ISO 16140-2:2016 validation of Kikkoman Biochemifa Company Easy Plate SA, for the enumeration of *Staphylococcus aureus* in a broad range of foods

MicroVal study number: 2021LR105

Method/Kit name: Easy Plate SA

Report version: MCS/ILS Summary report

MicroVal Expert Laboratory: Campden BRI

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

Foreword

The protocol is prepared in Accordance with ISO 16140-2:20016 and the most recent version of the MicroVal Technical Committee for interpretation on ISO 16140-2.

Company: Kikkoman Biochemifa Company

Expert Laboratory: Campden BRI

Method/Kit name: Easy Plate SA

Validation standard: Microbiology of the food chain— Method validation

- Part 1: Vocabulary (ISO 16140-1:2016)
- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)

Reference method: ISO 6888-1: 2021 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase - positive staphylococci (Staphylococcus aureus and other species) - Part 1 - Technique using Baird-Parker agar medium.

Scope of validation: Broad range of foods

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
- $\overline{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Incl/Excl Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- n number of samples
- na not applicable
- neg negative (target not detected)
- ng no growth
- nt not tested
- RT Relative Trueness
- SD standard deviation of differences
- 10^{-1} dilution 10-fold dilution of original food
- 10^{-2} dilution 100-fold dilution of original food

And, in *S. aureus* studies

- BPW Buffered Peptone Water
- PSD Peptone Salt Diluent
- MRD Maximum Recovery Diluent
- NA Nutrient Agar
- PCA Plate Count Agar
- BPA Baird Parker Agar

Table of Contents

1 Introduction

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

Alternative method

Easy Plate SA is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate SA method is intended to indicate the level of *Staphylococcus aureus* in food and beverage products. After incubation, *S. aureus* appears as blue colonies on the growth medium contained in the Easy Plate SA plate. Easy Plate SA is selective for *S. aureus* therefore other coagulase positive Staphylococci are not included in the scope of this validation.

Reference method is: ISO 6888-1: 2021 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase - positive staphylococci (*Staphylococcus aureus* and other species) - Part 1 - Technique using Baird-Parker agar medium.

Although the scope of the reference method is for *Staphylococcus* spp. it is noted that in previous MicroVal validation studies specific to coagulase positive *S. aureus,* ISO 6888-1 has been used as the reference method.

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

Categories included:

- Milk and dairy products (combined category raw and heat processed Milk and dairy products)
- Produce and fruits (combined category fresh and processed)
- Fishery products Combined category: raw, RTE, RTRH, RTC
- Meat and poultry products (RTE/RTRH)
- Multi-component foods or meal components

Criteria evaluated during the MCS/ILS study:

- Relative Trueness study
- Accuracy profile study
- Limit of Quantification study(LOQ)
- Inclusivity and exclusivity study
- Interlaboratory study

The final conclusion on the Validation Study is summarised below:

The alternative method Easy Plate SA shows comparable performance to the reference method ISO 4833-1:2013 for the enumeration of *Staphylococcus aureus* in a broad range of foods.

2 Method protocols

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

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

2.1 Reference method

A flow diagram of the reference method is shown in Annex A for reference. The plates were incubated for the minimum allowed time in the reference method of 24±2h i.e. 22h at 37±1°C with re-incubation for a further 22h at 37±1°C

Rabbit Plasma Fibrinogen (RPF Agar) was used as the confirmation step for the reference method following 9.4.3 of ISO 6888-1: 2021.

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

2.2 Alternative method

See the flow diagram in Annex A

The kit insert for the alternative method (Easy Plate SA) is given in Annex B.

The alternative method principle is based on chromogenic media. Easy Plate SA is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate SA method is intended to indicate the level of *Staphylococcus aureus* in food and beverage products. After incubation for 24h ±2h at 37°C, *S. aureus* appears as blue colonies on the growth medium contained in the Easy Plate SA plate. In this validation study, the minimum incubation time of 22h will be used for incubation of the Easy Plate SA. Easy Plate SA is selective for *S. aureus* therefore other coagulase positive Staphylococci are not included in the scope of this validation.

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 Methods Comparison Study

3.1 Sample preparation

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

The samples was prepared for analysis and diluted in Accordance with ISO 6887 (all parts) unless specified differently in the alternative method.

See Table 1 for specific preparations used in the validation study.

3.2 Relative trueness study

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

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

3.2.1 Number of samples

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

Table 1. List of Categories, Types, number of samples analysed and results obtained from testing within the relative trueness study.

Category	Types	Items	No of samples	Sample diluent used
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Raw milk and dairy products	Raw milk, raw milk cheese	5	6887-5
	Pasteurised milk and milk based products	Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk)	5	6887-5
	Dry milk products	Milk powders and powder for milk based desserts	5	6887-5
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon filet, tuna, bonito	5	6887-3
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods, semi-dried fish	5	6887-3
	Crustaceans	Shrimp, crab	5	6887-3
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot, radish sprouts, alfalfa	5	6887-4
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	6887-4
	Heat treated fruit and vegetables	Past smoothies/juice, blanched frozen vegetables	5	6887-4
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, egg and cress sandwich	5	6887-1, 6887-4
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products, quiche	5	6887-1, 6887-4
	Mayonnaise based deli-salads	Vegetable salad, egg mayonnaise	5	6887-1, 6887-4
Meat and poultry products (RTE/RTRH)	cooked meat and poultry products	Cooked cured hams, pate, cooked poultry,	5	6887-2
	Fermented or dried products	Salami, chicken sausage	5	6887-2
	Raw cured products	Dry cured hams, smoked turkey products	5	6887-2

75 samples were analysed, leading to 75 explotiable results

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

3.2.2 Test sample preparation

No naturally contaminated samples were found in pre-screening studies. It was therefore necessary to use artificial contamination procedures. Artificial procedures used a range of seeding protocols and strains in order to examine a wide range of different conditions.

Seeding

Samples were inoculated with *S. aureus* 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.

Spiking

Spiked samples were inoculated with heat treated strains of *S. aureus* (10min at 55°C). The injury level achieved was at least 0.5logs. The level of injury was assessed by plating the heat treated strain on a non-selective agar e.g. Nutrient Agar and a selective agar relevant to the organism.

Fifteen *S. aureus* isolates were used for artificial inoculations. These cultures preferably originated from comparable sample types as the ones to be inoculated. Each particular strain was used to contaminate up to 5 different items.

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

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.

3.2.3 Protocols applied during the validation study

A single protocol was applied for the study.

Reference method plates were incubated at 37±1ºC for 24±2h initially followed by a re-incubation for a further 24±2h at 37±1ºC.

Alternative method was incubated at 37±1ºC for 24±2h.

In all cases the minimum incubation times were used for the alternative method.

Confirmation was carried out on the reference method was by stabbing up to 5 typical colonies onto RBPF agar to check for coagulase activity. The plates were incubated at 37±1ºC for 24±2h to check for the presence of a precipitation halo indicating a positive result. For the alternative method, a confirmation was not needed for samples that were artificially contaminated.

3.2.4 Test results

All raw data per category are given in Excel file 2021LR105: tab Relative Trueness.

The samples were analysed by the reference and the alternative methods in order to have at least 15 interpretable results per category, and at least 5 interpretable results per tested type by the two methods.

3.2.5 Calculation and interpretation of relative trueness study

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x).
Figures 1 to 7 shows the scatter plots for the individual categories and Figure 8 for all categories.

Figure 1 - Scatter plot of the reference method versus alternative method results for Milk and dairy products (combined category raw and heat processed Milk and dairy products)

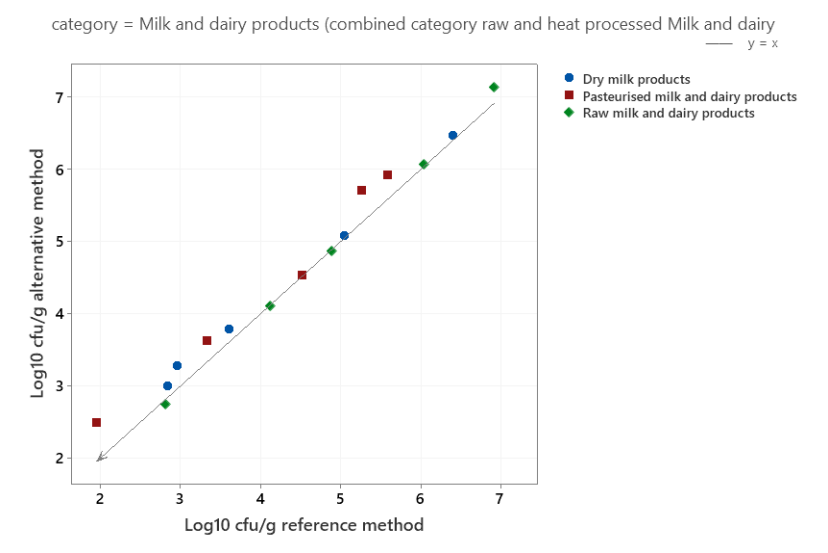


Figure 2 - Scatter plot of the reference method versus alternative method results for Fishery products, Combined category: RTE and RTRH

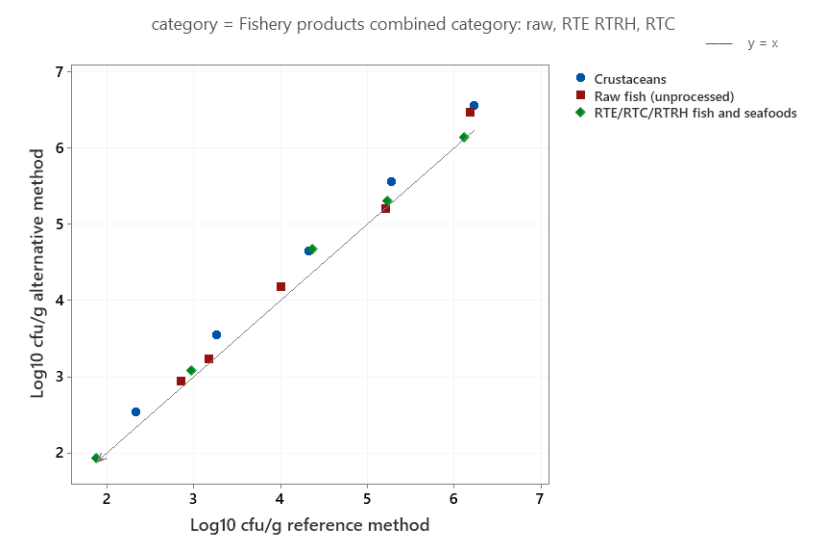


Figure 3 - Scatter plot of the reference method versus alternative method results for Produce and fruits (combined category fresh and processed)

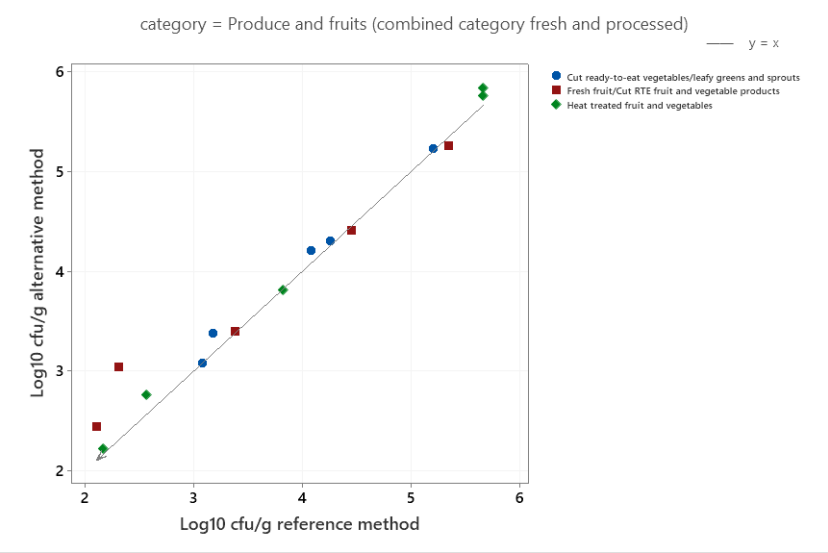


Figure 4 - Scatter plot of the reference method versus alternative method results for RTE/RTRH Meat and poultry (Combined category)

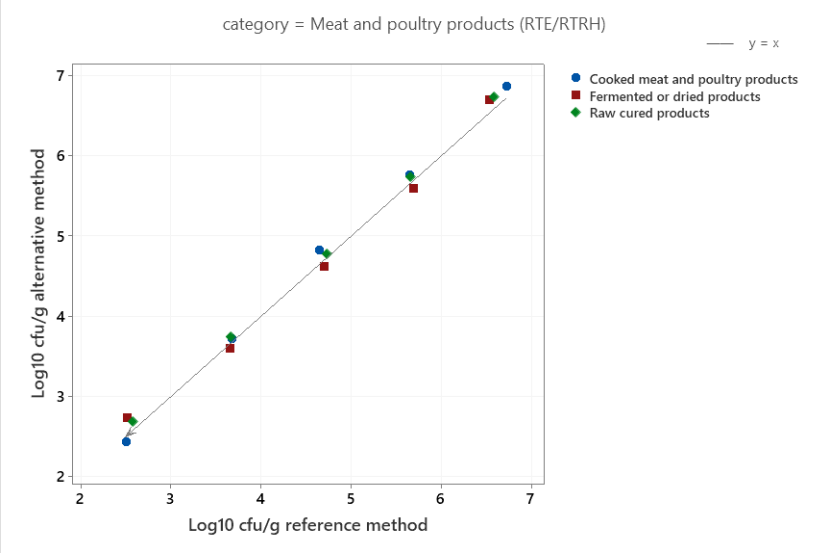


Figure 5 - Scatter plot of the reference method versus alternative method results for Multicomponent foods

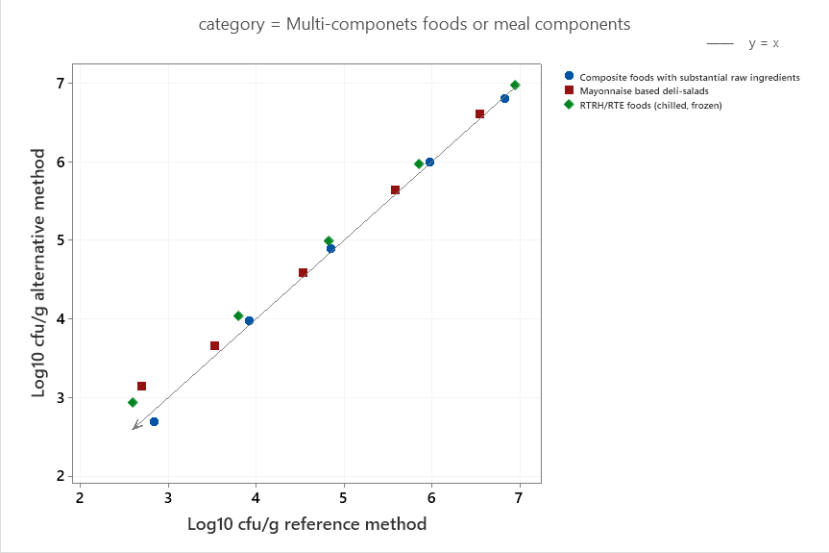
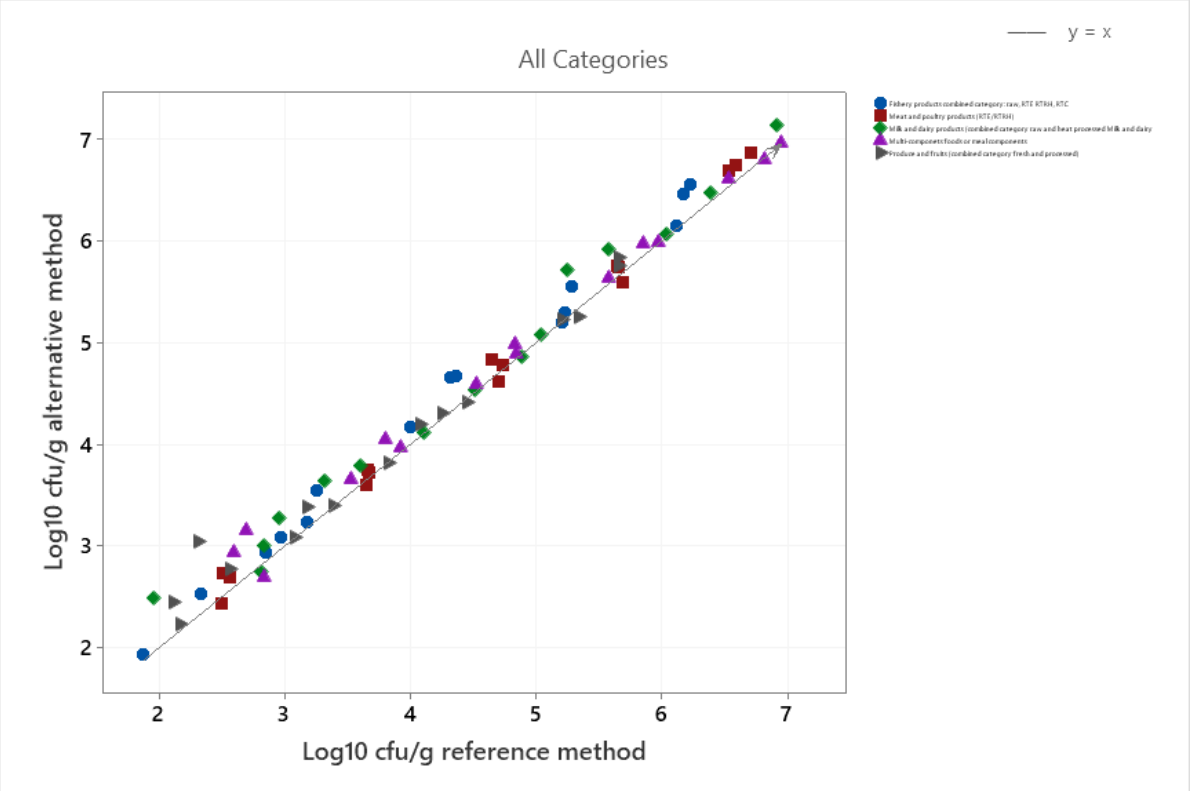


Figure 6 - Scatter plot of the reference method versus alternative method results for all categories



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results.

The data in the scatter plots show no obvious disagreement across all the samples.

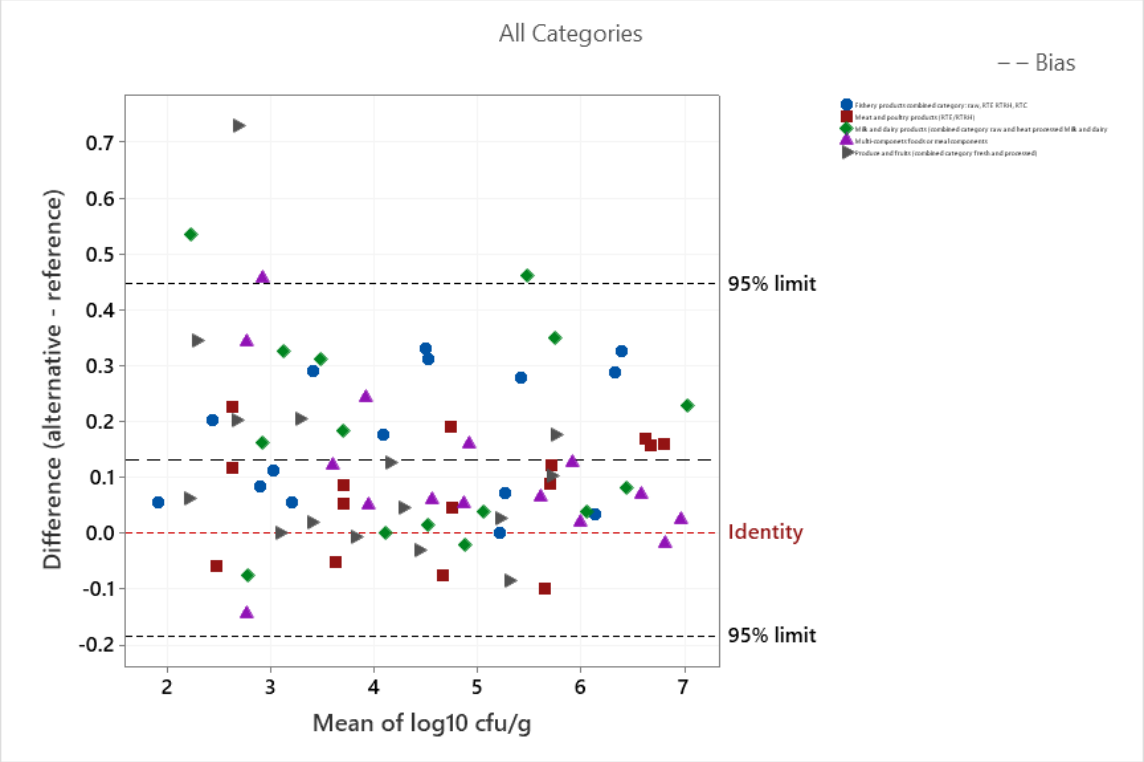
A summary of the calculated values per category is provided in Table 2

Table 2 - Summary of the calculated values per category

Category	**N**	$\bar{D}$	**SD**	**95% Lower limit**	**95% Upper limit**
Milk and dairy products	15	0.1736	0.1211	-0.0946	0.4418
Fishery products	15	0.0747	0.1035	-0.1547	0.3040
Produce and fruit	15	0.1747	0.1873	-0.2402	0.5897
Raw and RTC Meat and poultry	15	0.1077	0.1478	-0.2198	0.4351
Multicomponent	15	0.1271	0.2005	-0.3171	0.5713
All categories	**75**	0.1316	0.1571	-0.1836	0.4467

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

Figure 7 - The Bland-Altman difference plot for all the samples



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

Table 3 – data which are outside of the Accepted limits

Category	item	Code	Reference method Log cfu/g	Alternative method Log cfu/g	Mean Log cfu/g	Difference (Alternative-reference)	Lower/ upper limits
Produce and fruits (combined category fresh and processed)	Watermelon	C6	2.3118	3.0414	2.6766	0.7296	0.4467
Milk and dairy products (combined category raw and heat processed Milk and dairy	cheese 'n' prawns cheese spread	A6	1.9542	2.4886	2.2214	0.5343	0.4467
	Vanilla ice cream	A9	5.2553	5.7160	5.4856	0.4607	0.4467
Multi-component foods or meal components	Coleslaw with real mayo	D11	2.6902	3.1461	2.9182	0.4559	0.4467

Comments

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 are 4 in 75 data values which lie outside the CLs (Table 3) which is in line with expectations. There are no trends to the outlying data which represented three of the five categories tested. The samples covered a diverse range of foods items inoculated with a selection of isolates and therefore these results show good agreement between the two methods for enumeration of *S. aureus* with a slight negative bias for the alternate method with an overall bias from all the categories of 0.1316.

3.2.6 RT conclusions

The relative trueness of the Alternative method is satisfied as there was a good agreement between the reference method and alternative method in the scatterplots and Bland Altman analyses.

3.3 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. As per ISO 16140-2:2016 guidelines, this study was conducted using artificially contaminated samples.

3.3.1 Categories, sample types and strains

Five 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 4)

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

The tested categories, types and items in the accuracy profile study are provided in Table 4

Table 4. Categories, types, items, strains and inoculation levels for Accuracy profile study, greyed out cells are naturally contaminated.

Category	Types	Strain	Item	Target Level* cfu/g	Test portions
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Pasteurised dairy products	*S.aureus* CRA 1215 Isolated from cheese	Chilled custard	100-250	5
				25000-55000	5
				3000000 -5500000	5
			Cream cheese	70-200	5
				25000 -50000	5
				3500000- 7500000	5
Produce and fruits (combined category fresh and processed)	Fresh produce	*S.aureus* CRA1242 Outbreak isolate	Baby spinach	100-350	5
				10000- 30000	5
				1500000-2500000	5
			Vegetable juice	100-200	5
				10000-50000	5
				1000000-2000000	5
Meat and poultry products (RTE/RTRH)	RTE meats	*S.aureus* CRA 1217 Isolated from cooked beef	Pastrami	150-350	5
				15000-40000	5
				2500000-5000000	5
			Cooked sliced chicken roll	100-350	5
				10000-45000	5
				2000000-8000000	5
Fishery products Combined category: raw, RTE, RTRH, RTC	Cooked fish products e.g. prawns	*S.aureus* CRA 1208 Isolated from smoked fish	Fresh cooked prawns	1000- 17000	5
				14000-24000	5
				100000-700000	5
			Smoked salmon	70-200	5
				7000—45000	5
				600000-1500000	5
Multi component foods or	Composite foods with raw	*S.aureus* CRA 3097	Pasta salad	350-600	5
				2500-25000	5
				700000-1500000	5

Category	Types	Strain	Item	Target Level* cfu/g	Test portions
meal components	/processed ingredients	Isolated from pasta	Sandwich spread	400-700	5
				5500-15000	5
				650000-1500000	5

For all matrices used in the study, the 100g samples were inoculated and stored at 2-8°C for 48-72h prior to analysis.

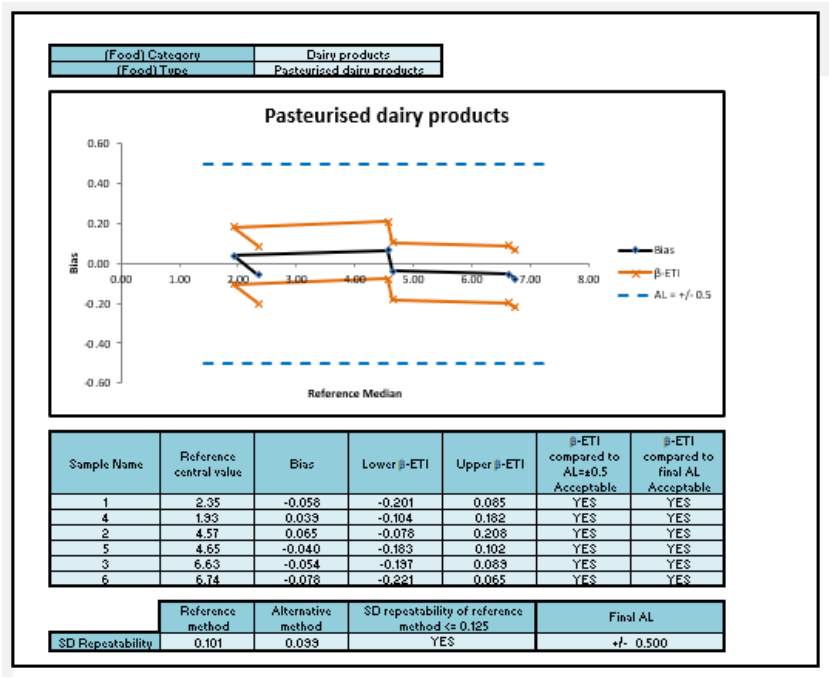
3.3.2 Calculations and interpretation of Accuracy profile study

All results are tabulated, calculated and interpreted According to ISO 16140-2. The statistical results are shown in Figures 8 to 14.

All raw data per category are given in Excel file 2021LR105: tab Accuracy Profile

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 of dairy products (Pasteurised dairy products) for Easy Plate SA method
Matrices used = chilled custard (1-3) and cream cheese (3-6)

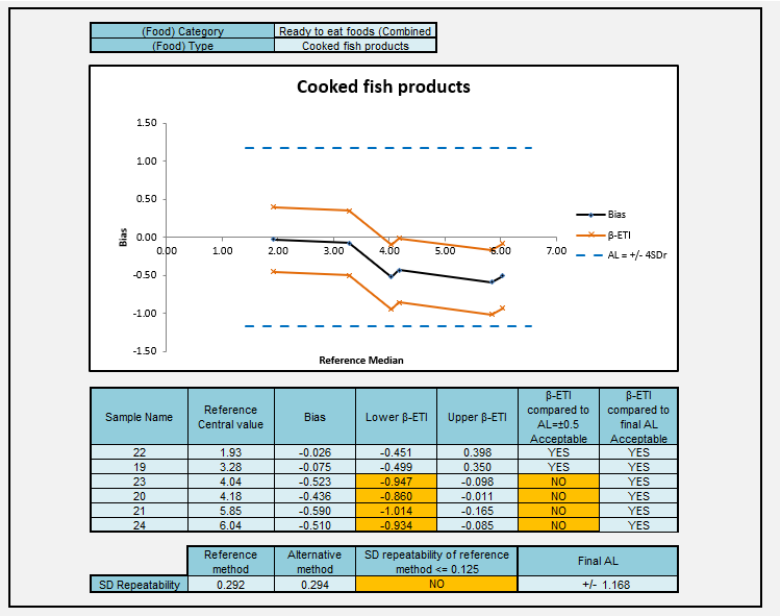


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
1	2.35	-0.058	-0.201	0.085	YES	YES
4	1.93	0.039	-0.104	0.182	YES	YES
2	4.57	0.065	-0.078	0.208	YES	YES
5	4.65	-0.040	-0.183	0.102	YES	YES
3	6.63	-0.054	-0.197	0.089	YES	YES
6	6.74	-0.078	-0.221	0.065	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.101	0.039	YES	+/- 0.500

Figure 9: Accuracy profile of Fishery products (Cooked fish products) for Easy Plate SA method

Matrices used = fresh cooked prawns (19-21) and smoked salmon (22-24)



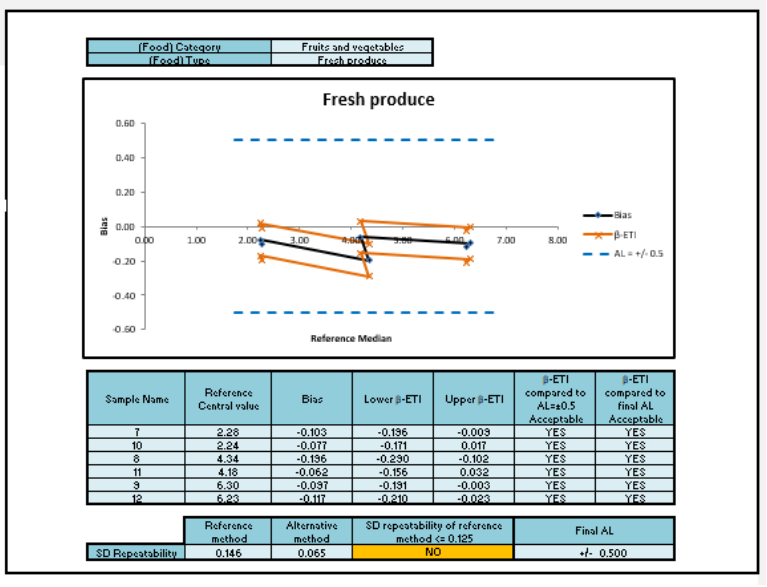
(Food) Category	Ready to eat foods (Combined
(Food) Type	Cooked fish products

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
22	1.93	-0.026	-0.451	0.398	YES	YES
19	3.28	-0.075	-0.499	0.350	YES	YES
23	4.04	-0.523	-0.947	-0.098	NO	YES
20	4.18	-0.436	-0.860	-0.011	NO	YES
21	5.85	-0.590	-1.014	-0.165	NO	YES
24	6.04	-0.510	-0.934	-0.085	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.292	0.294	NO	+/- 1.168

Figure 10: Accuracy profile for Fresh produce for Easy Plate SA method

Matrices used = baby spinach (7-9) and green smoothie (10-12)



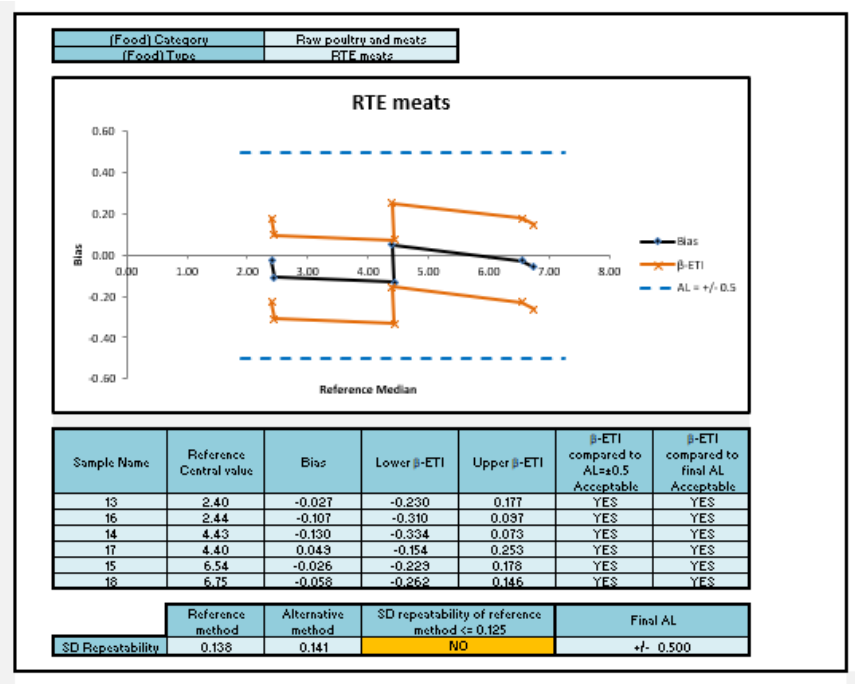
(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7	2.28	-0.103	-0.196	-0.009	YES	YES
10	2.24	-0.077	-0.171	0.017	YES	YES
8	4.34	-0.196	-0.290	-0.102	YES	YES
11	4.18	-0.062	-0.156	0.032	YES	YES
9	6.30	-0.097	-0.191	-0.003	YES	YES
12	6.23	-0.117	-0.210	-0.023	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.146	0.065	NO	+/- 0.500

Figure 11: Accuracy profile for RTE/RTRH Meat and poultry for Easy Plate SA method

Matrices used = pastrami (13-15) and chicken roll (16-18)



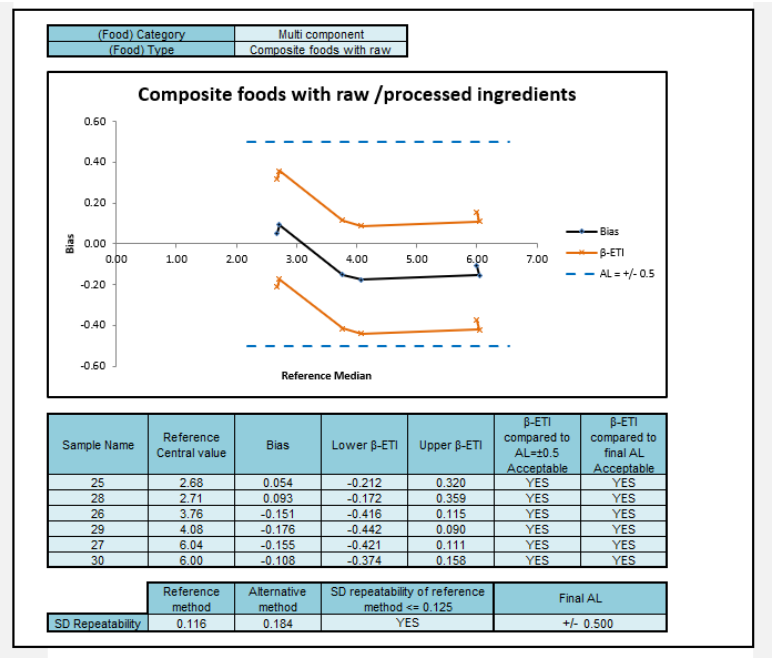
(Food) Category	Raw poultry and meats
(Food) Type	RTE meats

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
13	2.40	-0.027	-0.230	0.177	YES	YES
16	2.44	-0.107	-0.310	0.097	YES	YES
14	4.43	-0.130	-0.334	0.073	YES	YES
17	4.40	0.049	-0.154	0.253	YES	YES
15	6.54	-0.026	-0.229	0.178	YES	YES
18	6.75	-0.058	-0.262	0.146	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.138	0.141	NO	+/- 0.500

Figure 12: Accuracy profile for Multicomponent (composite foods with raw/processed ingredients) for Easy Plate SA method

Matrices used = Pasta salad (25-27) and sandwich spread (28-30)



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

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25	2.68	0.054	-0.212	0.320	YES	YES
28	2.71	0.093	-0.172	0.359	YES	YES
26	3.76	-0.151	-0.416	0.115	YES	YES
29	4.08	-0.176	-0.442	0.090	YES	YES
27	6.04	-0.155	-0.421	0.111	YES	YES
30	6.00	-0.108	-0.374	0.158	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.116	0.184	YES	+/- 0.500

Four of the five categories met the AL of 0.5log (dairy, fresh produce, RTE/RTRH meat and multicomponent foods). Two categories (Fishery products and multicomponent foods) required the new AL to be calculated. All data met the new AL values of 1.168.

Additional analysis of the category that had recalculates AL showed that 4 out of the 12 ß-ETI values for fishery products exceeded the 0.5 AL. For fishery products the values outside the AL were for the lower ETI for fresh cooked prawns and smoked salmon inoculated at medium and high levels. The SD repeatability of both methods was very similar being 0.292 for the reference method and 0.294 for the alternative method.

A negative bias was observed in the fishery products category for the medium and high levels tested in the study. An explanation for this could be that the reference method has a longer incubation time being plated for 24h longer, enabling the growth of the more stressed cells inoculated into the product.

3.3.3 Conclusion Accuracy profile study

The Accuracy of the Alternative method (Easy Plate SA) is satisfied as all categories met the 0.5log AL or the re-calculated AL.

3.4 Inclusivity and exclusivity study

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

3.4.1 Protocols

Inclusivity:

Fifty strains of *S. aureus* were analysed in the study. The analysis was carried out once with the Alternative method, the Reference method and a non-selective method. All inclusivity strains were grown overnight in a non-selective broth and enumerated on the reference method and alternative method at a level 10- 100 times greater than the minimum level of detection following protocols described in Annex A.

Exclusivity:

Thirty strains of non-target organisms was analysed. The analysis was carried out once with the Alternative method, the Reference method and a non-selective method. All exclusivity strains were grown in a suitable non-selective broth under optimal conditions of growth for at least 24 h and diluted to an appropriate level before testing begins.

Results inclusivity and exclusivity study

Summary data on inclusivity and exclusivity are given in Annex F. Raw data is given in Excel file 2020LR91 Quantitative data file: tabs Inclusivity and Exclusivity.

Inclusivity

For the inclusivity study, all 50 strains of *S. aureus* gave typical colonies on the alternate and reference method. Three isolates gave typical colonies on the reference and alternate media however did not give a positive reaction with the coagulase test. The level enumerated on the reference method and alternative method were similar with no negative or positive bias shown.

Exclusivity

For the exclusivity strains, 29 out of the 30 isolates tested gave the expected results on the Alternative method and 22 by the reference method. 1 strain was detected by the Alternative method (*Staphylococcus cohnii,* CRA 6714) and 8 by the Reference method *Staphylococcus caprae* (CRA 265), *Staphylococcus carnosus* (CRA 284) *Staphylococcus cohnii, (*CRA 6714) *Staphylococcus hyicus* (CRA 254), *Staphylococcus intermedius* (CRA 254) *Staphylococcus piscifermentans* (CRA 5929), *Staphylococcus sciuri* (CRA 6690) and *Staphylococcus warneri* (CRA 262). Four of the isolates giving colonies on BP agar were coagulase negative (*Staphylococcus caprae* CRA 265, *Staphylococcus carnosus*, *Staphylococcus piscifermentans*, and *Staphylococcus warneri*.

One isolate analysed in the exclusivity panel *Staphylococcus cohnii* (CRA 6714) was coagulase positive

Conclusion

All 50 *S. aureus* strains were correctly identified following the alternative method.

29 out of 30 non target strains were correctly identified as non- *S. aureus* following the alternative method detection and confirmation procedures.

The alternative method Easy Plate SA gave comparable performance to the reference method and is therefore selective and specific to *S. aureus*. Data from the study showed that the Easy Plate SA was more selective for *S. aureus* than the reference method.

3.5 Limit of quantification (LOQ)

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

The alternate method is based on visible colonies.

The LOQ did not have to be calculated for the alternative method in this study.

3.6 Conclusion (MCS)

- The alternative method Easy Plate SA for enumeration of Staphylococcus aureus shows satisfactory results for Accuracy profile.
- The alternative method Easy Plate SA for enumeration of Staphylococcus aureus shows satisfactory results for relative trueness
- Easy Plate SA shows comparable performance to the reference method for enumeration of Staphylococcus aureus in a broad range of foods, pet foods and animal feeds and environmental samples

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 organization

Collaborators

Samples were sent to 12 laboratories.

Matrix and strain used

Smoked salmon was inoculated with *Staphylococcus aureus* CRA 1208 (isolated from smoked fish)

Sample preparation

Samples were prepared and inoculated on 9 November 2022 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 below

Table 5 : 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

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 a frozen condition on 9 November 2022 and were received within 24 h to 72 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs. On receipt at the laboratories, the samples were stored frozen at ≤-18°C and defrosted prior to analysis as recommended in ISO 6887-1. The analyses was started on Monday 15 November 2022. Stability studies had been conducted to show that the required level of target organisms would be present after 7 and 8 days frozen storage. The expert lab analysed a set of samples on Monday 15 November 2022.

Analysis of Samples

Collaborative study laboratories and the expert laboratory carried out the analyses on Monday 14 November 2022. The analyses by the reference method and the alternative method were performed on the same day.

Experimental parameters controls

Detection of Staphylococcus aureus in the matrix before inoculation

In order to ensure absence of *Staphylococcus aureus* in the matrix, the reference method was performed on five portions (10 g) before the inoculation. All the results were negative.

Strain stability during transport

Duplicate samples inoculated at low, medium and high levels were tested for enumeration of *S. aureus* after 6 and 7 days storage at -18°C. Samples were thawed under controlled conditions prior to analysis. The data shows good stability under the storage regime tested (Table 6).

Table 6 - Staphylococcus aureus stability in the matrix

	Reference method cfu/g						Alternative method cfu/g					
	Low level		Medium level		*High level*		Low level		Medium level		*High level*	
Day	a	b	a	b	a	b	a	b	a	b	a	b
0	3.30E+03	3.10E+03	2.90E+04	3.10E+04	1.70E+06	2.20E+06	1.40E+03	1.40E+03	1.90E+04	1.00E+04	8.10E+05	8.90E+05
6	1.80E+03	2.30E+03	3.00E+04	3.00E+04	2.00E+06	2.50E+06	1.20E+03	1.40E+03	1.40E+04	1.60E+04	7.40E+05	1.20E+06
7	3.50E+03	2.70E+03	2.90E+04	2.50E+04	2.40E+06	1.40E+06	1.60E+03	1.60E+03	2.40E+04	1.30E+04	9.9E+05	1.30E+06

Logistic conditions

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, the receipt dates are given in Table 7.

Table 7 - Sample temperatures at receipt

Collaborator	Temperature measured by probe (°C)	Receipt date and time	State of the package and samples at receipt	Analysis date
1	10.4	10/11/2022,15:20	Good condition,	14/11/22
2	11.5	10/11/22, 14:00	Acceptable	14/11/22
3	1.4	10/11/22, 14:30	Good condition,	14/11/22
4	6.5	10/11/22, ca 10.15	No issues	14/11/22
5	No temperature data supplied			14/11/22
6	6.8	10/11/22, 12:00	sample still frozen	14/11/22
7	13	10/11/22, 14:10	satisfactory	14/11/22
8	7.2	10/11/22, 13:30	data logger not	14/11/22
9	12.1	11/11/22, 15:00	samples not found	14/11/22
10	No temperature data supplied			14/11/22
11	8.9	10/11/22,12:30	temp control defrosted	14/11/22
12	9.9	10/11/22, no time given	water vial not	14/11/22

No issues were encountered during the transport or at receipt for 12 out of 12 collaborators. All the samples were delivered on time and in appropriate conditions to 11 laboratories. The parcel delivered to lab 9 was held at ambient for longer prior to frozen storage therefore the results were excluded from the analysis

Temperatures during shipment and at receipt were all correct at all 11 other labs participating in the study.

A further lab (Lab 10) was removed as the data revealed cross contamination can occurred between the samples tested

The temperature curves are given in Annex I.

Calculation and summary of data

The raw data are given in Annex H.

MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 8.

Table 8 – Results obtained by the expert lab.

Level	Reference method cfu per g	Alternative method cfu per g
Blank	<10	<10

Level	Reference method cfu per g	Alternative method cfu per g
Low	330	210
Low	1300	130
Medium	6.10E+05	4.40E+05
Medium	7.10E+05	4.70E+05
High	5.80E+06	4.00E+06
High	5.80E+06	4.60E+06

Results obtained by the collaborative laboratories.

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

The results obtained by the collaborators are shown in Table 17.

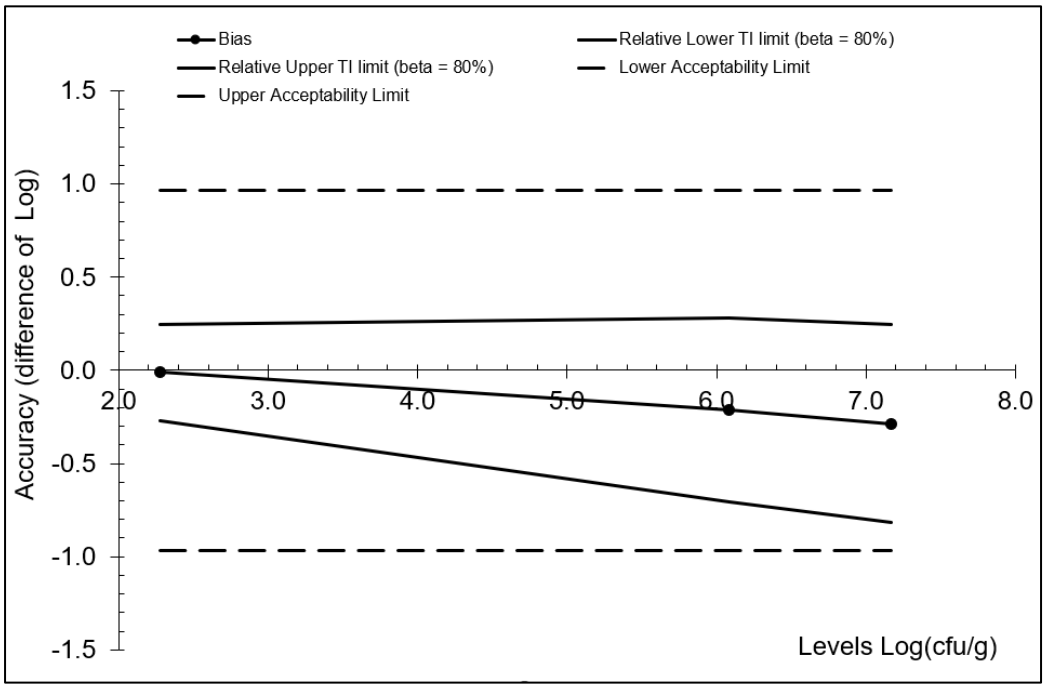
The accuracy profile plot is shown in Figure 13 and the statistical analysis of the data shown in Table 10.

Table 9: Summary of the results of the interlaboratory study per analyte level

Collaborator	Level	Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	2.34	2.46	2.30	2.45
2	low	2.48	2.30	2.43	2.20
3	low	2.81	2.53	2.26	2.45
4	low	2.15	2.11	2.08	2.15
5	low	2.38	2.41	2.28	2.28
6	low	2.32	2.04	2.46	2.36
7	low	2.04	2.00	2.32	2.46
8	low	2.36	2.58	2.32	2.38
9	low	2.93	2.20	2.23	2.41
10	low	2.15	4.18	2.15	2.18
11	low	2.20	2.26	2.04	1.78
12	low	2.38	2.15	2.11	2.38
1	medium	6.76	6.66	5.90	5.85
2	medium	5.87	5.86	5.73	5.75
3	medium	6.86	6.15	7.00	6.86
4	medium	6.38	6.15	6.48	6.81

5	medium	6.04	5.94	5.41	5.53
6	medium	5.80	5.90	5.83	5.94
7	medium	6.45	6.15	5.87	5.73
8	medium	5.98	5.99	5.83	5.86
9	medium	6.92	6.83	5.83	5.71
10	medium	5.76	6.38	5.51	5.51
11	medium	6.00	6.08	5.96	6.00
12	medium	5.64	5.81	5.45	5.71
1	high	7.62	7.73	6.85	6.83
2	high	6.92	6.81	6.79	6.72
3	high	7.81	8.00	7.08	8.00
4	high	7.66	7.48	7.81	7.72
5	high	7.04	7.04	6.48	6.69
6	high	6.99	6.92	6.89	6.87
7	high	7.95	7.11	6.91	6.81
8	high	7.00	6.93	6.81	6.81
9	high	6.9	6.8	6.84	6.86
10	high	8.4	7.0	7.2	6.9
11	high	7.15	7.08	6.97	6.94
12	high	6.72	6.89	6.41	6.63
1	blank	<1		<1	
2	blank	<1		<1	
3	blank	<1		<1	
4	blank	<1		<1	
5	blank	<1		<1	
6	blank	<1		<1	
7	blank	<1		<1	
8	blank	<1		<1	
9	blank	<1		<1	
10	blank	<1		<1	
11	blank	<1		<1	

Figure 13. Accuracy profile for Easy Plate SA from the ILS



A review of the accuracy profile and statistical analysis revealed that there was a negative bias of -0.286 observed for the high levels of contamination in the ILS samples. A root cause analysis was carried out to determine possible reasons for this.

The same batches of media were used for all participants in the study and the time and temperature used for the incubation of the plates was correct.

It was noted that the reproducibility of the low level samples was significantly higher than at the other levels being 0.2041 for the reference method and 0.220 for the Easy Plate SA, compared to <0.1-0.19 recorded for both the low and medium levels of contamination.

This study was run jointly with the Easy Plate AC and heat treated samples were used during the study due to the high level of background seen in the batch of smoked salmon used for the study. The higher variability in bacterial load could be explained by the difference in kill of the natural flora in the sample that would impact on the level of organisms able to grow at 30°C during the analysis. During the Easy Plate AC study, it was noted that the low-level samples contained 10^3 cfu per g, which was approximately 1 log higher than 10^2 cfu per g recorded for *S. aureus*. These results indicate that viable natural flora was present after the heat treatment.

Table 10. Statistical analysis of the ILS data according to the ISO spreadsheet

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	2.278	6.080	7.170			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	2.266	5.868	6.884	2.278	6.080	7.170
Repeatability standard deviation (sr)	0.118	0.112	0.081	0.111	0.108	0.211
Between-labs standard deviation (sL)	0.139	0.321	0.353	0.130	0.292	0.296
Reproducibility standard deviation (sR)	0.182	0.340	0.362	0.171	0.311	0.364
Corrected number of dof	12.058	8.924	8.411	12.061	9.021	11.145
Coverage factor	1.414	1.455	1.465			
Interpolated Student t	1.356	1.384	1.391			
Tolerance interval standard deviation	0.1900	0.3572	0.3809			
Lower TI limit	2.009	5.373	6.354			
Upper TI limit	2.524	6.362	7.414			
Bias	-0.012	-0.213	-0.286			
Relative Lower TI limit (beta = 80%)	-0.270	-0.707	-0.816			
Relative Upper TI limit (beta = 80%)	0.245	0.282	0.244			
Lower Acceptability Limit	-0.97	-0.97	-0.97			
Upper Acceptability Limit	0.97	0.97	0.97			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.293					

TRUE
FALSE

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

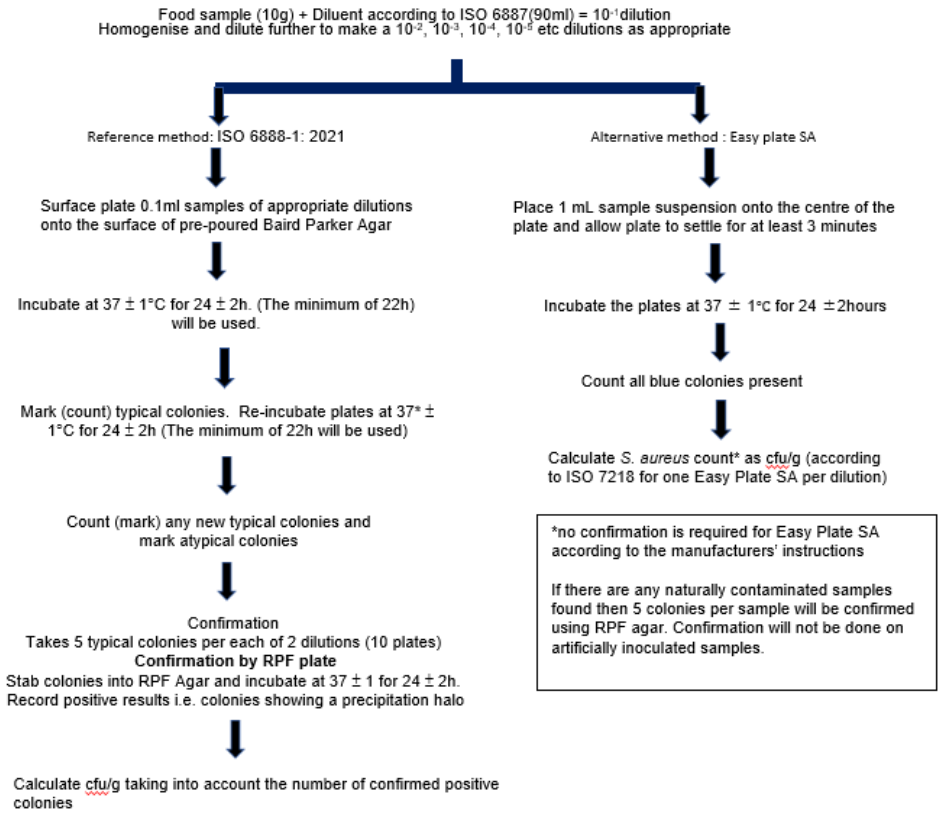
5 Overall conclusions of the MCS/ILS study

- The alternative method Easy Plate SA for enumeration of *Staphylococcus aureus* shows satisfactory results for relative trueness.
- The alternative method Easy Plate SA for enumeration of *Staphylococcus aureus* shows satisfactory results for accuracy profile.
- The alternative method Easy Plate SA for enumeration of *Staphylococcus aureus* is selective and specific.
- The alternative method Easy Plate SA for enumeration of *Staphylococcus aureus* shows satisfactory performance in the ILS.

Date 04/07/23

Signature Suzanne Jordan

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



n.b The minumum incubation time of 22h was used for the alternative method during the validation study.

ANNEX B: Kit insert

Please refer to separate pdf document

ANNEX C: Raw data per category – screening of samples

Sample screening data for RT study - screening data

Sample number	Item	Reference method ISO 6888-1 (2021)			Alternative method Easy Plate SA	
		Count -1 1ml	Count -1 0.1ml	Result cfu per g	Count -1	Result cfu per g
1	Raw milk	**0**	**0**	**<10**	**0**	**<10**
2	Raw salmon filet	**0**	**0**	**<10**	**0**	**<10**
3	Smoked salmon	**0**	**0**	**<10**	**0**	**<10**
4	Frozen seafood	**0**	**0**	**<10**	**0**	**<10**
5	Semi-dried fish	**0**	**0**	**<10**	**0**	**<10**
6	Cooked Shrimp	**0**	**0**	**<10**	**0**	**<10**
7	Crab meat	**0**	**0**	**<10**	**0**	**<10**
8	Bagged pre-cut lettuce	**0**	**0**	**<10**	**0**	**<10**
9	Shredded carrot	**0**	**0**	**<10**	**0**	**<10**
10	Chicken Sausages	**0**	**0**	**<10**	**0**	**<10**
11	Salami	**0**	**0**	**<10**	**0**	**<10**
12	Smoked salmon	**0**	**0**	**<10**	**0**	**<10**
14	Raw milk cheese	**0**	**0**	**<10**	**0**	**<10**
15	Tuna chunks	**0**	**0**	**<10**	**0**	**<10**
16	RTC Rice	**0**	**0**	**<10**	**0**	**<10**
17	Vegetable salad	**0**	**0**	**<10**	**0**	**<10**

ANNEX D: Calculations and interpretation of relative trueness

Items	Sample number	log(Ref)	log(Alt)	Mean	Difference
Crustaceans					
prawn cocktail	B11	2.330	2.531	2.431	0.201
brown shrimp	B12	3.255	3.544	3.400	0.289
Lemon & Garlic King Prawns	B13	4.322	4.653	4.488	0.331
Big & Juicy Crayfish Tails	B14	5.279	5.556	5.418	0.278
Chilli & Lime King Prawns	B15	6.230	6.556	6.393	0.326
Raw fish (unprocessed)					
Diced salmon fillets	B1	2.851	2.934	2.893	0.084
Cod loin	B2	3.176	3.230	3.203	0.054
skinless basa fillets	B3	4.000	4.176	4.088	0.176
Wild Salmon	B4	5.204	5.204	5.204	0.000
Cornish hake	B5	6.176	6.462	6.319	0.286
RTE/RTC/RTRH fish and seafoods					
Frozen Tuna steaks	B10	6.114	6.146	6.130	0.032
smoked haddock loin	B6	1.875	1.929	1.902	0.054
Honey Roast Salmon Flakes	B7	2.967	3.079	3.023	0.112
Ready to eat smoked mackerel fillets	B8	4.362	4.672	4.517	0.310
Creamy Herring	B9	5.230	5.301	5.266	0.071
Cooked meat and poultry products					
Dry cured pork	P1	2.498	2.439	2.469	-0.059
Pork pate	P2	3.672	3.724	3.698	0.052
Cooked ham	P3	4.643	4.833	4.738	0.189
Wafer thin turkey ham	P4	5.643	5.763	5.703	0.120
Wafer thin roast chicken slices	P5	6.716	6.875	6.796	0.159
Fermented or dried meat and poultry products					
Beef Jerky	P10	6.531	6.699	6.615	0.167
Kabanos dried chicken	P6	2.512	2.736	2.624	0.225
Kabanosy dried meat	P7	3.653	3.602	3.628	-0.051
German style salami	P8	4.699	4.623	4.661	-0.076
Chicken cocktail sausages	P9	5.690	5.591	5.641	-0.099
Raw cured meat and poultry products					
Prosuitto crudo	P11	2.568	2.686	2.627	0.118
Italian pepperoni	P12	3.663	3.748	3.705	0.085
Smoked chicken leg	P13	4.732	4.778	4.755	0.046
Chorizo	P14	5.653	5.740	5.697	0.087
Ox tongue	P15	6.591	6.748	6.670	0.157
Dry milk products					

Items	Sample number	log(Ref)	log(Alt)	Mean	Difference
Milk powder	A11	2.833	2.994	2.913	0.162
Pancake mix	A12	2.954	3.279	3.116	0.325
Dessert powder	A13	3.602	3.785	3.694	0.183
Scone powder	A14	5.041	5.079	5.060	0.038
Instant Custard Powder 72g	A15	6.398	6.477	6.438	0.079
Pasteurised milk and dairy products					
Skimmed milk	A10	5.580	5.929	5.755	0.350
cheese 'n' prawns	A6	1.954	2.489	2.221	0.534
Protein shake strawberry	A7	3.322	3.633	3.478	0.311
Double cream	A8	4.519	4.531	4.525	0.013
Vanilla ice cream	A9	5.255	5.716	5.486	0.461
Raw milk and dairy products					
Raw milk (AMP)	A1	2.813	2.736	2.775	-0.077
Raw milk (straightfromthecow)	A2	4.114	4.114	4.114	0.000
Raw milk cheese 1	A3	4.892	4.869	4.881	-0.023
Raw milk cheese 2	A4	6.041	6.079	6.060	0.038
No.1 Mountain Comté AOP	A5	6.919	7.146	7.033	0.227
Composite foods with substantial raw ingredients					
Triple grain salad	D1	2.836	2.690	2.763	-0.146
Prawn layered salad	D2	3.922	3.971	3.947	0.049
Cheese and spring onion sandwich	D3	4.845	4.898	4.871	0.053
Egg mayo sandwich	D4	5.982	6.000	5.991	0.018
Egg and cress sandwich	D5	6.826	6.806	6.816	-0.020
Mayonnaise based deli-salads					
Coleslaw with real mayo	D11	2.690	3.146	2.918	0.456
Potato salad with mayo	D12	3.531	3.653	3.592	0.122
Cheese coleslaw	D13	4.531	4.591	4.561	0.060
Egg mayonnaise	D14	5.580	5.643	5.612	0.064
Chicken, tomato and basil pasta with egg yolk and pasturized egg mayo	D15	6.544	6.613	6.578	0.069
RTRH/RTE foods (chilled, frozen)					
Spinach and tomato quiche	D10	6.954	6.978	6.966	0.023
Chicken tomato & basil pasta	D6	2.590	2.933	2.761	0.343
Curry cod fillet & chips	D7	3.799	4.041	3.920	0.242
Roasted Vegetable Couscous	D8	4.833	4.991	4.912	0.159
Savers Chicken Curry	D9	5.857	5.982	5.920	0.125
Cut ready-to-eat vegetables/leafy greens and sprouts					
Shredded iceberg lettuce	C1	3.079	3.079	3.079	0.000
Peeled Brussel sprouts	C2	4.079	4.204	4.142	0.125

Items	Sample number	log(Ref)	log(Alt)	Mean	Difference
Baby spinach	C3	3.176	3.380	3.278	0.204
Fine beans	C4	4.255	4.301	4.278	0.046
Sprouted salad topper	C5	5.204	5.230	5.217	0.026
Fresh fruit/Cut RTE fruit and vegetable products					
Super blue raw smoothie	C10	5.342	5.255	5.299	-0.087
Watermelon	C6	2.312	3.041	2.677	0.730
Blueberries	C7	2.104	2.447	2.275	0.343
Smooth freshly squeezed orange juice	C8	3.380	3.398	3.389	0.018
Super green raw smoothie	C9	4.447	4.415	4.431	-0.032
Heat treated fruit and vegetables					
Green smoothie	10F	2.1614	2.2227	2.1920	0.0613
wonder green juice	C12	2.561	2.763	2.662	0.202
Orange juice burst	C13	3.820	3.813	3.816	-0.007
Vegetable juice	C14	5.663	5.763	5.713	0.101
Brilliant beetroot juice	C15	5.663	5.839	5.751	0.176

ANNEX E: Summary tables Accuracy profile study

Dairy

(Food) Category 1	Dairy products
(Food) Type 1	Pasteurised dairy products

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
1	Chilled custard	low	140	185	282	223	230	136	195	170	205	209
4	Cream cheese	low	85	75	85	150	180	70	93	80	109	168
2	Chilled custard	intermediate	3.70E+04	3.50E+04	5.00E+04	3.30E+04	5.30E+04	4.00E+04	2.80E+04	4.30E+04	4.30E+04	4.50E+04
5	Cream cheese	intermediate	3.40E+04	4.50E+04	4.50E+04	3.80E+04	4.50E+04	4.50E+04	3.50E+04	4.40E+04	2.80E+04	4.10E+04
3	Chilled custard	high	4.40E+06	4.30E+06	3.90E+06	4.90E+06	3.50E+06	3.80E+06	3.50E+06	3.40E+06	5.50E+06	3.90E+06
6	Cream cheese	high	7.30E+06	4.90E+06	5.20E+06	5.50E+06	5.80E+06	5.60E+06	3.60E+06	3.50E+06	4.60E+06	5.60E+06

2 Fresh produce

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

			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7	Baby spinach	low	170	190	215	120	305	1.50E+02	1.27E+02	1.77E+02	1.09E+02	1.55E+02
10	Green smoothie	low	180	145	150	173	193	1.45E+02	1.60E+02	1.40E+02	1.30E+02	2.00E+02
8	Baby spinach	intermediate	1.10E+04	1.90E+04	2.50E+04	2.20E+04	2.90E+04	1.30E+04	1.20E+04	1.60E+04	1.40E+04	1.50E+04
11	Green smoothie	intermediate	1.50E+04	1.30E+04	1.10E+04	1.70E+04	4.80E+04	1.10E+04	1.30E+04	1.20E+04	1.40E+04	1.50E+04
9	Baby spinach	high	2.20E+06	1.90E+06	2.00E+06	1.70E+06	2.50E+06	1.90E+06	1.60E+06	1.50E+06	1.20E+06	2.00E+06
12	Green smoothie	high	1.70E+06	1.30E+06	1.80E+06	1.10E+06	1.80E+06	1.40E+06	1.30E+06	1.30E+06	1.40E+06	1.30E+06

RTE/RTRH meat and poultry

(Food) Category 3	RTE/RTRH meat and poultry
(Food) Type 3	RTE meats

			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
13	Pastrami	low	250	205	305	265	250	191	190	318	235	382
16	Cooked sliced chicken roll	low	230	275	350	287	197	175	140	215	335	255
14	Pastrami	intermediate	3.9E+04	2.7E+04	3.0E+04	2.5E+04	2.0E+04	2.0E+04	2.1E+04	1.8E+04	1.9E+04	2.6E+04
17	Cooked sliced chicken roll	intermediate	3.0E+04	1.3E+04	4.5E+04	2.5E+04	2.4E+04	2.8E+04	1.3E+04	3.5E+04	2.8E+04	2.5E+04
15	Pastrami	high	3.5E+06	2.5E+06	4.5E+06	3.3E+06	6.7E+06	3.3E+06	2.5E+06	3.5E+06	2.9E+06	4.5E+06
18	Cooked sliced chicken roll	high	3.1E+06	3.8E+06	7.6E+06	6.6E+06	5.6E+06	2.0E+06	2.7E+06	5.6E+06	5.2E+06	4.9E+06

Fish

(Food) Category 4		Ready to eat foods									
(Food) Type 4		Cooked fish products									
			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
22	Smoked salmon	low	75	145	85	180	75	80	80	60	145	80
19	Fresh cooked prawns	low	1.90E+03	1.70E+04	1.80E+03	1.90E+03	1.30E+03	1.60E+03	1.60E+03	1.50E+03	1.60E+04	1.20E+03
23	Smoked salmon	intermediate	1.10E+04	7.80E+03	7.40E+03	4.50E+04	4.10E+04	3.30E+03	1.90E+03	2.50E+03	1.30E+04	1.20E+04
20	Fresh cooked prawns	intermediate	1.40E+04	2.00E+04	1.30E+04	2.50E+04	1.50E+04	4.70E+03	5.50E+03	4.80E+03	6.60E+03	5.50E+03
21	Fresh cooked prawns	high	4.50E+05	7.20E+05	5.70E+05	1.50E+06	7.00E+05	1.40E+05	1.80E+05	1.40E+05	2.30E+05	2.00E+05
24	Smoked salmon	high	5.50E+05	3.50E+05	1.40E+06	1.10E+06	1.40E+06	3.20E+05	6.00E+04	3.60E+05	3.40E+05	3.50E+05

Multicomponent foods

(Food) Category 5		Multi component									
(Food) Type 5		Composite foods with raw /processed									
			Reference method					Alternative method				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
25	Pasta salad	low	463	477	575	355	555	586	570	540	486	477
28	Sandwich spread	low	457	590	513	407	570	695	636	545	490	640
26	Pasta salad	intermediate	5.80E+03	4.10E+03	1.20E+04	3.90E+03	6.60E+03	4.10E+03	2.90E+03	2.30E+04	2.50E+03	5.40E+03
29	Sandwich spread	intermediate	1.30E+04	1.20E+04	9.50E+03	6.40E+03	1.20E+04	1.40E+04	1.10E+04	8.00E+03	6.70E+03	5.60E+03
27	Pasta salad	high	1.00E+06	1.40E+06	1.20E+06	1.10E+06	9.90E+05	1.10E+06	1.10E+06	7.70E+05	6.50E+05	7.10E+05
30	Sandwich spread	high	1.00E+06	9.50E+05	1.20E+06	1.30E+06	7.00E+05	7.80E+05	6.80E+05	8.20E+05	1.20E+06	6.40E+05

ANNEX F Raw data from inclusivity and exclusivity

Inclusivity

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
1	267	Turkey	Not known	Industrial strain	T	70	4	**6.70E+08**	T	39	3	**3.80E+08**	T	70	8	**7.10E+08**	+
2	409	'Slow ' Cheddar Cheese	Not known	Industrial strain	T	79	6	**7.70E+08**	T	78	8	**7.80E+08**	T	69	11	**7.30E+08**	+
3	713	Human lesion	Not known	NCTC10788, ATCC 6538	T	101	6	**9.70E+08**	T	102	18	**1.10E+09**	T	113	13	**1.10E+09**	+
4	1197	Chicken	C	Industrial strain	T	54	4	**5.30E+08**	T	72	5	**7.00E+08**	T	96	7	**9.40E+08**	+
5	1208	Smoked fish	C	Industrial strain	T	174	25	**1.80E+09**	T	133	18	**1.40E+09**	T	T	19	**1.90E+09**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
6	1210	Smoked fish	C	Industrial strain	T	71	7	**7.10E+08**	T	66	5	**6.50E+08**	T	98	4	**9.30E+08**	weak +
7	1211	Shellfish	C and D	Industrial strain	T	91	5	**8.70E+08**	T	72	8	**7.30E+08**	T	84	8	**8.40E+08**	+
8	1213	Chicken	Not known	Industrial strain	T	53	4	**5.20E+08**	T	50	6	**5.10E+08**	T	86	3	**8.10E+08**	+
9	1214	Cooked beef	Not known	Industrial strain	T	94	15	**9.90E+08**	T	115	16	**1.20E+09**	T	89	9	**8.90E+08**	weak +
10	1215	Cheese	C	Industrial strain	T	122	12	**1.20E+09**	T	128	5	**1.20E+09**	T	149	11	**1.50E+09**	+
11	1216	Human abscess	C	NCTC 10655, ATCC 19095	T	82	8	**8.20E+08**	T	88	11	**9.00E+08**	T	77	18	**7.70E+08**	+
12	1217	Cooked beef	Not known	Industrial strain	T	64	2	**6.00E+08**	T	73	5	**7.10E+08**	T	75	5	**7.30E+08**	weak +

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
13	1219	Raw beef	C	Industrial strain	T	111	10	**1.10E+09**	T	121	9	**1.20E+09**	T	T	18	**1.80E+09**	+
14	1223	Chicken	A and E	Industrial strain	T	92	11	**9.40E+08**	T	55	5	**5.50E+08**	T	91	3	**8.50E+08**	+
15	1224	Margarine	D	Industrial strain	T	115	7	**1.10E+09**	T	70	5	**6.80E+08**	T	57	8	**5.90E+08**	weak +
16	1225	Cooked chicken	C and D	Industrial strain	T	54	6	**5.50E+08**	T	62	6	**6.20E+08**	T	69	8	**7.00E+08**	+
17	1227	Frozen cooked peeled prawns	B	Industrial strain	T	109	3	**1.00E+09**	T	118	17	**1.20E+09**	T	102	11	**1.00E+09**	+
18	1228	Frozen shrimp	A and B	Industrial strain	T	30	3	**3.00E+08**	T	33	6	**3.50E+08**	T	51	4	**5.00E+08**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
19	1230	Shellfish	C and D	Industrial strain	T	127	9	**1.20E+09**	T	77	5	**7.50E+08**	T	81	7	**8.00E+08**	+
20	1231	Food poisoning outbreak	A	Industrial strain	T	47	2	**4.50E+08**	T	78	4	**7.50E+08**	T	71	3	**6.70E+08**	+
21	16894	Food poisoning outbreak		NCIMB 11852	142	15	0	**1.40E+08**	112	11	3	**1.10E+08**	T	37	3	**3.60E+08**	+
22	1234	Food poisoning outbreak	E	Industrial strain	T	51	3	**4.90E+08**	T	81	5	**7.80E+08**	T	75	1	**7.50E+08**	+
23	1235	Cheese	Not known	Industrial strain	T	105	14	**1.10E+09**	T	102	12	**1.00E+09**	T	130	6	**1.20E+09**	+
24	1236	Cheese	C	Industrial strain	T	35	1	**3.30E+08**	T	59	6	**5.90E+08**	T	55	5	**5.50E+08**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
25	1238	Industrial isolate	A	Industrial strain	T	85	6	**8.30E+08**	43	2	0	**4.10E+07**	T	9	2	**9.10E+07**	+
26	1239	Raw pork	Not known	Industrial strain	119	13	1	**1.20E+08**	98	15	1	**1.50E+08**	T	25	2	**2.50E+08**	+
27	1240	Cheese	Not known	Industrial strain	T	125	18	**1.30E+09**	15	0	0	**1.50E+07**	T	2	0	**2.30E+07**	+
28	1241	Industrial isolate	D	Industrial strain	T	20	2	**2.00E+08**	T	40	6	**4.20E+08**	T	76	4	**7.30E+08**	-
29	1242	Food poisoning outbreak	A	Industrial strain	T	130	9	**1.30E+09**	37	4	0	**3.70E+07**	T	4	0	**3.80E+07**	+
30	1244	Cheese	C	Industrial strain	187	19	0	**1.90E+08**	150	21	1	**2.00E+08**	T	53	3	**5.10E+08**	+
31	1246	Pork sausage	Not known	Industrial strain	T	180	20	**1.80E+09**	21	0	0	**2.10E+07**	T	4	0	**3.20E+07**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
32	1446	Dairy product	A and D	Industrial strain	T	25	2	**2.50E+08**	T	54	6	**5.50E+08**	T	76	5	**7.40E+08**	+
33	16615	Not known		Industrial strain	T	21	2	**2.10E+08**	250	24	4	**2.50E+08**	T	18	2	**1.80E+08**	-
34	1991	Raw chicken	Not known	Industrial strain	164	20	2	**2.00E+08**	123	19	4	**1.90E+08**	T	21	4	**2.30E+08**	+
35	1992	Raw chicken	Not known	Industrial strain	125	17	0	**1.30E+08**	148	24	1	**1.60E+08**	T	23	3	2.40E+08	+
36	1993	Raw chicken	Not known	Industrial strain	189	18	4	**2.00E+08**	141	21	1	**2.00E+08**	T	23	2	**2.30E+08**	+
37	1994	Beef burger	C	Industrial strain	T	34	1	**3.20E+08**	T	56	3	**5.40E+08**	T	59	6	**5.90E+08**	+
38	2078	Milk powder	A and D	Industrial strain	T	14	3	**1.50E+08**	186	19	3	**2.00E+08**	T	53	7	**5.50E+08**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
39	2095	Milk powder	Not known	Industrial strain	T	124	10	**1.20E+09**	14	0	0	**1.40E+07**	T	2	0	**1.30E+07**	+
40	2096	Milk powder	Not known	Industrial strain	259	22	3	**2.30E+08**	148	15	0	**1.50E+08**	T	41	1	**3.80E+08**	+
41	3026	'Slow ' Cheddar Cheese	A	Industrial strain	156	12	3	**1.50E+08**	109	9	0	**1.10E+08**	T	25	3	**2.50E+08**	+
42	3097	Pasta	A	Industrial strain	139	10	0	**1.40E+08**	114	8	1	**1.10E+08**	T	13	0	**1.60E+08**	+
43	3098	Rice salad	Not known	Industrial strain	T	32	3	**3.20E+08**	T	28	3	**2.80E+08**	T	42	2	**4.00E+08**	+
44	16890	Fluid from septic arthritis	Not known	NCIMB 11787	115	10	0	**1.10E+08**	142	20	2	**1.50E+08**	T	16	0	**1.60E+08**	+

Number	CRA code	Source	Enterotoxin type	Culture collection identity if available	count on non selective agar (TSA)				Alternate Easy plate count (Easy Plate SA)				ISO reference method (ISO 6888-1:2021)				coagulase result
					-6	-7	-8	**cfu/ml**	-6	-7	-8	**cfu/ml**	-5	-6	-7	**cfu/ml**	
45	4105	unknown	A	NCIMB 12702, ATCC 25923	143	12	2	**1.40E+08**	122	13	3	**1.50E+08**	T	14	4	**1.60E+08**	+
46	5441	sheep	Not known	NCTC 1803	86	5	0	**8.30E+07**	80	8	1	**8.00E+08**	T	9	2	**1.10E+08**	+
47	5932	Pasta	Not known	Industrial strain	T	33	2	**3.20E+08**	T	29	2	**2.80E+08**	T	47	2	**4.50E+08**	+
48	5957	Pasta plant swab	Not known	Industrial strain	242	16	2	**2.30E+08**	139	15	1	**1.50E+08**	T	32	4	**3.30E+08**	+
49	15632	Not known	Not known	NCTC 6571, ATCC 9144	T	46	3	**4.50E+08**	T	38	1	**3.50E+08**	T	68	3	**6.50E+08**	+
50	15944	Ham	A	NCTC 10652	100	25	6	**1.00E+08**	73	6	2	**7.20E+07**	T	5	0	**9.50E+07**	+

Exclusivity

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
1	*Bacillus cereus*	1761	Dairy product	Industrial strain	54	2	0	0	5.1E+06	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
2	*Bacillus cereus*	4110	unknown	ATCC 10876, NCTC 7464	99	14	1	0	1.4E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
3	*Bacillus subtilis*	4112	unknown	ATCC 6633, NCTC 10400	80	3	0	0	7.5E+06	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
4	*Brochothrix thermospacta*	16019	Pork sausage	NCTC 10822	133	8	1	1	1.3E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
5	*Flavobacterium species*	4083	unknown	Industrial strain	T	T	49	2	4.6E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
6	*Enterobacter agglomerans*	1490	Raw mince	Industrial strain	T	155	5	0	1.1E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
7	*Enterococcus faecalis*	16049	Urine	NCIMB 13280, ATCC 29212	T	T	98	12	1.0E+09	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
8	*Enterococcus faecalis*	4113	unknown	NCTC 775	T	T	50	5	5.0E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
9	*Escherichia coli*	16041	Raw ground beef	Industrial strain	T	T	112	15	1.2E+09	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
10	*Lactobacillus brevis*	16628	unknown	NCTC13386	T	T	131	16	1.3E+09	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
11	*Lactobacillus gasseri*	6804	Human source	NCIMB 13081	T	81	1	0	1.3E+09	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
12	*Leuconostoc mesenteroides*	16022	Ham	Industrial strain	T	248	17	1	7.5E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
13	*Listeria monocytogenes*	1104	Soft cheese	Industrial strain	T	T	96	12	9.8E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
14	*Micrococcus luteus*	16258	unknown	NCTC 2665, ATCC 13507	T	134	4	0	1.3E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
15	*Pediococcus pentosaceus*	16030	Brine	Industrial strain	T	T	69	6	6.8E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
16	*Pseudomonas rhodesiae*	17344	unknown	Industrial strain	T	T	30	4	3.1E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
17	*Salmonella* Enteritidis	3505	Fish cakes	Industrial strain	nt	T	86	4	8.2E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
18	*Salmonella* Typhimurium	1960	Chicken	Industrial strain	nt	T	81	3	7.6E+08	0	0	0	<1.0E6	0	0	0	<1.0E6	n/a	n/a
19	*Staphylococcus caprae*	265	Goat	Industrial strain	nt	10	1	0	1.0E+07	0	0	0	<1.0E6	3	0	0	3.0E+06	-	<1.0E6
20	*Staphylococcus carnosus*	284	Fermented sausage	Industrial strain	nt	54	6	1	5.5E+07	0	0	0	<1.0E6	87	9	0	8.7E+07	-	<1.0E6
21	*Staphylococcus cohnii*	6714	unknown	Industrial strain	nt	190	26	3	2.0E+08	155	16	2	1.6E+08	T	17	6	2.1E+08	+	2.1E+08
22	*Staphylococcus epidemidis*	271	Human skin	Industrial strain	nt	74	9	2	7.5E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	-	n/a
23	*Staphylococcus hominis*	1527	Dried milk powder	Industrial strain	nt	10	0	0	1.0E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	-	n/a

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
24	*Staphylococcus hyicus*	281	Pig skin	Industrial strain	nt	97	5	0	9.3E+07	0	0	0	<1.0E6	79	14	0	8.5E+07	-	<1.0E6
25	*Staphylococcus intermedius*	254	pigeons	NCTC 11048, ATCC 29663	nt	85	7	0	8.4E+07	0	0	0	<1.0E6	29	3	0	2.9E+07	-	<1.0E6
26	*Staphylococcus piscifermentans*	5929	unknown	Industrial strain	nt	73	5	0	7.1E+07	0	0	0	<1.0E6	2	0	0	2.0E+06	-	<1.0E6
27	*Staphylococcus sciuri*	6690	unknown	Industrial strain	nt	T	48	4	4.7E+08	0	0	0	<1.0E6	3	1	0	3.0E+06	-	<1.0E6
28	*Staphylococcus simulans*	244	Human skin	Industrial strain	nt	94	9	2	9.4E+07	0	0	0	<1.0E6	0	0	0	<1.0E6	-	n/a
29	*Staphylococcus warneri*	262	German salami	Industrial strain	nt	26	3	0	2.6E+07	0	0	0	<1.0E6	27	1	0	2.5E+07	-	<1.0E6

Number	Organism	Campden strain code	Source	Culture collection identity if available	non selective count					count on alternative method easy plate SA				ISO reference method (ISO 6888-1:2021)					
					-5	-6	-7	-8	**count cfu/ml**	-6	-7	-8	count cfu/ml	-6	-7	-8	presumptive cfu/ml	Coagulase results	final result
30	*Staphylococcus xylosus*	266	Mettwurst sausage	Industrial strain	nt	85	6	0	8.30E+07	0	0	0	<1.00E6	0	0	0	<1.0E6	-	n/a
31	*Streptococcus lactis*	1509	Milk powder	Industrial strain	nt	93	5	1	8.90E+07	0	0	0	<1.00E6	0	0	0	<1.0E6	-	n/a

ANNEX G: Raw data from the ILS

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
1	1	Low	2.34	2.30	14/11/2022
	2	Medium	6.76	5.9	14/11/2022
	3	High	7.62	6.85	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.46	2.45	14/11/2022
	6	Medium	6.66	5.85	14/11/2022
	7	High	7.73	6.83	14/11/2022
2	1	Low	2.48	2.43	14/11/2022
	2	Medium	5.87	5.73	14/11/2022
	3	High	6.92	6.79	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.3	2.2	14/11/2022
	6	Medium	5.86	5.75	14/11/2022
	7	High	6.81	6.72	14/11/2022
3	1	Low	2.81	2.26	14/11/2022
	2	Medium	6.86	7.00	14/11/2022
	3	High	7.81	7.08	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.53	2.45	14/11/2022
	6	Medium	6.15	6.86	14/11/2022
	7	High	8.00	8.00	14/11/2022
4	1	Low	2.15	2.08	14/11/2022
	2	Medium	6.38	6.48	14/11/2022
	3	High	7.66	7.81	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.11	2.15	14/11/2022
	6	Medium	6.15	6.81	14/11/2022
	7	High	7.48	7.72	14/11/2022
5	1	Low	2.38	2.28	14/11/2022
	2	Medium	6.04	5.41	14/11/2022
	3	High	7.04	6.48	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.41	2.28	14/11/2022
	6	Medium	5.94	5.53	14/11/2022
	7	High	7.04	6.69	14/11/2022
6	1	Low	2.32	2.46	14/11/2022
	2	Medium	5.80	5.83	14/11/2022
	3	High	6.99	6.89	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.04	2.36	14/11/2022

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
	6	Medium	5.90	5.94	14/11/2022
	7	High	6.92	6.87	14/11/2022
7	1	Low	2.04	2.32	14/11/2022
	2	Medium	6.45	5.87	14/11/2022
	3	High	7.95	6.91	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.00	2.46	14/11/2022
	6	Medium	6.15	5.73	14/11/2022
	7	High	7.11	6.81	14/11/2022
8	1	Low	2.36	2.32	14/11/2022
	2	Medium	5.98	5.83	14/11/2022
	3	High	7.00	6.81	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.58	2.38	14/11/2022
	6	Medium	5.99	5.86	14/11/2022
	7	High	6.93	6.81	14/11/2022
9	1	Low	2.93	2.23	14/11/2022
	2	Medium	6.92	5.83	14/11/2022
	3	High	6.9	6.84	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.20	2.41	14/11/2022
	6	Medium	6.83	5.71	14/11/2022
	7	High	6.8	6.86	14/11/2022
10	1	Low	2.15	2.15	14/11/2022
	2	Medium	5.76	5.51	14/11/2022
	3	High	8.4	7.2	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	4.18	2.18	14/11/2022
	6	Medium	6.38	5.51	14/11/2022
	7	High	7.0	6.9	14/11/2022
11	1	Low	2.20	2.04	14/11/2022
	2	Medium	6.00	5.96	14/11/2022
	3	High	7.15	6.97	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.26	1.78	14/11/2022
	6	Medium	6.08	6.00	14/11/2022
	7	High	7.08	6.94	14/11/2022
Expert lab	1	Low	2.38	2.11	14/11/2022
	2	Medium	5.64	5.45	14/11/2022
	3	High	6.72	6.41	14/11/2022
	4	Blank	<1	<1	14/11/2022
	5	Low	2.15	2.38	14/11/2022
	6	Medium	5.81	5.71	14/11/2022

Laboratory	Sample code	Level	Reference method	Alternative Method	Date samples tested
	7	High	6.89	6.63	14/11/2022