.40E+08	8.6	T	39	4	3.90E+08	8.6	T	51	2	4.80E+08	8.7
Escherichia coli	1538	T	77	4	7.40E+08	8.9	T	73	7	7.90E+08	8.9	T	50	8	5.30E+08	8.7
Escherichia coli	11017	T	85	5	8.20E+08	8.9	T	76	9	7.70E+08	8.9	T	73	1	7.30E+08	8.9
Escherichia hermanii	7460	T	36	4	2.20E+08	8.3	T	23	1	2.20E+08	8.3	162	20	0	1.70E+08	8.2
Escherichia vulneris	1518	78	10	0	2.70E+07	7.4	27	0	0	2.70E+07	7.4	29	5	0	3.10E+07	7.5

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 16h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Hafnia alvei	1535	T	36	4	2.50E+08	8.4	T	25	2	2.50E+08	8.4	54	6	0	5.50E+07	7.7
Hafnia alvei	4936	T	95	8	9.40E+08	9.0	T	67	6	6.60E+08	8.8	1	1	0	1.00E+06	6.0
Klebsiella oxytoca	15926	T	52	2	4.90E+08	8.7	T	51	3	4.90E+08	8.7	T	58	3	5.50E+08	8.7
Klebsiella species	6762	T	58	6	5.80E+08	8.8	T	43	8	4.60E+08	8.7	T	57	4	5.50E+08	8.7
Klebsiella ozaene	4273	176	14	3	1.20E+08	8.1	120	11	1	1.20E+08	8.1	99	10	0	9.90E+07	8.0
Klebsiella aerogenes	8387	T	30	3	3.00E+08	8.5	T	30	6	3.30E+08	8.5	T	20	4	2.20E+08	8.3
Kluyvera ascorbata	17126	T	28	0	2.80E+08	8.4	T	30	0	3.00E+08	8.5	T	17	21	1.70E+08	8.2
Serratia fonticola	4613	T	30	4	3.10E+08	8.5	T	31	5	3.30E+08	8.5	153	8	0	1.50E+08	8.2
Serratia marcescens	16729	T	95	10	6.50E+08	8.8	T	67	4	6.50E+08	8.8	30	3	0	3.00E+08	8.5
Serratia fonticola	17098	T	44	3	4.30E+08	8.6	T	49	4	4.80E+08	8.7	T	39	2	3.70E+08	8.6

Inclusivity data for 18h incubation of CD CFR

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 18h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Citrobacter amalonaticus	6784	T	101	9	1.00E+09	9.0	T	77	8	7.70E+08	8.9	T	77	16	8.50E+08	8.9
Citrobacter amalonaticus	7458	T	104	8	1.00E+09	9.0	T	89	6	8.60E+08	8.9	T	41	5	4.20E+08	8.6
Citrobacter amalonaticus	7467	T	130	13	1.30E+09	9.1	T	88	13	9.20E+08	9.0	T	53	7	5.50E+08	8.7
Citrobacter amnigenus	7426	125	5	0	1.20E+08	8.1	107	7	0	1.10E+08	8.0	91	10	0	9.20E+07	8.0
Siccibacter turicensis	17681	120	8	0	9.40E+07	8.0	93	10	0	9.40E+07	8.0	86	5	2	8.30E+07	7.9
Citrobacter diversus	7119	T	90	10	9.10E+08	9.0	T	75	10	7.70E+08	8.9	T	56	8	5.80E+08	8.8
Citrobacter species	16262	T	121	11	1.20E+09	9.1	T	107	13	1.00E+09	9.0	T	68	10	7.10E+08	8.9
Cronobacter sazakii	17682	T	31	1	1.70E+08	8.2	T	18	1	1.70E+08	8.2	T	14	2	1.50E+08	8.2
Citrobacter freundii	6759	T	32	6	3.50E+08	8.5	T	30	3	3.00E+08	8.5	T	33	2	3.20E+08	8.5
Citrobacter youngae	NA	300	40	5	4.10E+08	8.6	T	33	3	3.30E+08	8.5	131	12	0	1.30E+08	8.1
Enterobacter aerogenes	4232	T	29	4	3.00E+08	8.5	T	52	0	5.20E+08	8.7	T	33	3	3.30E+08	8.5
Enterobacter agglomerans	1488	T	38	6	4.00E+08	8.6	T	46	6	4.70E+08	8.7	T	42	4	4.20E+08	8.6

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 18h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Enterobacter amingenus	16908	T	54	4	5.30E+08	8.7	T	46	6	4.70E+08	8.7	T	54	3	5.20E+08	8.7
Franconibacter helvectius	17678	T	22	4	3.20E+08	8.5	T	29	6	3.20E+08	8.5	T	15	2	1.50E+08	8.2
Enterobacter asburiae	17021	T	29	7	3.30E+08	8.5	T	43	4	4.30E+08	8.6	153	18	1	1.60E+08	8.2
Enterobacter cloacae	7547	T	92	8	9.10E+08	9.0	T	99	7	9.60E+08	9.0	T	87	10	8.80E+08	8.9
Enterobacter dispar	NA	T	50	1	4.60E+08	8.7	T	31	4	3.20E+08	8.5	93	15	1	1.50E+08	8.2
Enterobacter gergoviae	NA	T	60	7	6.10E+08	8.8	T	56	6	5.60E+08	8.7	T	37	5	3.80E+08	8.6
Enterobacter intermedius	NA	53	3	0	5.10E+07	7.7	77	9	3	7.80E+07	7.9	54	4	0	5.30E+07	7.7
Enterobacter intermedius	17023	44	7	3	4.60E+07	7.7	50	4	0	4.90E+07	7.7	52	6	0	5.30E+07	7.7
Enterobacter taylorae	7530	T	104	13	1.10E+09	9.0	T	80	10	8.20E+08	8.9	T	89	9	8.90E+08	8.9
Enterobacter xiangfangensis	NA	T	59	5	5.80E+08	8.8	T	60	5	5.90E+08	8.8	T	66	6	6.50E+08	8.8
Escherichia adecarboxylata	5501	T	47	9	5.10E+08	8.7	T	41	4	4.10E+08	8.6	T	41	1	3.80E+08	8.6
Escherichia vulneris	16260	209	27	2	2.60E+08	8.4	T= 175	22	0	2.20E+08	8.3	58	10	0	6.20E+07	7.8
Citrobacter freundii	40	T	63	8	6.50E+08	8.8	T	70	7	7.00E+08	8.8	218	44	0	4.40E+08	8.6

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 18h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Cronobacter sakazakii	16909	T	84	9	8.50E+08	8.9	T	67	4	6.50E+08	8.8	T	66	10	6.90E+08	8.8
Cronobacter turicensis lausannensis	17535	T	44	3	4.30E+08	8.6	T	37	3	3.60E+08	8.6	T	31	2	3.00E+08	8.5
Cronobacter universalis	17541	T	26	6	2.90E+08	8.5	T	35	2	3.40E+08	8.5	T	25	4	2.60E+08	8.4
Escherichia coli	1476	T	48	2	4.50E+08	8.7	T	18	3	1.90E+08	8.3	T	42	1	3.90E+08	8.6
Escherichia coli	2003	T	95	5	9.10E+08	9.0	T	114	13	1.20E+09	9.1	T	99	7	9.60E+08	9.0
Escherichia coli	2091	T	54	7	5.50E+08	8.7	T	62	3	5.90E+08	8.8	T	28	0	2.80E+08	8.4
Escherichia coli	2092	T	45	4	4.50E+08	8.7	T	35	2	3.40E+08	8.5	T	40	1	3.70E+08	8.6
Escherichia coli	11017	T	84	4	8.00E+08	8.9	T	93	12	9.50E+08	9.0	T	73	3	6.90E+08	8.8
Escherichia coli	15943	T	59	2	5.50E+08	8.7	T	56	3	5.40E+08	8.7	T	57	5	5.60E+08	8.7
Escherichia coli	16041	T	49	6	5.00E+08	8.7	T	50	2	4.70E+08	8.7	T	57	2	5.40E+08	8.7
Escherichia coli	1593	T	45	3	4.40E+08	8.6	T	39	4	3.90E+08	8.6	T	51	2	4.80E+08	8.7
Escherichia coli	1538	T	77	4	7.40E+08	8.9	T	73	7	7.90E+08	8.9	T	50	8	5.30E+08	8.7
Escherichia coli	11017	T	85	5	8.20E+08	8.9	T	76	9	7.70E+08	8.9	T	74	1	7.40E+08	8.9
Escherichia hermanii	7460	T	36	4	2.20E+08	8.3	T	23	1	2.20E+08	8.3	166	21	0	1.70E+08	8.2
Escherichia vulneris	1518	78	10	0	2.70E+07	7.4	27	0	0	2.70E+07	7.4	T	26	2	2.50E+08	8.4
Hafnia alvei	1535	T	36	4	2.50E+08	8.4	T	25	2	2.50E+08	8.4	T	46b, 36p	4	4.60E+08	8.7
Hafnia alvei	4936	T	95	8	9.40E+08	9.0	T	67	6	6.60E+08	8.8	T	33	3	3.30E+08	8.5
Klebsiella oxytoca	15926	T	52	2	4.90E+08	8.7	T	51	3	4.90E+08	8.7	T	60	3	5.70E+08	8.8

Organism	CRA code	Count results on PCA non selective					Count results on VRBA reference					Count results on 18h CD CFR				
		-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log	-6	-7	-8	cfu/g	log
Klebsiella species	6762	T	58	6	5.80E+08	8.8	T	43	8	4.60E+08	8.7	T	65	4	6.30E+08	8.8
Klebsiella ozaene	4273	176	14	3	1.20E+08	8.1	120	11	1	1.20E+08	8.1	122	17	0	1.20E+08	8.1
Klebsiella aerogenes	8387	T	30	3	3.00E+08	8.5	T	30	6	3.30E+08	8.5	T	20	4	2.20E+08	8.3
Kluyvera ascorbata	17126	T	28	0	2.80E+08	8.4	T	30	0	3.00E+08	8.5	T	17	21	1.70E+08	8.2
Serratia fonticola	4613	T	30	4	3.10E+08	8.5	T	31	5	3.30E+08	8.5	T	19	2	1.90E+08	8.3
Serratia marcescens	16729	T	95	10	6.50E+08	8.8	T	67	4	6.50E+08	8.8	30	5	0	3.20E+07	7.5
Serratia fonticola	17098	T	44	3	4.30E+08	8.6	T	49	4	4.80E+08	8.7	T	43	3	4.20E+08	8.6

Exclusivity data

Organism	CRA code	Counts obtained on Non-selective						Counts obtained on Reference method: VRBA			Counts obtained on Alternative method: CD CFR		
		-5	-6	-7	-8	cfu/ml	log	-1	-2	cfu/g	-1	-2	cfu/ml
Acinetobacter calcoaceticus	7421	T	103	15	1	1.10E+08	8.0	0	0	<10	0	0	<10
Acinetobacter lwoffii	7438	T	125	11	1	1.20E+08	8.1	0	0	<10	0	0	<10
Aliivibrio fisherii	16818	T	T	41	5	4.20E+08	8.6	0	0	<10	0	0	<10
Burkholderia gladioli	8175	69	11	1	0	7.30E+06	6.9	0	0	<10	0	0	<10
Buttiauxella agrestis	17110	T	68	11	0	7.20E+07	7.9	0	0	<10	0	0	<10
Buttiauxella warmboldiae	17112	T	259	18	0	2.50E+08	8.4	0	0	<10	0	0	<10
Lactobacillus bichneri	167	T	T	105	16	1.10E+09	9.0	0	0	<10	0	0	<10
Chryseobacterium hominis	4088	T	T	116	6	1.10E+09	9.0	0	0	<10	0	0	<10
Xanthomonas species	8119	T	68	7	0	6.80E+07	7.8	0	0	<10	0	0	<10
Ewingella americana	8086	174	14	0	0	1.70E+07	7.2	0	0	<10	0	0	<10
Gluconoacetobacter liquefaciens	16761	T	36	3	0	3.50E+07	7.5	0	0	<10	0	0	<10
Janthinobacterium agaricidamnosum	8191	T	241	27	3	2.70E+08	8.4	0	0	<10	0	0	<10
Listeria monocytogenes	1104	T	200	22	4	2.00E+08	8.3	0	0	<10	0	0	<10
Moraxella osloensis	17043	1	0	0	0	1.00E+05	5.0	0	0	<10	0	0	<10
Morganella morganii	269	T	60	3	1	5.70E+07	7.8	0	0	<10	0	0	<10
Pasteurella bettyae	8391	T	T	41	0	4.10E+08	8.6	0	0	<10	0	0	<10
Pediococcus pentasaceus	16030	T	T	83	4	7.90E+08	8.9	0	0	<10	0	0	<10
Photobacterium damselae	16821	T	15	1	0	1.50E+07	7.2	10*	2*	1.10E+02	0	0	<10
Proteus mirabilis	1588	T	T	60	11	6.50E+08	8.8	0	0	<10	0	0	<10
Proteus vulgaris	1581	T	T	89	8	8.80E+08	8.9	0	0	<10	0	0	<10
Providencia rettgeri	8386	T	T	84	8	8.40E+08	8.9	0	0	<10	0	0	<10

Organism	CRA code	Counts obtained on Non-selective						Counts obtained on Reference method: VRBA			Counts obtained on Alternative method: CD CFR		
		-5	-6	-7	-8	cfu/ml	log	-1	-2	cfu/g	-1	-2	cfu/ml
Pseudomonas aeruginosa	8299	T	80	7	1	7.90E+07	7.9	0	0	<10	0	0	<10
Pseudomonas fluorescens	15937	T	216	24	0	2.20E+08	8.3	0	0	<10	0	0	<10
Pseudomonas tolaassi	17347	T	T	31	4	3.20E+08	8.5	0	0	<10	0	0	<10
Salmonella Typhimurium	11634	T	T	117	14	1.20E+09	9.1	0	0	<10	0	0	<10
Salmonella enterica subsp Ealing	1362	T	T	122	10	1.90E+08	8.3	0	0	<10	0	0	<10
Staphylococcus aureus	4105	T	199	14	1	1.90E+08	8.3	0	0	<10	0	0	<10
Aeromonas salmonicida	8388	T	T	60	4	5.80E+08	8.8	0	0	<10	0	0	<10
Bacillus cereus	4110	T	34	3	0	3.40E+07	7.5	0	0	<10	0	0	<10
Shewella putrifaciens	16056	93	7	2	0	9.10E+06	7.0	0	0	<10	0	0	<10

*atypical colonies, confirmed negative on brilliant green lactose bile broth