2022LR113 Neogen Soleris Non-Fermenting Total Viable Count (NF-TVC) vial MCS ILS Summary Report 14/10/2024

Method Comparison Study Report for the ISO 16140-2:2016 validation of Neogen Soleris Non-Fermenting Total Viable Count (NF-TVC), for the detection of Total Viable Count at a threshold of 10 cfu per g in a broad range of foods

MicroVal study number: 2022LR113

Method/Kit name: Neogen Soleris® Non-Fermenting Total Viable Count (NF-TVC)

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Foreword

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

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Method/Kit name: Neogen Soleris® Non-Fermenting Total Viable Count (NF-TVC)

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 4833-1:2013 Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30°C by the pour plate technique

Scope of validation: Broad range of foods

Certification orgnization: LRQA

List of abbreviations

A(lt)	Alternative method
AL	Acceptability Limit
Art. Cont.	artificial contamination
CFU	Colony Forming Units
EL	Expert Laboratory
FP	False Positive
FPR	False Positive Ratio
g	Gram
h	Hour
ILS	Interlaboratory Study
LOD	Level of Detection
MCS	Method Comparison Study
min	minute
ml	millilitre
MR	(MicroVal) Method Reviewer
MVTC	MicroVal Technical Committee
NA	Negative Agreement
na	not applicable
ND	Negative Deviation
neg (-)	negative/no growth/no reaction/target not detected
NS	Non-Suspect growth
nt	not tested
PA	Positive Agreement
PD	Positive Deviation
pos (+)	positive/growth/target detected
PPNA	Presumptive Positive Negative Argreement (belongs to the False Positive results)
PPND	Presumptive Positive Negative Deviation (belongs to the False Positive results)
R(ef)	Reference method
RLOD	Relative Level of Detection
RT	Relative Trueness
S	Suspect growth
SE	Relative Sensitivity
SP	Relative Specificity
TP	True Positive

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of an alternative method for the detection of for the detection of Total Viable Count at a threshold of 10 cfu per g in a broad range of foods was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

The Total Viable Count (NF-TVC) vial (9 mL) is suitable for dilute-to-specification monitoring in a variety of food matrices. As organisms grow in the broth medium, the carbon dioxide (CO_2) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris® instrument.

The time to growth detection in the Soleris® system is correlated to the level of target organisms present in the sample, with higher levels of contamination having a shorter detection time. In this validation, the method was used to determine a defined threshold of product contamination of greater then 10 cfu per g. If successful, the alternative method would be used as a rapid screen for higher throughput product monitoring of a broad range of food products, reducing hands on analysis time as well as time to result.

The reference method used was:

ISO 4833-1:2013; Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30°C by the pour plate technique

Although the reference method is generally used to enumerate the level of total viable count, in this validation it was used to establish if levels of the target organism exceeded the defined detection threshold of 10 CFU per g. Thus, the best approach to this semi-quantitative validation was to use a Qualitative presence/absence approach, but to set the presence/absence limit at 10 CFU/g.

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

Categories included:

- Dairy (heat processed)
- Fishery products (raw and RTC)
- Fresh and processed produce
- Multicomponent foods or meal components
- Meat and poultry products (Raw and RTC)

Criteria evaluated during the study have been:

- Method Comparison Study (MCS)
  - Sensitivity study
  - Relative level of detection study
  - Inclusivity and exclusivity study

The **method comparison study conclusions** are:

- The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).
- The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for all categories tested.
- The alternative Neogen Soleris® NF-TVC detection method is selective and specific.

The **inter-laboratory study conclusions** are:

- The observed value for ND-PD is lower than the acceptability limits.
- There are no individual categories tested for the IL.
- The data and interpretations comply with the EN ISO 16140-2:2016 requirements.

This report corresponds to the method comparison study and gathers the observed data and interpretations according to the EN ISO 16140- 2:2016 standard and the MicroVal technical committee interpretations v2.5.

2 Method protocols

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

Although the Reference and Alternative methods were performed with the same test portion, the analysis does not share a common enrichment step, due to the requirement for pH adjustment in the alternative method. In addition, each method used in the study followed different principles. The Reference method detects growth of the target organisms in agar as part of a pour plate protocol. The Alternative method enables growth of the target organisms in a liquid medium to the set threshold of detection. Due to the differences in methodology, the validation was carried out as an unpaired study.

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method and the alternative method were done according to ISO 6887-series.

2.2 Alternative method

See the flow diagram of the alternative method in Annex B.

The alternative method principle is based on optical detection of microbial growth through the use of a medium containing dye indicators. As organisms grow in the broth medium, the carbon dioxide (CO_2) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris® instrument.

At defined time intervals during incubation, the soft agar plug at the bottom of the vial is monitored for colour changes which indicated growth of the target organism. Readings were collated by the software within the instrument that automatically analysed the data to provide the final results. An incubation time of 30 hours was selected as the incubation time for the test, although the Soleris® system was run for 48 hours to check for any slow growing strains. All samples with a detection time of < 30 hours were reported as detected, and any detection times over 30 hours have been reported as not detected.

Following incubation of the vial for the required time, positive vials were streaked to PCA and incubated for 24-72 hours at 30°C±1°C to check for the presence of colonies. The shortest incubation time of 24h used, unless otherwise specified. Confirmation was performed for the purpose of this certification study to verify postive results obtained in the vials, as opposed to a requirement of customers where confirmation is optional. Customers can perform confirmation for troubleshooting purposes. Streaking the vial on PCA can allow customers to isolate colonies for further identification.

2.3 Study design

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

Unless otherwise stated, sample preparations were done according to ISO 6887-series.

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

3 Method comparison study

3.1 Sensitivity Study

The sensitivity study (SE) is the ability of the method selected to detect the analyte by either the reference or the alternative method.

3.1.1 Categories and sample types
A total of 5 Categories were included in this validation study.
A minimum of 60 Items for each Category were tested by both the reference method and the alternative method in the sensitivity study, with a minimum of 30 positive samples per Category.
Each Category was made up of 3 Types, with at least 20 Items representative for that Type.

In the ideal situation, 10 samples (50 %) tested per type should be positive and 10 negative, but should range between 25 % and 75 %. For each category, at least 30 samples will have a positive result by the reference and/or the alternative method.

NF-TVC vial is a method for the detection of total viable count. The categories tested in the study were anticipated to be naturally contaminated with organisms capable of growing in a total viable count. During the study, a serial dilution series was prepared for each food item, and 3-4 dilutions within the dilution series were tested by both the reference and alternative methods. A single dilution (e.g. -6) was selected per category using pre-defined criteria to carry forward for statitical analysis. To enable compliance with the requirements of ISO 16140-2, a dilution that gave between 25%-75% positive samples for each type by the reference method for each sample type. The approach used in the current validation followed the study design for a previously certified TVC vial test, study reference 2021LR94.

On the occasion where two dilutions fulfil this criteria, the plate with the greater dilution factor was selected for the following reasons:

- To ensure that the correct number of positive and negative samples are achieved
- Higher dilutions have a lower count per plate (typically 1-10 cfu), which is closer to the limit of detection and is therefore a greater challenge for the method

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 (examples)	Preparations	Number of samples analysed
Pasteurised milk and dairy products	Pasteurised milk	Pasteurised milk	6887-5	20
	Pasteurised dairy products	Ice-cream, milk based drinks, cheese	6887-5	20

Category	Types	Items (examples)	Preparations	Number of samples analysed
	Dry milk products	Milk powder, dessert powder	6887-1, 6887-4	20
Raw Fishery products	Raw fish (unprocessed)	Raw salmon filet, tuna	6887-3	25
	Ready to cook	Fishcakes	6887-3	25
	Crustaceans	Shrimp, crab	6887-3	20
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	6887-4	20
	Fresh fruit/Cut RTE fruit	Cut fruits, freshly squeezed juice, smoothies	6887-4	20
	Heat treated fruit and vegetables	Pasteurised smoothies/juice, blanched frozen vegetables	6887-4	20
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, sandwiches	6887-2	20
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products,	6887-2	20
	Mayonnaise based deli-salads	Vegetable salad	6887-2	20
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	6887-2	20
	Raw processed meat	Frozen burger patties, pork meat balls, seasoned raw meat, lamb mince	6887-2	20
	RTC processed poultry	seasoned chicken, turkey meat balls	6887-2	20

310 samples were analyzed. The distribution of positive and negative samples per tested category and type is given respectively in Table 2.

Table 2 - Distribution per tested category and type

Category		Type		Number of positives*	Number of negatives
1	Milk and dairy products	a	Pasteurised milk	7	13
		b	Pasteurised dairy products	9	11
		c	Dry milk products	14	6
		Total		**30**	**30**
2	Fishery products (raw and RTC)	a	Raw fish (unprocessed)	16	9
		b	RTC fish	12	13
		c	Crustaceans	9	11
		Total		**37**	**33**
3	Fresh produce and fruits	a	Cut ready-to-eat vegetables/leafy greens and sprouts	13	7
		b	Fresh fruit/cut RTE fruit and vegetable products	14	6
		c	Heat treated fruit and vegetables	8	12
		Total		**35**	**25**
4	Multi-component foods or meal components	a	Composite foods with substantial raw ingredients	11	9
		b	RTRH/RTE foods (chilled, frozen)	13	7
		c	Mayonnaise based deli salads	7	13
		Total		**31**	**29**
5	Raw and ready to cook RTC meat and poultry	a	Raw poultry and meat cuts	9	11
		b	Raw processed meat	11	9
		c	RTC processed meat and poultry	14	6
		Total		**34**	**26**
Grand total				**167**	**143**

*Positive by at least one of the methods

3.1.2 Test sample preparation

100 % of the samples analysed in the study were naturally contaminated therefore artificial contaminations were not needed.

3.1.3 Confirmation protocols

Following 48 hours of incubation in the Soleris® instrument, each vial was streaked to PCA and incubated at 30±1°C for 24 hours to check for visible colonies.

3.1.4 Sensitivity study results

All raw data on the sensitivity study are given in Annex C.

Table 3 shows the summary of results of the reference method and the alternative methods for **all Categories**.

Table 4 shows the Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method).

Table 3 - Summary of sensitivity study results – *all categories*

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement (R+/A+) **PA = 134**	Positive deviation (R-/A+) **PD = 17**
Alternative method negative (A-)	Negative deviation (R+/A-) **ND = 18**	Negative agreement (R-/A-) **NA = 143**

Table 4 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method)

Category		**Type**		**PA**	**NA[1]**	**PD**	**ND[2]**	**PPNA[3]**	**PPND[3]**
1	**Milk and dairy products**	a	Pasteurised milk	7	13	0	0	0	0
		b	Pasteurised dairy products	8	11	1	0	0	0
		c	Dry milk products	14	6	0	0	0	0
		Total		**29**	**30**	**1**	**0**	**0**	**0**
2	**Fishery products (raw and RTC)**	a	Raw fish (unprocessed)	12	9	1	3	1	0
		b	RTC fish	10	13	0	2	0	0
		c	Crustaceans	4	11	3	2	0	0
		Total		**26**	**33**	**4**	**7**	**1**	**0**
3	**Fresh produce and fruits**	a	Cut ready-to-eat vegetables/leafy greens and sprouts	12	7	1	0	0	0
		b	Fresh fruit/cut RTE fruit and vegetable products	13	6	1	0	0	0
		c	Heat treated fruit and vegetables	2	12	1	5	0	0
		Total		**27**	**25**	**3**	**5**	**0**	**0**
4	**Multi-component foods or meal components**	a	Composite foods with substantial raw ingredients	6	9	2	3	0	0
		b	RTRH/RTE foods (chilled, frozen)	13	7	0	0	0	0
		c	Mayonnaise based deli salads	6	13	0	1	0	0
		Total		**25**	**29**	**2**	**4**	**0**	**0**

Category		Type		PA	NA[1]	PD	ND[2]	PPNA[3]	PPND[3]
5	Raw and ready to cook RTC meat and poultry	a	Raw poultry and meat cuts	7	11	1	1	0	0
		b	Raw processed meat	7	9	3	1	0	0
		c	RTC processed meat and poultry	13	6	1	0	0	0
		Total		**27**	**26**	**5**	**2**	**0**	**0**
Grand total				**134**	**143**	**15**	**18**	**1**	**0**

[1] NA: including PPNA, [2] ND: including PPND, [3] FP = PPNA + PPND

3.1.5 Sensitivity study calculations

The sensitivity study parameters as specified in Table 5 were calculated for all Categories and Types, and the overview is given in Table 6.

Table 5 – Formula to calculate the sensitivity parameters

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\%$
Relative trueness	$RT = \frac{(PA + NA)}{N} \times 100\%$
False positive ratio for the alternative method	$FPR = \frac{(FP)}{NA} \times 100\%$

Table 6 - Overview calculated sensitivity parameters per Category and Type

Category		Type		PA	NA	PD	ND	PPNA	PPND	Total	SE alt	SE ref	RT	FPR
1	Milk and dairy products	a	Pasteurised milk	7	13	0	0	0	0	20	100	100	100	0
		b	Pasteurised dairy products	8	11	1	0	0	0	20	100	100	100	0
		c	Dry milk products	14	6	0	0	0	0	20	100	100	100	0
		Total		**29**	**30**	**1**	**0**	**0**	**0**	**60**	**100**	**100**	**100**	**0**
2	Fishery products (raw and RTC)	a	Raw fish (unprocessed)	12	9	1	3	1	0	25	92	75	69	11
		b	RTC fish	10	13	0	2	0	0	25	100	87	87	0
		c	Crustaceans	4	11	3	2	0	0	20	100	85	88	0
		Total		**26**	**33**	**4**	**7**	**1**	**0**	**70**	**98**	**83**	**82**	**3**
3	Fresh produce and fruits	a	Cut ready-to-eat vegetables/leafy greens and sprouts	12	7	1	0	0	0	20	100	100	100	0
		b	Fresh fruit/cut RTE fruit and vegetable products	13	6	1	0	0	0	20	100	100	100	0
		c	Heat treated fruit and vegetables	2	12	1	5	0	0	20	100	71	72	0
		Total		**27**	**25**	**3**	**5**	**0**	**0**	**60**	**100**	**83**	**85**	**0**
4	Multi-component foods or meal components	a	Composite foods with substantial raw ingredients	6	9	2	3	0	0	20	100	75	79	0
		b	RTRH/RTE foods (chilled, frozen)	13	7	0	0	0	0	20	100	100	100	0
		c	Mayonnaise based deli salads	6	13	0	1	0	0	20	100	93	93	0
		Total		**25**	**29**	**2**	**4**	**0**	**0**	**60**	**100**	**88**	**89**	**0**
5	Raw and ready to cook RTC meat and poultry	a	Raw poultry and meat cuts	6	11	2	1	0	0	20	100	92	93	0
		b	Raw processed meat	7	9	3	1	0	0	20	100	90	92	0
		c	RTC processed meat and poultry	13	6	1	0	0	0	20	100	100	100	0
		Total		**26**	**26**	**6**	**2**	**0**	**0**	**60**	**100**	**93**	**94**	**0**
Grand total				**133**	**143**	**16**	**18**	**1**	**0**	**310**	**99**	**89**	**90**	**0.7**

[1] NA: including PPNA, [2] ND: including PPND, [3]FP = PPNA + PPND

3.1.6 Discordant results

Negative deviations are listed in Table 7.

Table 7 - Negative deviations

Category	Type	Sample	Sample no	Plate count for reference method
Fishery products (raw and RTC)	Raw fish (unprocessed)	Scottish tail salmon fillets	S42	1
Fishery products (raw and RTC)	Raw fish (unprocessed)	Sea bass fillets	T17	3
Fishery products (raw and RTC)	Raw fish (unprocessed)	Raw squid rings	F62	9
Fishery products (raw and RTC)	RTC fish	Creamy cod fishcakes	F7	2
Fishery products (raw and RTC)	RTC fish	Smoked haddock fishcakes	F13	6
Fishery products (raw and RTC)	Crustaceans	White crab 2	S5	1
Fishery products (raw and RTC)	Crustaceans	White & Brown Crab	S6	1
Fresh produce and fruits	Heat treated fruit and vegetables	Orange juice	T42	1
Fresh produce and fruits	Heat treated fruit and vegetables	Mango baby food	P46	1
Fresh produce and fruits	Heat treated fruit and vegetables	Apple, mango and banana baby food	P47	1
Fresh produce and fruits	Heat treated fruit and vegetables	Pear baby food	P49	1
Fresh produce and fruits	Heat treated fruit and vegetables	V8 vegetable juice	P60	2
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Cheese and onion sandwich	T50	2
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Tomato and basil pasta salad	C18	4
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Honey and mustard chicken pasta	C19	5
Multi-component foods or meal components	Mayonnaise based deli salads	Chicken caesar salad	T58	2
Raw and ready to cook RTC meat and poultry	Raw poultry and meat cuts	Fresh British turkey breast mini fillets	R10	1
Raw and ready to cook RTC meat and poultry	Raw processed meat	Pork shoulder in bbq sauce	T7	2

Positive deviations are listed in Table 8.

Table 8 - Positive deviations

Category	Type	Sample	Sample nº
Fishery products (raw and RTC)	Raw fish (unprocessed)	Salmon fillet	S41
Fishery products (raw and RTC)	Crustaceans	Cold water large prawns	T26
Fishery products (raw and RTC)	Crustaceans	White crab	S1
Fishery products (raw and RTC)	Crustaceans	Premium crab meat	S4
Fresh produce and fruits	Cut ready-to-eat vegetables/leafy greens and sprouts	Rocket salad	P20
Fresh produce and fruits	Fresh fruit/cut RTE fruit and vegetable products	Mango	P29
Fresh produce and fruits	Fresh fruit/cut RTE fruit and vegetable products	Tropical juice	P57
Multicomponent foods or meal components	Composite foods with substantial raw ingredients	Cheese and pickle sandwich	C13
Multi-component foods or meal components	Composite foods with substantial raw ingredients	tesco chicken and chorizo pasta salad	C14
Raw and ready to cook RTC meat and poultry	Raw poultry and meat cuts	Turkey thigh mince 7% fat	T4
Raw and ready to cook RTC meat and poultry	Raw processed meat	Fire pit sweet and smoky beef kebabs	T8
Raw and ready to cook RTC meat and poultry	Raw processed meat	Quarter pounder burgers	R37
Raw and ready to cook RTC meat and poultry	Raw processed meat	Venison burgers	R40
Raw and ready to cook RTC meat and poultry	RTC processed meat and poultry	British beef quarter pounders	R58
Milk and dairy products	Pasteurised dairy products	Soured cream	T67

There were 18 negative deviations, and 15 positive deviations observed in the sensitivity study. Further analysis revealed that the negative deviations were seen in 4 out of the 5 categories tested. Statistical analysis was performed to check the potential possibility of a link between the dilution used and the deviations obtained. The results demonstrated that there was no significant difference between the average dilutions available between negative and positive deviations (P=0.4). For all negative deviations, the level obtained on the reference method is between 1-9 cfu/plate. The method shows equivalent performance to the reference method at low levels in the RLOD portion of the study.

The ND-PD values were highest in the multicomponent foods and produce and fruits categories. Similar levels of negative and positive deviations were noted in the other three categories tested.

Most of the negative deviations seen in the produce and fruits category were obtained with heat processed fruit and vegetable samples. Vegetable juice was also tested in the RLOD study and achieved an RLOD of 0.640. The RLOD value for the vegetable juice was below the AL of 2.5 for an unpaired study and does not indicate systematic bias with the sample type. One possible explanation for the deviations could be the prescence of moulds, as mould colonies were observed in several heat processed fruit and vegetable samples tested. All 5 yeast and mould strains analysed in the inclusivity study were detected within 30 hours, although the incubation time and temperature is not optimised for yeasts and moulds. It is recommended to use a test specific to yeast and moulds if only yeast and moulds are expected in the samples to be analysed.

There were 11 samples (10 PA, 1 PD) that required the incubation time of the confirmation plate to be increased from 24 hours to 72 hours. These samples are identified in the raw data, and are from the fishery product category. One possible explanation for this is the organisms present in these samples are slower growing.

The analysis of discordant results according to ISO 16140-2:2016 for an unpaired study is given in Table 9.

Table 9 – Interpretation of the sensitivity study results (unpaired study)

Category	**Negative Deviations (ND[1])**	**Positive Deviations (PD)**	**ND-PD**	**Acceptability Limit (AL)**
Milk and dairy products	0	1	-1	3
Fishery products (raw and RTC)	7	4	3	3
Produce and fruits (combined fresh and heat processed)	5	3	2	3
Multi-component foods or meal components	4	2	2	3
Raw and ready to cook RTC meat and poultry	2	5	-3	3
Total	**18**	**15**	3	**5**

[1] ND: including PPND

3.1.7 Conclusion sensitivity study

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).

3.2 Relative level of detection study

The relative level of detection is the level of detection at P = 0,50 (LOD_{50}) of the alternative method divided by the level of detection at P = 0,50 (LOD_{50}) of the reference method.

3.2.1 Categories, sample types and strains

One sample type and one relevant target micro-organism for this sample type was chosen for each of the Categories in this validation study, as shown in Table 10. Canned products were selected for the RLOD study to enable a consistent low level of artificial contamination to be achieved.

Table 10 - List of selected types and strains per category, as tested within the relative level of detection study.

Category	Type	Item	Strain	Seeding or spiking procedure	Log injury
Dairy products (heat processed)	Dry dairy products	Milk powder	*Bacillus cereus* CRA 1724 isolated from Dried milk	Seeding – inoculated with a lyophilized culture and stored for >2 weeks at ambient	N/A
Fishery products (raw and RTC)	Canned ambient stable fish	Tuna Chunks in Sunflower Oil	*Pseudomonas fragi* CRA7222 Isolated from spoiled fish	Spiking – culture heat stressed at 55°C for 15 minutes	0.57
Produce and fruits (combined category fresh and processed)	Heat processed fruit and vegetables	Vegetable juice	*E.coli* CRA3379 isolated from spinach	Spiking – culture heat stressed at 55°C for 15 minutes	0.28
Raw and RTC Meat and poultry (Combined category)	Canned ambient stable meat	Chopped Ham & Pork	*Staphylococcus warneri* CRA 3198 Isolated from dry sausage	Spiking – culture heat stressed at 55°C for 15 minutes	0.77
Multicomponent foods or meal components	Ready to (re)heat food: ambient stable (canned)	Spaghetti hoops	*Hafnia alvei* CRA7417 isolated from pate	Spiking – culture heat stressed at 55°C for 15 minutes	0.65

3.2.2 Test sample preparations
Three levels of artificial contamination were prepared for each type:

- Negative control level: One non-inoculated in order to get 5 test portions,
- Low level: One inoculated between 8 and 12 CFU/g in order to get 20 test portions providing fractional recovery,
- Higher level: One inoculated between 15 and 25 CFU/sample in order to get 5 test portions contaminated at a higher level.

3.2.3 RLOD study results
The tabulated raw data on the RLOD study are given in Annex D.

The RLOD calculations were performed using the Excel spread sheet (version 3 15-08-2015) of the international standard as described in ISO 16140-2: 2016.

The RLOD per Category is given in Table 11.

Table 11 – Presentation of RLOD before and after confirmation of the alternative method results

Type (Category)	**RLOD using the alternative method results**	**RLOD using the confirmed alternative method results**
Dairy products (heat processed)	0.838	0.838
Fishery products (raw and RTC)	1.000	1.513
Produce and fruits (combined category fresh and processed)	0.640	0.640
Raw and RTC Meat and poultry	0.456	0.456
Multicomponent foods or meal components	1.161	1.161
Combined	**0.793**	**0.854**

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, 2.5 for unpaired studies, for all categories tested.

In addition, LOD50 values were calculated using the equations quoted in Wilrich and Wilrich (2009) Journal of AOAC International 92 (6) 1763-1772 downloaded from

www.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich.index.html

The LOD50 per Category is given in Tables 12 and 13.

Table 12 – LOD_{50} calculation for presumptive results

Results of the PODLOD calculations - with the LOD relating to d in cfu / g											
Matrix		Matrix effect	Log matrix effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu / g			$LOD_{95\%}$ = 95% limit of detection in cfu / g			Test statistic matrix effect
No. i	Designation Matrix$_i$	F_i	f_i	s_{fi}	Detection limit $d_{0.5,i}$	Lower conf. limit $d_{0.5,i,L}$	Upper conf. limit $d_{0.5,i,U}$	Detection limit $d_{0.95,i}$	Lower conf. limit $d_{0.95,i,L}$	Upper conf. limit $d_{0.95,i,U}$	$\lvert z_i \rvert$
1	Dairy	0.010	-4.567	0.259	6.671	3.977	11.191	28.831	17.186	48.366	2.9E-21
2	Fishery products	0.012	-4.399	0.256	5.638	3.382	9.400	24.367	14.616	40.625	2.1E-23
3	Vegetable juice	0.013	-4.317	0.257	5.194	3.110	8.676	22.449	13.439	37.497	2.8E-21
4	Meat and poultry	0.008	-4.772	0.263	8.192	4.842	13.860	35.405	20.926	59.901	3.1E-21
5	Multicomponent foods	0.021	-3.860	0.286	3.289	1.856	5.828	14.213	8.020	25.190	2.5E-21
Combined results		0.012	-4.413	0.115	5.717	4.543	7.194	24.708	19.636	31.091	5.6E-21
		The combined results are based on the data of matrices 1, 2, 3, 4 and 5									

Table 13 - LOD_{50} calculation for confirmed results

Results of the PODLOD calculations - with the LOD relating to d in cfu / g											
Matrix		Matrix effect	Log matrix effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu / g			$LOD_{95\%}$ = 95% limit of detection in cfu / g			Test statistic matrix effect
No. i	Designation Matrix$_i$	F_i	f_i	s_{fi}	Detection limit $d_{0.5,i}$	Lower conf. limit $d_{0.5,i,L}$	Upper conf. limit $d_{0.5,i,U}$	Detection limit $d_{0.95,i}$	Lower conf. limit $d_{0.95,i,L}$	Upper conf. limit $d_{0.95,i,U}$	$\lvert z_i \rvert$
1	Dairy	0.010	-4.567	0.259	6.671	3.977	11.191	28.831	17.186	48.366	2.9E-21
2	Fishery products	0.012	-4.399	0.256	5.638	3.382	9.400	24.367	14.616	40.625	2.1E-23
3	Vegetable juice	0.009	-4.668	0.260	7.384	4.393	12.411	31.912	18.986	53.639	3.0E-21
4	Meat and poultry	0.008	-4.772	0.263	8.192	4.842	13.860	35.405	20.926	59.901	3.1E-21
5	Multicomponent foods	0.021	-3.860	0.286	3.289	1.856	5.828	14.213	8.020	25.190	2.5E-21
Combined results		0.011	-4.484	0.115	6.142	4.881	7.728	26.544	21.094	33.402	5.7E-21
		The combined results are based on the data of matrices 1, 2, 3, 4 and 5									

3.2.4 Conclusion RLOD study

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, 2.5 for unpaired studies, for all categories tested.

3.3 Inclusivity/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.

3.3.1 Protocols

Inclusivity: 50 strains of target microorganisms (bacteria, yeasts and moulds) able to grow in aerobic conditions at 30°C were freshly cultured in an appropriate non-selective broth at 30°C. Dilutions were made in order for the vials to be inoculated at 10-100 times greater than the minimal level of detection (10-100 cfu in vial). The alternative method protocol was then performed (no sample material was added).

No exclusivity study was carried out for this method, as the vial detects all organisms capable of growing in an aerobic plate count, hence there are no non-target organisms.

3.3.2 Results inclusivity and exclusivity study
All raw data on inclusivity and exclusivity are given in Annex E.

A total of 50 strains were tested for **inclusivity**. All 50 isolates showed the expected positive result with detection times ranging from 10.9 to 29.6 hours.

3.3.3 Conclusion inclusivity and exclusivity study
The alternative Neogen Soleris® NF-TVC detection method is selective and specific.

4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for all categories tested.

The alternative Neogen Soleris® NF-TVC detection method is selective and specific.

5 Interlaboratory study

The interlaboratory 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.

5.1 Study organisation

Collaborators number

Samples were sent to 14 participants from 9 laboratories. The participants were located in seven countries: Switzerland, Spain, Netherlands, Ireland, France, United States and England. The participant details are shown in Annex F.

In addition to the 14 external participants, an internal participant from the analytical services team at Campden BRI, also took part in the study. This participant is in addition to the expert laboratory participant who was unfamiliar with the alternative method.

Matrix and strain used

A UHT soya-based dessert was inoculated with *Staphylococcus saprophyticus* CRA 314 isolated from a factory environment.

Samples

For each participant, 24 blind coded samples were prepared for detection of total viable count analysis by the reference and alternative methods.

Samples were prepared and inoculated on Monday 10th June 2024.

Inoculation

The targeted inoculation levels were the following:

- Level 0: 0 CFU/ g,
- Level 1: 11 CFU/ g, inoculation level providing as much as possible fractional positive recovery data;
- Level 2: 30 CFU/g

Following inoculation, samples were stored frozen for 24h before shipment to the participants.

Labelling and shipping

Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories taking part in the study.

A frozen water vial was added to the package in order for participants to record the temperature on arrival.

A temperature probe 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 48h to the involved laboratories. The temperature conditions had to stay lower or equal to 1°C during transport and sample storage in the labs.

Analyses

Collaborative study laboratories and the expert laboratory carried out the analyses on 17th June 2024 with the alternative and reference methods. The analyses by the reference method and the alternative method were performed on the same day.

5.2 Experimental parameters controls

5.2.1 Detection of total viable count in the matrix before inoculation
To detect the presence of total viable count the reference method was performed on eight portions (10 g) before the inoculation. All the results were negative indicating no background contamination in the samples.

5.2.2 Strain stability during transport
To test the stability of the strain in the matrix. 500cfu/g was inoculated into samples and tested after 7 days of storage, at two storage protocols, that mimic the conditions of storage and transit.

Storage protocol 1 – 24h frozen, 48h in temperature-controlled storage box, 4 days frozen

Storage protocol 2 – 24h chilled, 48h in temperature-controlled storage box, 4 days chilled

Table 14 – Total viable count stability in the matrix

Day	**Reference method (cfu/g)**	
	Replicate 1	**Replicate 2**
Day 0	260	150
Storage protocol 1 (Day 7)	100	40
Storage protocol 2 (Day 7)	70	80

The results show that there was a slight die-off after storage for 7 days.

Samples were then inoculated at lower levels to determine the detection of both methods before and after storage, the results are shown in Table 15.

Table 15 – Stability results

Day	Reference method						Alternative method					
	0cfu/g		10 cfu/g		25cfu/g		0cfu/g		10 cfu/g		25cfu/g	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Day 0	-	-	-	+	+	+	-	-	+	-	+	+
Storage protocol 1 (day 7)	-	-	+	-	+	+	-	-	-	-	+	+
Storage protocol 2 (day 7)	+	+	+	+	+	-	-	-	+	+	+	+

The results show fractional positives with both methods at day 7. Storage protocol 1 was used as it appears to reduce the risk of positive uninoculated samples.

5.2.3 Logistic conditions

The temperatures measured at reception by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 16.

Table 16 - Sample temperatures at receipt

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	State of the package and samples at the receipt	Analysis date
1	Sample did not arrive				
2	-0.1	Water control damaged	13/6/2024 14:20	Ok – except for water control	17/6/2024

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	State of the package and samples at the receipt	Analysis date
3A	-0.1	N/A – frozen water control	14/6/2024 14:00	Ok	17/6/2024
3B	0.9	N/A – frozen water control	14/6/2024 14:00	Ok	17/6/2024
4	-1.1	N/A – frozen water control	13/6/2024 Not listed	Ok	24/6/2024
5A	-4.6	N/A – frozen water control	13/6/2024 14:00	Ok	17/6/2024
5B	-1.1	N/A – frozen water control	13/6/2024 14:00	Ok	17/6/2024
6A	-3.0	N/A – frozen water control	13/6/2024 14:00	Ok	17/6/2024
6B	-1.6	N/A – frozen water control	13/6/2024 14:00	Ok	17/6/2024
7	-1.0	3°C	13/6/2024 10:00	Ok	17/6/2024
8A	-1.4	N/A – frozen water control	13/6/2024 14:50	Ok	17/6/2024
8B	-1.4	N/A – frozen water control	13/6/2024 14:50	Ok	17/6/2024
9A	Data not provided – test not completed				
9B	Data not provided – test not completed				

Participant 1 did not receive the samples due to customs issues. Participant 9A + 9B informed us that the samples were stored ambient on arrival and did not complete the testing.

Participant 4 received the samples on time, and stored them correctly, however, the set-up day was 7 days later than the other participants. This is outside the scope of the stability trials.

For the remainder of the participants, no problem was encountered during the transport or at receipt. Temperatures during shipment and at receipt were all correct.

5.3 Calculation and summary of data

5.3.1 MicroVal Expert laboratory results
The results obtained by the expert laboratory are given in Table 17.

Table 17 – Results obtained by the expert lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	6/8	6/8
L2	8/8	8/8

5.3.2 Results obtained by the collaborative laboratories

- Total Viable Count *detection*

12 out of 15 participants returned data for the study. The results obtained by the individual collaborators in the inter-laboratory study are summarised in Table 18 (reference method) and Table 19 (alternative method).

Table 18 - Positive results by the reference method (ALL the collaborators)

Participant	**Contamination level**		
	L0	**L1**	**L2**
2	0/8	6/8	8/8
3A	2/8	6/8	8/8
3B	1/8	7/8	8/8
4	5/8	2/8	4/8
5A	3/8	6/8	8/8
5B	2/8	5/8	8/8

Participant	Contamination level		
	L0	**L1**	**L2**
6A	0/8	6/8	8/8
6B	0/8	6/8	8/8
7	0/8	6/8	8/8
8A	0/8	6/8	8/8
8B	0/8	6/8	8/8
10	0/8	6/8	8/8
TOTAL	**P_0 = 13/96**	**P_1 = 69/96**	**P_2 =92/96**

Table 19 - Positive results (before and after confirmation) by the alternative methods (ALL the collaborators)

Participant	Contamination level					
	L0		***L1***		***L2***	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
2	0/8	0/8	6/8	6/8	8/8	8/8
3A	0/8	0/8	6/8	6/8	8/8	8/8
3B	0/8	0/8	6/8	6/8	8/8	8/8
4	1/8	1/8	6/8	6/8	8/8	8/8
5A	0/8	0/8	6/8	6/8	8/8	8/8
5B	0/8	0/8	6/8	6/8	8/8	8/8
6A	0/8	0/8	6/8	6/8	8/8	8/8

Participant	Contamination level					
	L0		***L1***		***L2***	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
6B	0/8	0/8	6/8	6/8	8/8	8/8
7	0/8	2/8	0/8	6/8	0/8	8/8
8A	0/8	0/8	6/8	6/8	8/8	8/8
8B	0/8	0/8	6/8	6/8	8/8	8/8
10	0/8	0/8	6/8	6/8	8/8	8/8
TOTAL	**P_0 = 1/96**	**CP_0 = 3/8**	**P_1 = 66/96**	**CP_1 = 72/96**	**P_2 = 88/96**	**CP_2 = 96/96**

Analysis of the data revealed issues were experienced in sample analysis for 2 of the participants (4 and 7) and these are detailed below.

Participant 4

It was noted that participant 4 stored their samples frozen for 2 weeks instead of 1 week stated in the ILS instructions. A 2-week storage time is beyond the scope of the stability trial which was carried out for 1 week. Data analysis showed that storage has potential impact on reference method. The number of positives for each method is shown below:

Level of contamination	Number of positives reference method	Number of positives alternative method
Level 1	2	6
Level 2	4	8

There are 5 positive blank samples for the reference method and 1 positive blank sample for the alternative method. Further investigations on the identification of the positive blank samples were inconclusive.

Due to the results of the investigations and the potential impact on the reference method, the results for participant 4 have been excluded from the analysis.

Participant 7

There was no detection time recorded with the Soleris instrument for any samples tested by participant 7. On visual inspection, the vials showed visible signs of growth (e.g. change of plug colour, turbidity in broth contained in the vial). In addition, the vials were streaked and fractional positive confirmation results were received.

Further investigation found that the graphs were not plotting, which indicated that the instrument was not recording measurements. The instrument was out of calibration and using a previous software version.

Due to the instrument issues, the results for participant 7 were not taken forward for the analysis.

Positive blank samples

For the remaining 10 participants in the study, four participants had up to 3 positive samples for the reference method. This translates to 10% positive results for the blank samples tested by the 10 participants taken forward for the analysis. Further investigations were carried out by the participants, including MALDI-ToF confirmations and/or images of the plates. Based on the results of these investigations, all remaining 10 participants have been included in the statistical analysis.

Conclusion

Participants 4 and 7 have been excluded. The remaining labs with positive blank samples have been retained due to the results of the investigations. Further information on these topics are included in the root cause analysis for reference.

5.3.3 Results of the collaborators retained for interpretation
The results obtained with 10 collaborators kept for interpretation are presented in Table 20 (reference method) and Table 21 (alternative method).

Table 20 - Positive results by the reference method (without participant 4 and 7)

Participant	Contamination level		
	L0	L1	L2
2	0/8	6/8	8/8
3A	2/8	6/8	8/8
3B	1/8	7/8	8/8
5A	3/8	6/8	8/8

Participant	Contamination level		
	L0	L1	L2
5B	2/8	6/8	8/8
6A	0/8	6/8	8/8
6B	0/8	6/8	8/8
8A	0/8	6/8	8/8
8B	0/8	6/8	8/8
10	0/8	6/8	8/8
TOTAL	**P_0 = 8/80**	**P_1 = 60/80**	**P_2 = 80/80**

Table 21 - Positive results (before and after confirmation) by the alternative methods (without participant 4 and 7)

Collaborator	Contamination level					
	L0		***L1***		***L2***	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
2	0/8	0/8	6/8	6/8	8/8	8/8
3A	0/8	0/8	6/8	6/8	8/8	8/8
3B	0/8	0/8	6/8	6/8	8/8	8/8
5A	0/8	0/8	6/8	6/8	8/8	8/8
5B	0/8	0/8	6/8	6/8	8/8	8/8
6A	0/8	0/8	6/8	6/8	8/8	8/8
6B	0/8	0/8	6/8	6/8	8/8	8/8
8A	0/8	0/8	6/8	6/8	8/8	8/8

Collaborator	Contamination level					
	L0		***L1***		***L2***	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
8B	0/8	0/8	6/8	6/8	8/8	8/8
10	0/8	0/8	6/8	6/8	8/8	8/8
TOTAL	**P_0 = 0/8**	**CP_0 =0/8**	**P_1 = 60/80**	**CP_1 = 60/80**	**P_2 =80/80**	**CP_2 = 80/80**

5.3.4 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are the following (See Table 22).

Table 22 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	90%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	100%

N - number of all L0 tests

P_0 - total number of false-positive results obtained with the blank samples before confirmation

CP_0 - total number of false-positive results obtained with the blank samples

5.3.5 Calculation of the sensitivity (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (L1).

A summary of the results of the collaborators retained for interpretation and obtained with the reference and the alternative methods for Level 1 is provided in Table 23.

Table 23 - Summary of the obtained results with the reference method and the alternative method for Level 1 and/or Level 2

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 60**	Positive deviation (R-/A+) **PD = 0**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 1**	Negative agreement (A-/R-) **NA = 19**

Based on the data summarized in Table 10, the values of sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account the confirmations, are the following (See Table 24).

Table 24 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \; x \; 100\% =$	98.4
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \; x \; 100\% =$	100
Relative trueness	$RT = \frac{(PA+NA)}{N} \; x \; 100\% =$	98.75
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \; x \; 100\% =$	0

5.3.6 Interpretation of data

The negative deviations are listed in Table 25.

Table 25 - Negative deviations for Level 1

Sample nº	Reference method result	Alternative method results	Inoculation (CFU/ml)
Participant 3B	+	ND	11

For an **unpaired study design**, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) shall not be higher than the AL. The AL is defined as $[(\text{ND} - \text{PD})_{max}]$ and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{\text{ref}} = \frac{P_x}{N_x}$$

where

P_x = number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all the collaborators

N_x = number of samples tested at level x (L_1 or L_2) with the reference method by all the collaborators

$$(p+)_{\text{alt}} = \frac{CP_x}{N_x}$$

where

CP_x = number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all the collaborators;

N_x = number of samples tested at level x (L_1 or L_2) with the alternative method by all the collaborators.

$$(\text{ND-PD})_{\text{max}} = \sqrt{3N_x \times \left((p+)_{\text{ref}} + (p+)_{\text{alt}} - 2\left((p+)_{\text{ref}} \times (p+)_{\text{alt}}\right)\right)}$$

where

N_x = number of samples tested for level x (L_1 or L_2) with the reference method by all the collaborators.

In this study, fractional recovery was observed at Level 1. The calculations, according to the EN ISO 16140-2:2016, are shown in Table 26.

Table 26 - Calculations

	Level 1
N_X	80
$(p+)_{ref}$	0.76
$(p+)_{alt}$	0.75
AL = (ND - PD) max	18
ND - PD	1
Conclusion	**Pass**

The ISO 16140-2 (2016) requirements are fulfilled as (ND - PD) is below the Acceptability Limit.

5.3.7 Evaluation of the RLOD between laboratories

The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 06.07.2015. The results are used only for information (see Table 1).

Table 1 - RLOD

Results by method									
Method	Method effect F_i	Log method effect f_i	SD of log method effect s_{fi}	$LOD_{50\%}$ = 50% limit of detection in cfu per sample size			$LOD_{95\%}$ = 95% limit of detection in cfu per sample size		
				Detection limit $d_{0.5,i}$	Lower conf. limit $d_{0.5,i,L}$	Upper conf. limit $d_{0.5,i,U}$	Detection limit $d_{0.95,i}$	Lower conf. limit $d_{0.95,i,L}$	Upper conf. limit $d_{0.95,i,U}$
Reference	0.143	-1.945	0.118	4.85	3.83	6.13	20.95	16.56	26.51
Alternative	0.139	-1.973	0.117	4.98	3.95	6.29	21.54	17.06	27.20
Conclusions	The methods are not significantly different at the 5% significance level (change in deviance of the model with method effects to the null model D_{method} = 0.03 with 1 degree of freedom, p-value 0.86).								
	The relative limit of detection (RLOD) of the alternative method, as compared to the reference method, is 1.03 with a 90% confidence interval of 0.78 - 1.35.								

5.4 Root cause analysis – interlaboratory study

A root cause analysis was carried out to investigate issues that occurred during the ILS. The two issues included in the root cause analysis were the number of external study participants and the positive blank samples obtained with the reference method.

5.4.1 Recruitment of Participants

There was difficulty in recruiting the required number of participants for the ILS due to the nature of the method (i.e. instrument-based). The study was arranged on two previous occasions however they were cancelled due to a lack of participants.

On this occasion, 14 external participants were recruited for the study included in the current report. The parcels for 13 out of 14 participants arrived in time and 2 participants did not complete the testing. As a result, 11 external datasets were received from the 14 participants recruited for the ILS.

From the 11 external datasets that were received, there were 2 datasets that required further investigation.

Participant 7

No detection time was recorded with the Soleris instrument for any samples analysed in the ILS. However, the vials were streaked, and fractional positive confirmation results were received for level 1. The vials also showed visible signs of growth (e.g. change of plug colour, turbidity in broth, as shown in Figure 1.

Further investigation found that the graphs were not plotting, indicating that the instrument was not recording measurements. The instrument was out of calibration and was using a previous software version. All other participants in the study were using the same software version.

Due to the instrument issues, the results for participant 7 were excluded from the analysis.

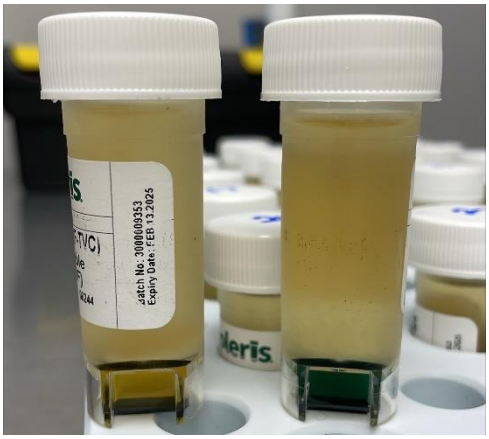


Figure 1 - Image taken by participant showing (left) positive vial and (right) negative vial

Participant 4

It was noted that participant 4 stored their samples frozen for 2 weeks instead of 1 week stated in the ILS instructions. The impact of the increased storage time was further investigated below

The plan for preparation, storage and testing of the samples was as follows:

Monday 10th June	Tuesday 11th June	Wednesday 12th June – Thursday 13th June	Monday 17th June
Samples were inoculated, then frozen	Samples were packaged and shipped	Samples arrived in participating laboratories. Participants froze the samples until Analysis.	Participants defrosted under controlled conditions and began testing.

Participant 7 stored the samples for an additional week before testing on Monday 24th June which was beyond the scope of the stability trial. The storage had a potential impact on reference method, the number of positives for each method is shown below:

- L1 2/8 ref, 6/8 alt
- L2 4/8 ref, 8/8 alt

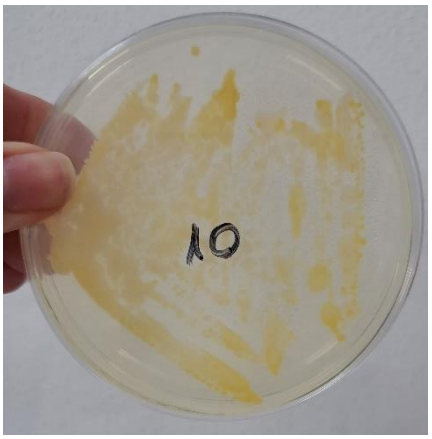


Figure 2 – Alternative confirmation plate for Participant 7, sample 10 which shows contamination of a blank sample with colony morphology similar to that of the inoculating strain

In addition to the potential stability issue, there were 5 positive blank samples for the reference method, and 1 positive blank sample for the alternative method. The participant provided images of the reference plates and alternative confirmation plates. The images were analysed by the expert laboratory for colony morphology. Figure 2 shows the positive blank sample for the alternative method, which has similar morphology to the inoculating strain *(Staphylococcus saprophyticus)*. The investigation carried out on the images provided by the participant were inconclusive, however it indicates that there could have been contamination between inoculated and uninoculated samples.

Due to the results of the investigations and the potential impact of additional storage on the reference method, the results for participant 4 have been excluded.

Use of an internal participant

Due to the reduction in datasets received compared to the number of participants that were recruited and the exclusion of 2 datasets, there were 9 external datasets remaining for analysis. The minimum number of datasets required for the analysis is 10.

In addition to 14 external participants, an internal participant from the analytical services team at Campden BRI, also took part in the study. This participant was in addition to the expert laboratory participant. The internal participant was not involved in the preparation of samples and had not performed the method before the interlaboratory study therefore could be considered to be an independent participant.

The decision-making process to include the internal dataset for the analysis was guided by the relevant definitions within ISO16140-1 (2016) and ISO 16140-2 (2016) concerning the organisation of the ILS and the participants taking part in the study.

In section 2.13 of ISO16140-1 (2016), a collaborator is defined as an individual laboratory technician who works completely independently for the other collaborators using a different set of blind samples or test portions.

The organising laboratory is defined in section 2.45 of ISO16140-1 (2106), as an expert independent laboratory with the responsibility for managing all of the technical and statistical analysis involved in the validation study including the method comparison study and interlaboratory study.

Section 5.2.2 of ISO 16140-2 (2016) outlines the measurement protocol of the ILS, which states that that the technicians involved in the preparation of the samples used in the ILS shall not take part in the testing of those samples within the interlaboratory study

During the study, the samples analysed at the organising laboratory were tested by a technician who had not been involved in the preparation of the samples. As this set of samples were analysed by a technician who was not responsible for the preparation of the samples or the data or statistical analysis of the samples, it is proposed that this data set could be considered as independent from the other collaborators.

The statistical analysis was performed on 9 external datasets and 1 internal dataset performed by analytical services.

The expert laboratory dataset was not included in the analysis.

5.4.2 Positive blank samples

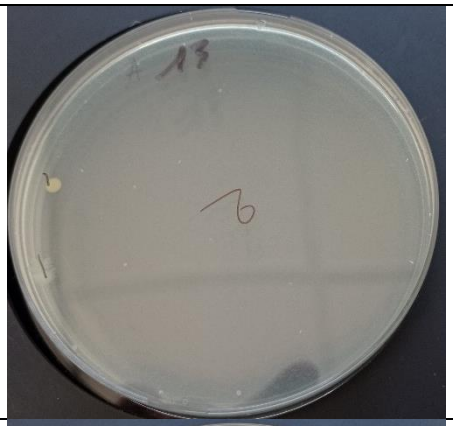
Of the 10 datasets included in the analysis, 4 participants had up to 3 blank samples with a positive result. 8 out of 80 uninoculated samples were positive for the reference method only. The number of positive blank samples per participant is shown in Table 28.

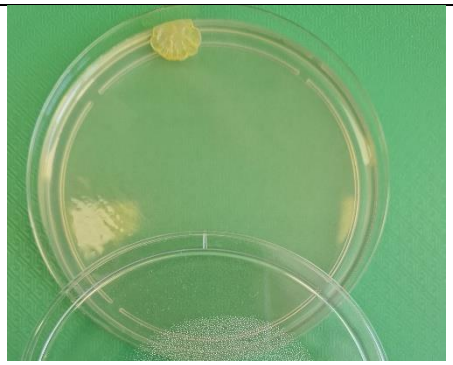
Table 28 – Number of positive uninoculated samples obtained by each participant

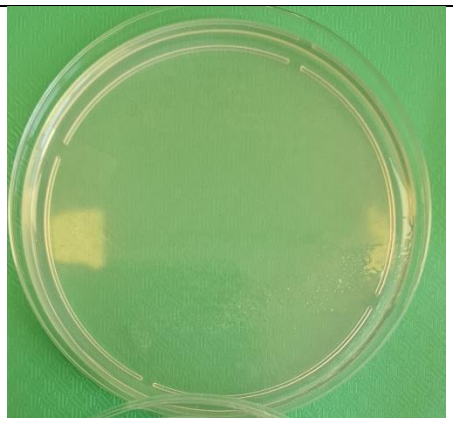
Participant	Contamination level
	L0
2	0/8
3A	2/8
3B	1/8
5A	3/8
5B	2/8
6A	0/8
6B	0/8
8A	0/8
8B	0/8
10	0/8
TOTAL	**P_0 = 8/80**

Further investigations were carried out for participants with positive blank samples. Participants with positive blank samples were asked to take images of plates and carry out MALDI-ToF confirmations, if available. The results of the investigations are shown in Table 29.

Table 29 – Investigation of positive blank samples

Participant	Sample	Count on plate	Image of reference plate taken by the participant	MALDI-ToF result
3A	13	2		Colony A - *Bacillus cereus* 2.23 Colony B – *Corynebacterium mucifaciens* 1.98 (lower confidence score)
	22	2		Colony A – *Bacillus cereus* 2.3 Colony B – *Bacillus cereus* 2.3
3B	1	2		Colony A – *Bacillus cereus* 2.3 Colony B – *Bacillus cereus* 2.11

5A	1	1		N/A
	13	1		N/A
	16	1		N/A

5B	7	11 (small)		N/A
	10	2		N/A

The investigations showed minimal contamination on each plate for the reference method. The colony morphologies/identifications obtained indicate that these samples were not contaminated with the inoculating strain. In conclusion, the blank samples with non target organsims present were retained for statistical analysis as they were considered to be a not detected result.

Root cause analysis – positive blank samples

The scope of the alternative method is the detection of total viable count. Positive blank samples were obtained with the reference method only. For each sample, 1ml of the 1 in 10 dilution was plated on 1x petri dish for the reference method.

Some potential causes for the positive blank samples are listed in Table 30.

Table 30 – Potential root causes for positive blank samples

Potential Cause	Reasons for/against potential cause	Likeliness of potential cause to produce positive blank samples
Competency/familarity of reference method	Participants were asked to complete a survey on how frequently they perform the reference method. 2/4 affected participants have completed the survey and responded that they are performing the reference method on a daily/weekly basis. The results of the remaining participants will be presented at the MVTC.	Unlikely
Sterility of media	1 participant found 1 bottle of PCA that was visibly contaminated. The contaminated bottle was not used in the study, although there is possibility that more bottles could have been contaminated. The occurance of positive blank samples was limited to 2 laboratories.	Likely
Handling of samples	Images/confirmations show that the contamination observed in the positive blank samples is different to the inoculating strain. This indicates that inoculated and uninoculated samples have not been contaminated with each other.	Unlikely
Aseptic technique	Images show colonies on the surface of the plate, this indicates that the samples could have been contaminated after the sample was plated e.g. when pouring media. The occurance of positive blank samples was limited to 2 laboratories.	Possible – when pouring media

Sample was naturally contaminated	The sample selected is a UHT product, and therefore less likely to be naturally contaminated. Natural contamination was not observed in screening and the stability study. Samples were prepared to minimise potential contamination: • Weighed in a laminar flow • Uninoculated samples were frozen immediately after weighing • Inoculated samples were weighed on the day of inoculation Images from the participants indicate that contamination was typically on the surface of the plate, this does not indicate natural contamination of the sample.	Unlikely

Two other validation studies involving the detection of low levels of total viable count have been completed, 2021LR100 NF105 Commercial Sterility vials and 2021LR94 Certablue TVC vials. False positive blank samples were also found during the interlaboratory study for both validation studies. Both of these studies were accepted by the MVTC following review of the results and potential causes of the positve blank samples. For the Certablue TVC vials, the contamination was found in the alternative method only. As a result of discussions at the MVTC, a warning was added to the kit insert to emphasize the risk of cross contamination.

5.4.3 Summary

14 external participants were recruited for the current ILS and 11 external datasets were received. After initial review, 2 external datasets were excluded and the statistical analysis was carried out with 9 external dataset + 1 internal dataset. The internal dataset was carried out by a member of the analytical services team who was not involved in the validation study and had not previously used the NF TVC vial method. In this case it was considered that the internal dataset included in the study was independent.

Positive samples were obtained by the reference method in 10% of blank samples tested. The contaminated samples were limited to 4 participants in 2 laboratories. Further investigations and a root cause analysis were carried out, which identified that the contamination was likely due to the sterility of PCA used or aseptic technique. The impact of this on the validity of the results is minimal.

In conclusion, the interlaboratory study data and interpretations comply with the ISO 16140-2:2016 requirements.

6 CONCLUSION

The **method comparison study conclusions** are:

- The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).
- The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for all categories tested.
- The alternative Neogen Soleris® NF-TVC detection method is selective and specific.

The **inter-laboratory study conclusions** are:

- The observed value for ND-PD is lower than the acceptability limits.
- There are no individual categories tested for the IL.
- The data and interpretations comply with the EN ISO 16140-2:2016 requirements.

The Neogen NF-TVC vial is considered equivalent to the ISO standard.

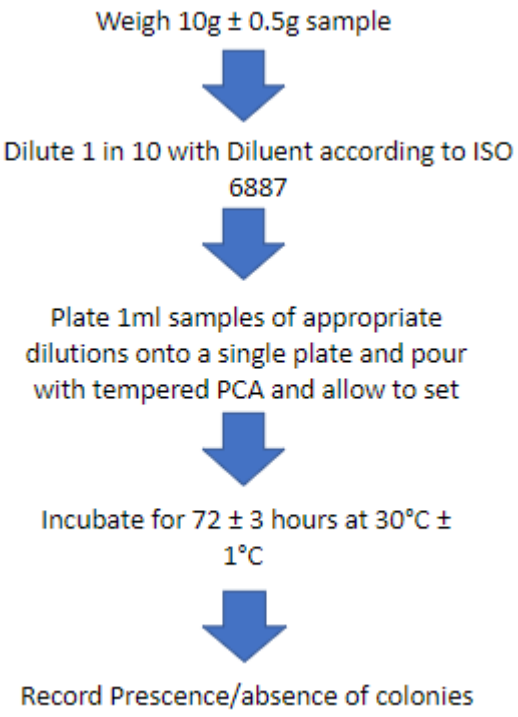
Date,

17/10/2024

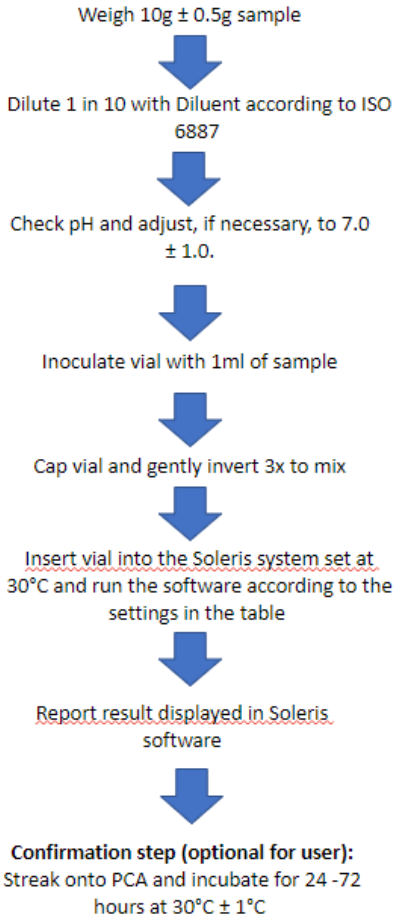
Signature,

Alice Foxall – Project Manager, Molecular Microbiology and Methods

ANNEX A: Flow diagram of the reference method



ANNEX B: Flow diagram of the alternative method



Software settings

Threshold	Skip	Shuteye	Temperature	Test duration
10	1	30	30°C	30 hours

The confirmation step is **not required** for the user, and was performed for purposes of this certification only

ANNEX C: Raw data from the sensitivity study

Milk and dairy products							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T61	Pasteurised milk	Semi skimmed milk 1	-6	-	-	-	NA
T62	Pasteurised milk	Semi skimmed milk 2	-6	-	-	-	NA
T63	Pasteurised milk	Jersey milk	-6	+	+	+	PA
T64	Pasteurised milk	Whole milk	-6	-	-	-	NA
T65	Pasteurised milk	Skimmed milk	-6	+	+	+	PA
D6	Pasteurised milk	Semi skimmed milk that tastes like whole	-6	-	-	-	NA
D7	Pasteurised milk	West country whole milk	-6	+	+	+	PA
D8	Pasteurised milk	Organic skimmed milk	-6	-	-	-	NA
D9	Pasteurised milk	Jersey milk taste the difference	-6	+	+	+	PA
D10	Pasteurised milk	Organic fresh semi skimmed milk	-6	-	-	-	NA
D11	Pasteurised milk	Fresh whole milk fresher for longer	-6	-	-	-	NA
D12	Pasteurised milk	Fresh skimmed milk fresher for longer	-6	-	-	-	NA
D13	Pasteurised milk	British whole milk so organic	-6	+	+	+	PA
D14	Pasteurised milk	Skimmed milk tastes like semi skimmed	-6	-	-	-	NA
D15	Pasteurised milk	British semi skimmed milk so organic	-6	+	+	+	PA
D16	Pasteurised milk	Fresh semi skimmed milk 2l fresher for longer	-6	-	-	-	NA
D17	Pasteurised milk	British skimmed milk	-6	-	-	-	NA
D18	Pasteurised milk	British whole mik	-6	-	-	-	NA
D19	Pasteurised milk	British semi skimmed milk	-6	-	-	-	NA
D20	Pasteurised milk	British whole milk	-6	+	+	+	PA

Milk and dairy products							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T66	Pasteurised dairy products	Fresh whipping cream	-4	-	-	-	NA
T67	Pasteurised dairy products	Soured cream	-4	+	+	-	PD
T68	Pasteurised dairy products	Fat free cottage cheese, onion & chive	-4	+	+	+	PA
T69	Pasteurised dairy products	Butter milk	-4	+	+	+	PA
T70	Pasteurised dairy products	Light original soft cheese	-4	-	-	-	NA
D26	Pasteurised dairy products	Chocolate milkshake	-4	+	+	+	PA
D27	Pasteurised dairy products	Strawberry milkshake	-4	-	-	-	NA
D28	Pasteurised dairy products	Milk chocolate chip ice crea	-4	+	+	+	PA
D29	Pasteurised dairy products	Caramel and vanilla ice cream	-4	-	-	-	NA
D30	Pasteurised dairy products	Vanilla ice cream	-4	-	-	-	NA
D31	Pasteurised dairy products	Ricotta cheese	-4	-	-	-	NA
D32	Pasteurised dairy products	Mozzarella cheese	-4	+	+	+	PA
D33	Pasteurised dairy products	Garlic and herb soft french cream cheese	-4	-	-	-	NA
D34	Pasteurised dairy products	Liquid cheese original	-4	-	-	-	NA
D35	Pasteurised dairy products	Organic double free range cream	-4	+	+	+	PA
D36	Pasteurised dairy products	Jersey double cream taste the difference	-4	+	+	+	PA
D37	Pasteurised dairy products	Double organic cream	-4	+	+	+	PA
D38	Pasteurised dairy products	Cream	-4	-	-	-	NA
D39	Pasteurised dairy products	British double cream	-4	-	-	-	NA
D40	Pasteurised dairy products	Clotted cream	-4	-	-	-	NA
T71	Dry milk products	Dried skim milk	-1	-	-	-	NA
T72	Dry milk products	Milk shake powder	-1	+	+	+	PA

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Milk and dairy products							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T73	Dry milk products	Skimmed milk powder	-1	-	-	-	NA
T74	Dry milk products	Non-fat skim milk	-1	+	+	+	PA
T75	Dry milk products	Non-fat skim milk	-1	+	+	+	PA
D46	Dry milk products	Strawberry flavoured milk powder	-1	-	-	-	NA
D47	Dry milk products	Vanilla flavoured milk powder	-1	-	-	-	NA
D48	Dry milk products	Banana milkshake powder tub	-1	-	-	-	NA
D49	Dry milk products	Chocolate milkshake powder tub	-1	+	+	+	PA
D50	Dry milk products	Strawberry milkshake powder tub	-1	-	-	-	NA
D51	Dry milk products	Chocolate flavour dessert	-1	+	+	+	PA
D52	Dry milk products	Butterscotch flavour dessert	-1	+	+	+	PA
D53	Dry milk products	Strawberry flavour dessert	-1	+	+	+	PA
D54	Dry milk products	Custard powder	-1	+	+	+	PA
D55	Dry milk products	Instant custard powder no added sugar	-1	+	+	+	PA
D56	Dry milk products	Instant custard powder 2	-1	+	+	+	PA
D57	Dry milk products	Instant custard powder 3	-1	+	+	+	PA
D58	Dry milk products	Strawberry milk powder	-1	+	+	+	PA
D59	Dry milk products	Chocolate milk powder	-1	+	+	+	PA
D60	Dry milk products	Speculoos whey powder	-1	+	+	+	PA

Fishery products							
Samples in bold required 72h incubation for confirmation result							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
S41	Raw fish	Salmon fillet	-8	+	+	-	PD
S42	Raw fish	Scottish tail fillets	-8	-	-	+	ND
S43	Raw fish	Yellowfin Tuna Steaks	-8	-	-	-	NA
S44	Raw fish	Icelandic cod fillets	-8	-	-	-	NA
S45	Raw fish	Scottish Loch trout fillets	-8	-	-	-	NA
T16	Raw fish	Basa fillets	-8	+	+	+	PA
T17	Raw fish	Sea bass fillets	-8	-	-	+	ND
T18	**Raw fish**	**Smoked basa fillets**	**-8**	**+**	**+**	**+**	**PA**
T19	Raw fish	Smoked Norwegian haddock fillets	-8	+	+	+	PA
T20	Raw fish	Salmon fillets	-8	+	+	+	PA
F61	Raw fish	Wild salmon fillets	-8	-	-	-	NA
F62	Raw fish	Raw squid rings	-8	-	-	+	ND
F63	**Raw fish**	**Cod fillets**	**-8**	**+**	**+**	**+**	**PA**
F64	Raw fish	White fish fillets	-8	+	+	+	PA
F65	**Raw fish**	**Boneless Seabass Fillets**	**-8**	**+**	**+**	**+**	**PA**
F66	**Raw fish**	**Mussels**	**-8**	**+**	**+**	**+**	**PA**
F67	Raw fish	Salmon tails	-8	+	+	+	PA
F68	Raw fish	Scottish salmon fillets	-8	-	-	-	NA
F69	Raw fish	Mild and delicate Scottish smoked salmon	-8	-	-	-	NA

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Fishery products							
Samples in bold required 72h incubation for confirmation result							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
F70	**Raw fish**	**Skin on cod fillets**	**-8**	+	+	+	**PA**
F16	**Raw fish**	**Cod fillets 2**	**-8**	+	+	+	**PA**
F17	Raw fish	Smoked haddock fillets	-8	+	+	+	PA
F18	Raw fish	Wild salmon fillets	-8	-	-	-	NA
F19	Raw fish	Norweigian Flaky cod fillets	-8	+	-	-	PPNA
F20	Raw fish	White fish fillets	-8	-	-	-	NA
S61	RTC fish	Smoked kipper fillets w/ butter	-8	-	-	-	NA
S62	RTC fish	Mediterranean tomato fish bakes	-8	-	-	-	NA
S63	RTC fish	Cod & spinach gratin	-8	-	-	-	NA
S64	**RTC fish**	**Smoked haddock fishcakes w/ cheddar & leek sauce**	**-8**	+	+	+	**PA**
S65	RTC fish	Chunky cod fish fingers	-8	-	-	-	NA
T21	RTC fish	King prawn flavour tails	-8	-	-	-	NA
T22	RTC fish	Breaded Cod Fishcakes	-8	-	-	-	NA
T23	RTC fish	Cod fishcakes with roasted tomato and mozzarella	-8	-	-	-	NA
T24	**RTC fish**	**Golden breaded cod fillets**	**-8**	+	+	+	**PA**
T25	RTC fish	Smoked haddock fishcakes with cheddar and leek	-8	-	-	-	NA
F6	**RTC fish**	**Salmon fishcakes**	**-8**	+	+	+	**PA**
F7	RTC fish	Creamy cod fishcakes	-8	-	-	+	ND
F11	RTC fish	Crisp lightly dusted Basa fillets	-8	+	+	+	PA
F13	RTC fish	Smoked haddock fishcakes with melting cheddar centre	-8	-	-	+	ND
F14	RTC fish	Breaded white fish fillets	-8	-	-	-	NA
F15	RTC fish	Salmon fishcakes with melting hollandaise centre	-8	+	+	+	PA
F32	**RTC fish**	**Cod & Chorizo Fishcakes**	**-8**	+	+	+	**PA**
F33	RTC fish	Hot Smoked Salmon Fillets	-8	-	-	-	NA
F34	**RTC fish**	**Smoked Haddock Fishcakes**	**-8**	+	+	+	**PA**

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Fishery products							
Samples in bold required 72h incubation for confirmation result							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
F35	RTC fish	Finest Prawn Cocktail	-8	-	-	-	NA
F36	RTC fish	Salmon Spinach & Lemon Fishcakes	-8	-	-	-	NA
F37	RTC fish	Cod Fishcakes	-8	+	+	+	PA
F38	RTC fish	Flipper Dippers	-8	+	+	+	PA
F39	RTC fish	Honey Roast Salmon Flakes	-8	-	-	-	NA
F40	RTC fish	Thai Inspired Salmon & Cod Fishcakes	-8	+	+	+	PA
T26	**Crustaceans**	**Cold water large prawns**	**-6**	**+**	**+**	**-**	**PD**
S1	Crustaceans	White Crab	-6	+	+	-	PD
T28	Crustaceans	Garlic & herb king prawns	-6	-	-	-	NA
T29	Crustaceans	Soy, ginger &chilli king prawns	-6	-	-	-	NA
T30	Crustaceans	Raw & peeled jumbo king prawns	-6	-	-	-	NA
S2	Crustaceans	Brown Crab	-6	-	-	-	NA
F10	Crustaceans	Cold water large prawns	-6	-	-	-	NA
S3	Crustaceans	Crab	-6	-	-	-	NA
S4	Crustaceans	Premium Crab meat	-6	+	+	-	PD
S5	Crustaceans	White Crab 2	-6	-	-	+	ND
S6	Crustaceans	White & Brown Crab	-6	-	-	+	ND
S7	Crustaceans	Crayfish tails	-6	-	-	-	NA
S8	Crustaceans	Crab meat shredded	-6	-	-	-	NA
F54	Crustaceans	Raw Chilli & Lime Marinated King Prawns	-6	+	+	+	PA
F55	Crustaceans	Raw Headless King Prawns	-6	-	-	-	NA
S9	Crustaceans	Canadian cooked lobster	-6	-	-	-	NA
F57	Crustaceans	Lemon & Pepper King Prawns	-6	+	+	+	PA
S10	Crustaceans	Organic Raw King Prawns	-6	-	-	-	NA
F59	Crustaceans	Raw King Prawns	-6	+	+	+	PA

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Fishery products							
Samples in bold required 72h incubation for confirmation result							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
F60	Crustaceans	Raw & peeled red shrimp	-6	+	+	+	PA

Fresh produce and fruits							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T31	Cut ready-to-eat vegetables/leafy greens	Italian wild rocket	-8	-	-	-	NA
T32	Cut ready-to-eat vegetables/leafy greens	Babyleaf salad	-8	-	-	-	NA
T33	Cut ready-to-eat vegetables/leafy greens	Fine beans & tenderstem broccoli	-8	-	-	-	NA
T34	Cut ready-to-eat vegetables/leafy greens	Mixed leaf salad	-8	-	-	-	NA
T35	Cut ready-to-eat vegetables/leafy greens	Butterhead salad	-8	-	-	-	NA
P6	Cut ready-to-eat vegetables/leafy greens	Chopped spinach	-8	+	+	+	PA
P7	Cut ready-to-eat vegetables/leafy greens	Edamae stir dry	-8	+	+	+	PA
P8	Cut ready-to-eat vegetables/leafy greens	Baby vegetable stir dry	-8	+	+	+	PA
P9	Cut ready-to-eat vegetables/leafy greens	Fine beans and tenderstem broccoli	-8	+	+	+	PA
P10	Cut ready-to-eat vegetables/leafy greens	Mixed vegetable stir fry	-8	-	-	-	NA
P11	Cut ready-to-eat vegetables/leafy greens	Hot and spicy stir fry	-8	+	+	+	PA
P12	Cut ready-to-eat vegetables/leafy greens	Florette baby leaf & rocket	-8	+	+	+	PA
P13	Cut ready-to-eat vegetables/leafy greens	Mixed pepper stir fry veg mix	-8	-	-	-	NA
P14	Cut ready-to-eat vegetables/leafy greens	Tenderstem stir fry veg mix	-8	+	+	+	PA
P15	Cut ready-to-eat vegetables/leafy greens	Organic rocket salad	-8	+	+	+	PA
P16	Cut ready-to-eat vegetables/leafy greens	Oriental vegetable stir dry	-8	+	+	+	PA
P17	Cut ready-to-eat vegetables/leafy greens	Wild rocket	-8	+	+	+	PA
P18	Cut ready-to-eat vegetables/leafy greens	Spinach, watercress and rocket salad	-8	+	+	+	PA
P19	Cut ready-to-eat vegetables/leafy greens	Cut babygem lettuce	-8	+	+	+	PA

Fresh produce and fruits							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
P20	Cut ready-to-eat vegetables/leafy greens	Cut peppers and lettuce mix	-8	+	+	-	PD
T36	Fresh fruit/cut RTE fruit products	Blueberries	-5	-	-	-	NA
T37	Fresh fruit/cut RTE fruit products	Strawberries	-5	-	-	-	NA
T38	Fresh fruit/cut RTE fruit products	Berry fruit salad	-5	-	-	-	NA
T39	Fresh fruit/cut RTE fruit products	Apple banana strawberry and grape	-5	-	-	-	NA
T40	Fresh fruit/cut RTE fruit products	Melon kiwi and strawberry	-5	-	-	-	NA
P26	Fresh fruit/cut RTE fruit products	Apples, mango strawberry and raspberry	-5	+	+	+	PA
P27	Fresh fruit/cut RTE fruit products	Mango	-5	+	+	+	PA
P28	Fresh fruit/cut RTE fruit products	Pomegrante seeds	-5	+	+	+	PA
P29	Fresh fruit/cut RTE fruit products	Mango	-5	+	+	-	PD
P30	Fresh fruit/cut RTE fruit products	Grapes and berries	-5	+	+	+	PA
P31	Fresh fruit/cut RTE fruit products	Rainbow fruit platter	-5	+	+	+	PA
P32	Fresh fruit/cut RTE fruit products	Watermelon fingers	-5	+	+	+	PA
P33	Fresh fruit/cut RTE fruit products	Melon stone fruit and raspberry	-5	+	+	+	PA
P34	Fresh fruit/cut RTE fruit products	Fruit platter	-5	+	+	+	PA
P35	Fresh fruit/cut RTE fruit products	Melon and pineapple fingers	-5	+	+	+	PA
P36	Fresh fruit/cut RTE fruit products	Melon	-5	+	+	+	PA
P37	Fresh fruit/cut RTE fruit products	Mango and watermelon fingers	-5	+	+	+	PA
P38	Fresh fruit/cut RTE fruit products	Apple pineapple and grape	-5	+	+	+	PA
P39	Fresh fruit/cut RTE fruit products	Mango pineapple and passionfruit	-5	-	-	-	NA
P40	Fresh fruit/cut RTE fruit products	Melon medley	-5	+	+	+	PA

Fresh produce and fruits							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T41	Heat treated fruit and vegetables	Apple and Raspberry juice	-1	-	-	-	NA
T42	Heat treated fruit and vegetables	Pure orange juice	-1	-	-	+	ND
T43	Heat treated fruit and vegetables	Apple and pear juice	-1	-	-	-	NA
T44	Heat treated fruit and vegetables	Multivitamin boost fruit juice	-1	-	-	-	NA
T45	Heat treated fruit and vegetables	Pure orange and mango fruit juice	-1	+	+	+	PA
P46	Heat treated fruit and vegetables	Organic Mangoes Baby Food Pouch	-1	-	-	+	ND
P47	Heat treated fruit and vegetables	Organic Squeezy Fruit Pouch Apple Mango & Banana	-1	-	-	+	ND
P48	Heat treated fruit and vegetables	Organic Sweet Potatoes Food Pouch	-1	+	+	+	PA
P49	Heat treated fruit and vegetables	Organic Pears Food Pouch	-1	-	-	+	ND
P50	Heat treated fruit and vegetables	Organic Squeezy Fruit Apple Strawberry & Blueberry Food	-1	-	-	-	NA
P51	Heat treated fruit and vegetables	Organic Prunes Baby Food Pouch	-1	-	-	-	NA
P52	Heat treated fruit and vegetables	Organic Bananas Food Pouch	-1	-	-	-	NA
P53	Heat treated fruit and vegetables	Squeezy Fruit Pouch Apple & Strawberry	-1	-	-	-	NA
P54	Heat treated fruit and vegetables	Organic Carrots Baby Food Pouch	-1	-	-	-	NA
P55	Heat treated fruit and vegetables	Strawberry & Banana Fruit Smoothie	-1	-	-	-	NA
P56	Heat treated fruit and vegetables	Tangy & Sweet Orange Juice with Bits	-1	-	-	-	NA
P57	Heat treated fruit and vegetables	Tropical Juice	-1	+	+	-	PD
P58	Heat treated fruit and vegetables	Pineapple Coconut & Banana Fruit Smoothie	-1	-	-	-	NA
P59	Heat treated fruit and vegetables	Mango & Passion Fruit Smoothie	-1	-	-	-	NA
P60	Heat treated fruit and vegetables	Vegetable juice	-1	-	-	+	ND

Multicomponent foods							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T46	Composite foods with substantial raw ingredients	Chicken triple sandwich	-7	+	+	+	PA
T47	Composite foods with substantial raw ingredients	Chicken and sweetcorn sandwich	-7	+	+	+	PA
T48	Composite foods with substantial raw ingredients	Tuna and cucumber sandwich	-7	+	+	+	PA
T49	Composite foods with substantial raw ingredients	Egg and cress sandwich	-7	-	-	-	NA
T50	Composite foods with substantial raw ingredients	Cheese and onion sandwich	-7	-	-	+	ND
C6	Composite foods with substantial raw ingredients	Spicy chicken pasta	-7	-	-	-	NA
C7	Composite foods with substantial raw ingredients	Coronation chicken pasta salad	-7	-	-	-	NA
C8	Composite foods with substantial raw ingredients	Southern fried chicken pasta	-7	+	+	+	PA
C9	Composite foods with substantial raw ingredients	Onion bhaji sandwich	-7	-	-	-	NA
C10	Composite foods with substantial raw ingredients	Spinach pinenut pasta	-7	-	-	-	NA
C11	Composite foods with substantial raw ingredients	Chicken and bacon pasta	-7	-	-	-	NA
C12	Composite foods with substantial raw ingredients	Feta and slow roast tomato pasta	-7	-	-	-	NA
C13	Composite foods with substantial raw ingredients	Cheese and pickle sandwich	-7	+	+	-	PD
C14	Composite foods with substantial raw ingredients	Chicken and chorizo pasta salad	-7	+	+	-	PD
C15	Composite foods with substantial raw ingredients	Chicken and bacon pasta	-7	+	+	+	PA
C16	Composite foods with substantial raw ingredients	Chicken tomato and basil pasta salad	-7	-	-	-	NA
C17	Composite foods with substantial raw ingredients	Cheese and tomato pasta	-7	-	-	-	NA
C18	Composite foods with substantial raw ingredients	Tomato and basil pasta salad	-7	-	-	+	ND
C19	Composite foods with substantial raw ingredients	Honey and mustard chicken pasta	-7	+	+	+	ND

Multicomponent foods							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
C20	Composite foods with substantial raw ingredients	Tuna & sweetcorn pasta	-7	+	+	+	PA
T51	RTRH/RTE foods (chilled, frozen)	Lasagne	-6	+	+	+	PA
T52	RTRH/RTE foods (chilled, frozen)	Tomato and mozzarella pasta bake	-6	+	+	+	PA
T53	RTRH/RTE foods (chilled, frozen)	Macaroni cheese	-6	+	+	+	PA
T54	RTRH/RTE foods (chilled, frozen)	Tikka masala & pilau rice	-6	+	+	+	PA
T55	RTRH/RTE foods (chilled, frozen)	Spicy chicken pasta	-6	-	-	-	NA
C26	RTRH/RTE foods (chilled, frozen)	Ham & mushroom tagliatelle	-6	+	+	+	PA
C27	RTRH/RTE foods (chilled, frozen)	Chicken hotpot	-6	+	+	+	PA
C28	RTRH/RTE foods (chilled, frozen)	Cottage pie	-6	+	+	+	PA
C29	RTRH/RTE foods (chilled, frozen)	Shepards pie	-6	-	-	-	NA
C30	RTRH/RTE foods (chilled, frozen)	Tomato & mozzarella penne bake	-6	+	+	+	PA
C31	RTRH/RTE foods (chilled, frozen)	Chicken curry & rise	-6	-	-	-	NA
C32	RTRH/RTE foods (chilled, frozen)	Sausage & mash	-6	-	-	-	NA
C33	RTRH/RTE foods (chilled, frozen)	Spaghetti Bolognese	-6	+	+	+	PA
C34	RTRH/RTE foods (chilled, frozen)	Italian beef lasagne	-6	-	-	-	NA
C35	RTRH/RTE foods (chilled, frozen)	Chicken korma with pilau rice	-6	+	+	+	PA
C36	RTRH/RTE foods (chilled, frozen)	Beef stew & dumplings	-6	+	+	+	PA
C37	RTRH/RTE foods (chilled, frozen)	Chicken & bacon pasta bake	-6	+	+	+	PA
C38	RTRH/RTE foods (chilled, frozen)	Spaghetti & meatballs	-6	-	-	-	NA
C39	RTRH/RTE foods (chilled, frozen)	Spaghetti carbonara	-6	+	+	+	PA

Multicomponent foods							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
C40	RTRH/RTE foods (chilled, frozen)	Macaroni and cheese	-6	-	-	-	NA
T56	Mayonnaise based deli salads	Triple grain salad mayonnaise	-8	-	-	-	NA
T57	Mayonnaise based deli salads	Party salad mayonnaise	-8	-	-	-	NA
T58	Mayonnaise based deli salads	Chicken Caesar salad	-8	-	-	+	ND
T59	Mayonnaise based deli salads	Ham egg and coleslaw salad	-8	-	-	-	NA
T60	Mayonnaise based deli salads	Chicken & bacon salad	-8	+	+	+	PA
C45	Mayonnaise based deli salads	Cesear salad	-8	+	+	+	PA
C47	Mayonnaise based deli salads	Chicken and bacon Caesar salad	-8	+	+	+	PA
C48	Mayonnaise based deli salads	Celery fruit nut salad	-8	-	-	-	NA
C49	Mayonnaise based deli salads	Tuna and sweetcorn salad	-8	-	-	-	NA
C50	Mayonnaise based deli salads	Falafel and rice salad	-8	-	-	-	NA
C51	Mayonnaise based deli salads	Chicken rice bowl	-8	-	-	-	NA
C52	Mayonnaise based deli salads	Bang bang chicken salad	-8	+	+	+	PA
C53	Mayonnaise based deli salads	Chicken, broccoli and almond salad	-8	+	+	+	PA
C54	Mayonnaise based deli salads	Hummus salad	-8	-	-	-	NA
C55	Mayonnaise based deli salads	Prawn layered salad	-8	-	-	-	NA
C56	Mayonnaise based deli salads	Baby potato & free-range egg salad	-8	-	-	-	NA
C57	Mayonnaise based deli salads	Chicken & bacon Caesar pasta salad	-8	-	-	-	NA
C58	Mayonnaise based deli salads	Baby potato salad	-8	-	-	-	NA
C59	Mayonnaise based deli salads	Cauliflower & spicy rice w/ mayonnaise	-8	+	+	+	PA

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Multicomponent foods							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
C60	Mayonnaise based deli salads	Chicken & bacon pasta salad	-8	-	-	-	NA

Raw and Ready to Cook Meat and Poultry							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T1	Raw poultry and meat cuts	Chicken breast fillets	-7	-	-	-	NA
T2	Raw poultry and meat cuts	Lean diced beef	-7	-	-	-	NA
T3	Raw poultry and meat cuts	Fresh lamb chops	-7	+	+	+	PA
T4	Raw poultry and meat cuts	Turkey thigh mince 7% fat	-7	+	+	-	PD
T5	Raw poultry and meat cuts	Pork loin steaks	-7	-	-	-	NA
R6	Raw poultry and meat cuts	Turkey breast fillet portions	-7	+	+	+	PA
R7	Raw poultry and meat cuts	Turkey burgers	-7	+	+	+	PA
R8	Raw poultry and meat cuts	Turkey breast steaks	-7	-	-	-	NA
R9	Raw poultry and meat cuts	Turkey streaks	-7	-	-	-	NA
R10	Raw poultry and meat cuts	Turkey breast mini fillets	-7	-	-	+	ND
R11	Raw poultry and meat cuts	Chicken skin on drumsticks	-7	+	+	+	PA
R12	Raw poultry and meat cuts	Fresh small whole chicken	-7	-	-	-	NA
R13	Raw poultry and meat cuts	Fresh chicken thigh fillets skinless + boneless	-7	-	-	-	NA
R14	Raw poultry and meat cuts	Chicken breast mini fillets	-7	-	-	-	NA
R15	Raw poultry and meat cuts	Chicken skin on thighs	-7	+	+	+	PA
R16	Raw poultry and meat cuts	Chicken breast 2	-7	-	-	-	NA
R17	Raw poultry and meat cuts	Chicken breast fillets 3	-7	+	+	+	PA
R18	Raw poultry and meat cuts	British pork shoulder steaks	-7	-	-	-	NA
R19	Raw poultry and meat cuts	Thick cut british pork chops	-7	-	-	-	NA
R20	Raw poultry and meat cuts	Diced beef	-7	+	+	+	PA

Raw and Ready to Cook Meat and Poultry							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T6	Raw processed meat	BBQ pork riblets	-7	-	-	-	NA
T7	Raw processed meat	Pork shoulder in a bbq sauce	-7	-	-	+	ND
T8	Raw processed meat	Sweet and smoky beef kebabs	-7	+	+	-	PD
T9	Raw processed meat	Beef burgers	-7	+	+	+	PA
T10	Raw processed meat	Pork sausages	-7	+	+	+	PA
R26	Raw processed meat	Turkey gluten free sausages	-7	-	-	-	NA
R27	Raw processed meat	2% fat turkey mince	-7	+	+	+	PA
R28	Raw processed meat	Turkey meatballs	-7	+	+	+	PA
R29	Raw processed meat	British pork stir fry	-7	-	-	-	NA
R30	Raw processed meat	British pork belly slices	-7	-	-	-	NA
R31	Raw processed meat	Pork tenderloin fillet	-7	+	+	+	PA
R32	Raw processed meat	Smoked gammon steaks	-7	+	+	+	PA
R33	Raw processed meat	Slow cooked pulled pork	-7	-	-	-	NA
R34	Raw processed meat	Chinese style pork	-7	-	-	-	NA
R35	Raw processed meat	5% fat beef mince	-7	+	+	+	PA
R36	Raw processed meat	10% fat meatballs	-7	-	-	-	NA
R37	Raw processed meat	Quarter pounder burgers	-7	+	+	-	PD
R38	Raw processed meat	Caramelised onion beef burgers	-7	-	-	-	NA
R39	Raw processed meat	Wagyu beef burgers	-7	-	-	-	NA
R40	Raw processed meat	Venison burgers	-7	+	+	-	PD

Raw and Ready to Cook Meat and Poultry							
Sample code	**Type**	**Sample used**	**Dilution selected**	**Soleris result**	**PCA confirmation result from Soleris vial**	**Reference result**	**Agreement**
T11	RTC meat and poultry	Breaded chicken goujons	-4	-	-	-	NA
T12	RTC meat and poultry	Turkey meatballs	-4	-	-	-	NA
T13	RTC meat and poultry	BBQ roast chicken wings	-4	+	+	+	PA
T14	RTC meat and poultry	Chicken kiev bites	-4	+	+	+	PA
T15	RTC meat and poultry	Southern fried breaded chicken mini fillets	-4	-	-	-	NA
R46	RTC meat and poultry	Chicken quarter pounder	-4	-	-	-	NA
R47	RTC meat and poultry	Pork & herb sausage patties	-4	+	+	+	PA
R48	RTC meat and poultry	Garlic chicken kievs	-4	+	+	+	PA
R49	RTC meat and poultry	Garlic butter chicken kievs	-4	+	+	+	PA
R50	RTC meat and poultry	Buttered chicken breast joint	-4	+	+	+	PA
R51	RTC meat and poultry	Chicken wings side	-4	+	+	+	PA
R52	RTC meat and poultry	Wild garlic chicken kiev with salted butter	-4	+	+	+	PA
R53	RTC meat and poultry	Breaded garlic chicken kiev	-4	+	+	+	PA
R54	RTC meat and poultry	Garlic breast kievs whole fillet	-4	+	+	+	PA
R55	RTC meat and poultry	Cheese & bacon chicken kievs	-4	+	+	+	PA
R56	RTC meat and poultry	Beef & herb meatballs	-4	-	-	-	NA
R57	RTC meat and poultry	Beef meatballs	-4	-	-	-	NA
R58	RTC meat and poultry	British beef quarterpounders	-4	+	+	-	PD
R59	RTC meat and poultry	Turkey mini kievs	-4	+	+	+	PA
R60	RTC meat and poultry	Cheese and bacon chicken kievs	-4	+	+	+	PA

ANNEX D: Raw data from the RLOD study

Sample code	Level	Inoculation (cfu/g)	Soleris alternative method			Reference method
			Soleris result (detection time)	Confirmation result (+/-)	Alternative final result (+/-)	
Category - Dairy products (heat processed)						
R1	Blank	N/A	ND	-	-	-
R2			ND	-	-	-
R3			ND	-	-	-
R4			ND	-	-	-
R5			ND	-	-	-
R6	Low	11	ND	-	-	+
R7			ND	-	-	-
R8			ND	+	-	+
R9			10.3	+	+	+
R10			14.3	+	+	+
R11			11.6	+	+	-
R12			11.7	+	+	-
R13			ND	-	-	+
R14			ND	-	-	-
R15			10.4	+	+	+
R16			11.4	+	+	+
R17			13	+	+	-
R18			10.4	+	+	-
R19			14.3	+	+	-
R20			ND	+	-	+
R21			ND	-	-	+
R22			11.2	+	+	+
R23			10.1	+	+	+
R24			13.5	+	+	+
R25			11.7	+	+	+
R26	High	25	12.7	+	+	+
R27			11.7	+	+	-
R28			23.7	+	+	+
R29			10.9	+	+	+
R30			12.6	+	+	+

Sample code	Level	Inoculation (cfu/g)	Soleris alternative method			Reference method
			Soleris result (detection time)	Confirmation result (+/-)	Alternative final result (+/-)	
Category - Raw and RTC fishery products						
R31	Blank	N/A	ND	-	-	-
R32			ND	-	-	-
R33			ND	-	-	-
R34			ND	-	-	-
R35			ND	-	-	-
R36	Low	12	ND	-	-	-
R37			ND	-	-	-
R38			ND	-	-	-
R39			ND	-	-	-
R40			25.7	-	-	+
R41			13.4	+	+	+
R42			22.8	+	+	+
R43			13.5	+	+	+
R44			14	+	+	+
R45			14.9	+	+	+
R46			13.9	+	+	+
R47			13.2	+	+	+
R48			14.7	+	+	+
R49			26.3	-	-	+
R50			20.8	-	-	+
R51			12.3	+	+	+
R52			ND	-	-	+
R53			13.5	+	+	-
R54			15.1	+	+	+
R55			14.6	+	+	+
R56	High	24	12.8	+	+	+
R57			13.1	+	+	+
R58			13.2	+	+	+
R59			13.1	+	+	+
R60			13.1	+	+	+

Sample code	Level	Inoculation (cfu/g)	Soleris alternative method			Reference method
			Soleris result (detection time)	Confirmation result (+/-)	Alternative final result (+/-)	
Category - Produce and fruits (combined category: fresh and processed)						
R61	Blank	N/A	ND	-	-	-
R62			ND	-	-	-
R63			ND	-	-	-
R64			ND	-	-	-
R65			ND	-	-	-
R66	Low	11	15.4	+	+	+
R67			15	+	+	+
R68			15.7	+	+	+
R69			17	+	+	+
R70			15.4	+	+	+
R71			16.9	+	+	+
R72			15.1	+	+	+
R73			15.4	+	+	+
R74			15.6	+	+	+
R75			15.7	+	+	+
R76			14.8	+	+	+
R77			16.1	+	+	+
R78			15.9	+	+	+
R79			ND		-	+
R80			16.4	+	+	-
R81			ND	-	-	-
R82			15.5	+	+	-
R83			ND	-	-	+
R84			ND	-	-	-
R85			ND	-	-	+
R86	High	23	15	+	+	+
R87			15.9	+	+	+
R88			15.5	+	+	+
R89			17.3	+	+	+
R90			16.4	+	+	+

Sample code	Level	Inoculation (cfu/g)	Soleris alternative method			Reference method
			Soleris result (detection time)	Confirmation result (+/-)	Alternative final result (+/-)	
Category - Raw and RTC meat and poultry (combined)						
R91	Blank	N/A	ND	-	-	-
R92			ND	-	-	-
R93			ND	-	-	-
R94			ND	-	-	-
R95			ND	-	-	-
R96	Low	11	ND	-	-	+
R97			ND	-	-	-
R98			ND	-	-	-
R99			ND	-	-	-
R100			ND	-	-	-
R101			23.4	+	+	-
R102			23.3	+	+	+
R103			21.7	+	+	-
R104			26.3	+	+	-
R105			25.6	+	+	+
R106			22.8	+	+	+
R107			ND	-	-	+
R108			ND	-	-	-
R109			25.2	+	+	-
R110			ND	-	-	+
R111			24.1	+	+	-
R112			29.5	+	+	-
R113			25.6	+	+	+
R114			22.5	+	+	+
R115			ND	-	-	-
R116	High	22	23.2	+	+	+
R117			22.4	+	+	+
R118			23	+	+	+
R119			22.5	+	+	+
R120			22.6	+	+	+

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Sample code	Level	Inoculation (cfu/g)	Soleris alternative method			Reference method
			Soleris result (detection time)	Confirmation result (+/-)	Alternative final result (+/-)	
Category - Multicomponent foods or meal components						
R121	Blank	N/A	ND	-	-	-
R122			ND	-	-	-
R123			ND	-	-	-
R124			ND	-	-	-
R125			ND	-	-	-
R126	Low	11	17.5	+	+	+
R127			18.4	+	+	+
R128			16.8	+	+	-
R129			19.4	+	+	+
R130			17.7	+	+	+
R131			17.5	+	+	+
R132			15.5	+	+	+
R133			ND	-	-	-
R134			ND	-	-	-
R135			16.1	+	+	+
R136			16.9	+	+	+
R137			17.5	+	+	+
R138			20.3	+	+	-
R139			17.1	+	+	+
R140			15.8	+	+	-
R141			16	+	+	+
R142			16.4	+	+	-
R143			17.1	+	+	+
R144			17.3	+	+	-
R145			16.8	+	+	+
R146	High	28	15.9	+	+	+
R147			15.7	+	+	+
R148			17.1	+	+	+
R149			16.1	+	+	+
R150			16.8	+	+	+

ANNEX E: Raw data on inclusivity and exclusivity

Code	Genus	species	CRA Number	Origin	Cfu/plate	Alternative method result	
						Soleris result	Detection time (h)
1	*Raoultella*	*terrigena*	17343	raw milk	30	D	14.1
2	*Enterobacter*	*cloacae*	1472	dried milk	108	D	10.9
3	*Klebsiella*	*oxytoca*	8387	Water	10	D	14.7
4	*Kluyvera*	*ascorbata*	17126	industrial	20	D	14.6
5	*Escherichia*	*adecarboxylata*	5501	Skim milk powder	23	D	12
6	*Klebsiella*	*trevisanii*	NCIMB 8606	Ropy cream	18	D	12.8
7	*Pantoea*	*agglomerans*	17030, NCIMB 702072	Pasteurised milk	4	D	19.7
8	*Aeromonas*	*salmonicida*	8388, NCTC 8049	tin of milk with a fishy odour	2	D	22.9
9	*Escherichia*	*coli*	1476	Dried milk	31	D	12.6
10	*Rahnella*	*aqualtilis*	16911	drinking water	40	D	19.3
11	*Bacillus*	*weihenstephanensis*	16578	Pasteurised milk	3	D	15.6
12	*Lysinibacillus*	*sphaericus*	7746	unknown	1	D	15.1
13	*Bacillus*	*coagulans*	16586	Sterilised milk	51	D	28.6
14	*Clostridium*	*perfringens*	15911, NCTC 8239	salt beef	129	D	16.4
15	*Staphylococcus*	*delphini*	16891	factory isolate	11	D	16.8
16	*Leuconostoc*	*mesenteroides*	17490	Green ham	51	D	12.6
17	*Buttiauxella*	*agrestis*	17110	Pond water	72	D	14.2
18	*Citrobacter*	*youngae*	NCTC 13709	Meat scraps	87	D	12.3
19	*Moraxella*	*osloensis*	17043	milk	46	D	24.5
20	*Paraburkholderia*	*cepaciae*	16779	soft drinks environment	68	D	12.8
21	*Staphylococcus*	*carnosus*	284	goat's milk	20	D	13
22	*Listeria*	*ivanovii*	1123	soft cheese	9	D	19.8
23	*Streptococcus*	*thermophilus*	16045, NCIMB 8510	Pasteurised milk	20	D	15.8
24	*Lactobacillus*	*acidophilus*	7675	Dairy product	30	D	16.5
25	*Carnobacterium*	*divergens*	3910	Brie	15	D	15

2022LR113 Neogen Soleris Non-Fermenting Total Viable Count (NF-TVC) vial MCS ILS Summary Report 14/10/2024

Code	Genus	species	CRA Number	Origin	Cfu/plate	Alternative method result	
						Soleris result	Detection time (h)
26	*Staphylococcus*	*saprophyticius*	8999	distilled water environmental	34	D	18.9
27	*Micrococcus*	*luteus*	3503	Air sample	8	D	16.7
28	*Enterococcus*	*faecalis*	1513	Dried milk powder	40	D	15.1
29	*Staphylococcus*	*cohnii*	272	skin	83	D	25.1
30	*Enterococcus*	*faecium*	16866	Uncooked Sausage	65	D	14.9
31	*Staphylococcus*	*aureus*	409/3026	Slow cheese	52	D	14.9
32	*Staphylococcus*	*epidermidis*	314	runway & can seam	7	D	20.9
33	*Pediococcus*	*pentosaceus*	16030	Brine	85	D	28.5
34	*Listeria*	*monocytogenes 1/2a*	1100	Stilton	50	D	22.1
35	*Listeria*	*innocua*	3130	Cheese factory	39	D	22
36	*Listeria*	*fleischmanii subspecies fleishmanii*	16876	Swiss hard cheese	105	D	21.9
37	*Streptococcus*	*parauberis*	17214	Raw mince	31	D	11.9
38	*Staphylococcus*	*hominis*	16828	unknown	5	D	21.1
39	*Staphylococcus*	*warneri*	3198	Dry sausage	11	D	22.4
40	*Lactococcus*	*lactis*	16029	Green ham	35	D	25.4
41	*Micrococcus*	*roseus*	7775	water	14	D	25.5
42	*Streptococcus*	*lactis*	1511	dried milk powder	7	D	16.6
43	*Enterococcus*	*pseudoavium*	16852	Cow udder - bovine mastitis	25	D	13.9
44	*Candida*	*krussei*	CRA629	Yogurt base	1	D	26.6
45	*Kluyveromyces*	*marxianus*	CRA 6749	Dairy isolate	25	D	16.4
46	*Torulaspora*	*delbrukeii*	CRA16154	Spoiled yogurt	13	D	24.2
47	*Geochium*	*condidum*	14398	Factory isolate	7	D	16.2
48	*Fusarium*	*solani*	16976	Factory isolate	25	D	26.9
49	*Debracmyces*	*hansenii*	15969	Environment	49	D	29.6
50	*Kluyveromyces*	*lactis*	MUCL 28769	gassy cheese	12	D	14.4

Key D = detected

ANNEX F: Collaborators in ILS

Laboratories	Country	Address	Number of collaborators
NQAC Konolfingen	Switzerland	Bern, Switzerland	1
Aliminter	Spain	Murcia, Spain	1
Kraft Heinz Elst	Netherlands	Elst, Netherlands	2
Dairygold	Ireland	Cork, Ireland	1
Danone - Aqualab	France	Évian, France	2
Q labs	United States	Ohio, US	1
Muller Yoghurts & Desserts	England	Market Drayton, England	2
Kraft Heinz Kitt Green	England	Wigan, England	2
PZ Cussons	England	Manchester, England	2
Campden BRI – Microbiology Analytical Services	England	Gloucestershire, UK	1