2021 LR100

Soleris commercial sterility testing vials (NF105) summary report

Method Comparison and ILS Study Report for the ISO 16140-2:2016 validation of Soleris commerical sterilty testing vials (NF105), for the determination of commercial sterility in UHT treated milk and dairy alternative drinks

MicroVal study number: 2021 LR100

Method/Kit name: Neogen Soleris commercial sterility testing vials (NF105)

Report MCS/ILS: summary report

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2021 LR100

Soleris commercial sterility testing vials (NF105) summary report

Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v.2.5

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Method/Kit name: Neogen Soleris commercial sterility testing vials (NF105)

Validation standard: Microbiology of the food chain— Method validation

Part 1: Vocabulary (ISO 16140-1:2016) and

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

Reference method: Codex Code of Hygienic practice for milk and milk products (CAC/RCP 57-2004). Annex B Microbioicidal control measures Section 2.2 process management for UHT treatment using ISO 4833-1:2013 for the plate count at 30°C

Scope of validation: UHT treated milk and dairy alternative drinks

Certification organization: Lloyd's Register

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

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1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method for the detection of commerical sterility in a single food category - UHT treated milk and dairy alternative drinks, was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

The Soleris commercial sterility testing vials (NF105). These are medium vials which contain a broth able to grow aerobic, mesophilic microorganisms (bacteria, yeasts and moulds) present in UHT liquid products such as milk that are able to grow on an aerobic plate count at 30°C. As growth of the organisms occurs, acid is produced which is detected by a pH indicator dye resulting in a colour change from green to yellow. This change in colour migrates into a soft agar plug at the base of the vial which is read by the optical sensors in the Soleris system. The vials are read in real time by LED light passing through the agar plug to a photo diode detector with the instrument.

The time to growth detection in the Soleris system is correlated to the level of microorganisms present in the sample, with higher levels of contamination having a shorter detection time. This detection time is based on a calibration curve developed for bacteria capable of growing in a total viable count. In this validation, the method was used to determine a defined threshold of product contamination of greater than ≤ 10cfu per 0.1ml as defined in the European Directive 92/46 annex C chapter 2 for ultra-high temperature (UHT) milk**.** If successful, the alternative method would be used as a rapid screen for commercial sterility monitoring of UHT treated milk and dairy drinks, reducing hands on analysis time as well as time to result.

As the method is for the detection of all microorgansism present in the liquid products, it is not proposed to carry out a confirmation of positive samples.

The reference method used was: Codex Code of Hygienic practice for milk and milk products (CAC/RCP 57-2004). Annex B Microbioicidal control measures Section 2.2 process management for UHT treatment using ISO 4833-1:2013 for the plate count at 30°C

Scope of the validation study is: UHT treated milk and dairy alternative drinks – up to 1L cartons

Category included:

- UHT treated milk and dairy alternative drinks

Criteria evaluated during the study have been:

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

Summarized, the conclusions on the Method Comparison study are:

In the sensitivity study, the observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for both incubation times (48h and 72h pre-incubation).

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for the single category tested at both pre incubation levels (48h and 72h).

The alternative Neogen Soleris commercial sterility testing vials detection method is selective and specific.

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

The data shows no significant differences between the reference method and the alternative method.

The ND-PD value meets the AL criteria of $ND\text{-}PD_{max}$ L_1 level and L_2 level.

The data and interpretations comply with the EN ISO 16140-2:2016 requirements.

The Neogen Soleris commercial sterility testing vial (NF105) is considered equivalent to the Reference method.

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 most recent version of the MicroVal technical committee interpretations.

2 Method protocols

The Method Comparison Study was carried out using cartons of UHT treated product material up to 1L in volume.

As there was no shared initial (pre)-enrichment step for the reference and the alternative method, different test portions, but coming from the same batch of product (Item), was used for the two methods. All resulting data was treated as unpaired data (EN-ISO 16140-2).

2.1 Reference method

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

The European Directive 92/46 was selected as it is required for random sampling checks of final product to determine that an effective heat treatment has been carried out on sterilised and UHT milk products. This method has a sterility pass/fail criterion of 10 colonies per 0.1ml. For this study, the result of the reference method was interpreted as:

Presence of >10 colonies per 0.1ml = positive result, sterility fail

Presence of ≤10 colonies per 0.1ml = negative result, sterility pass

Sample preparation for the reference method was done according to ISO 6887-series after the initial sample pre incubation step was carried out as outlined in European Directive 92/46 annex C chapter 2 for ultra-high temperature (UHT) milk. During this study, the dairy samples were plated onto milk plate count agar (mPCA) as required by ISO 4833-1:2013. The plant based products were plated onto PCA. In this study, 1ml aliquots of a 10^{-1} sample dilution were plated out to detect the presence of 10 colonies per 0.1ml =100 cfu per ml.

2.2 Alternative method

The flow diagram of the alternative method is given in Annex B.

Sample preparation for the alternative method was carried following the Sterility Testing Vials kit insert following protocol 2 Direct inoculation (presence/absence).

See the commercial sterility Testing Vials kit insert in Annex C.

The alternative method principle is based on growth of non-fermenting bacteria, yeasts and moulds present in UHT liquid products such as milk. As growth of the organisms occurs, acid is produced which is detected by a pH indicator dye resulting in a colour change from green to yellow. This change in colour migrates into a soft agar plug at the base of the vial which is read by the optical sensors in the Soleris system. The vials are read in real time by LED light passing through the agar plug to a photo diode detector with the instrument Neogen Soleris Next Generation (SNG-INS32), with the software version Neogen Fusion (Version 1.6 or newer).

The time to growth detection in the Soleris system is correlated to the level of microorganisms present in the sample, with higher levels of contamination having a shorter detection time.

The test involves the pre-incubation of the 1L carton of UHT treated product at 30°C for 48-72h. A 5ml aliquot of the milk was then placed in the vial which was pH adjusted to pH 7.1 - 7.5 if required. The vial was then incubated in the instrument for 48-72h and readings were taken during the incubation period to determine if a colour change has occurred in the NF-105 (5 mL) vials. For this study,the shortest incubation time of 48h for the pre-incubation was used. Samples that gave a negative result after 48h were reincubated up to the full 72h pre-incubation period to check that the samples were truly negative. The Soleris results was interpreted as follows:

Detection time recorded = positive result, sterility fail

No detection time recorded = negative result, sterility pass

2.3 Study design

As the reference and alternative methods have a different incubation time (15 days for the reference method compared to 48-72h for alternative method, they do not have a common enrichment procedure. Therefore different samples were tested and the data generated were analysed as an unpaired data study.

3 Method comparison study

The Method Comparison Study were carried out using 1L UHT treated liquids (dairy and dairy alternative drinks) where possible.

See Table 1 for specific preparations used in the validation 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

One Category was included in this validation study.

A minimum of 60 Items were tested by both the reference method and the alternative method in the sensitivity study, with a minimum of 30 positive samples per Category.

The Category was made up of 3 Types, with at least 20 Items representative for that Type.
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	Type	Alternative method protocol	Test portion size*	Number of samples
UHT treated milk and dairy alternative drinks	A UHT Dairy liquid products	Neogen Soleris commercial sterility testing vials (NF105)* protocol 2 Direct inoculation	Up to 1L volume cartons	20
	B UHT plant drinks	Neogen Soleris commercial sterility testing vials (NF105)* protocol 2 Direct inoculation	Up to 1L volume cartons	20
	C UHT flavoured milks	Neogen Soleris commercial sterility testing vials (NF105)* protocol 2 Direct inoculation	Up to 1L volume cartons	20

* If different between reference method and alternative method mention both.

60 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**		**Positive samples***	**Negative samples**	**Total**
UHT treated milk and dairy alternative drinks	a	UHT Dairy liquid products	10	10	20
	b	UHT plant drinks	10	10	20
	c	UHT flavoured milks	10	10	20
Total			**30**	**30**	**60**

*Positive by at least one of the methods

3.1.2 Test sample preparation

As this study involves sterility testing, naturally contaminated samples were not used due to the nature of the product being tested and the level of contamination being detected. In this study, artificially contaminated samples were used to achieve the required number of positive samples. The items in this study were inoculated with a range of microorganims including vegetative cells, yeasts moulds

and spores. At the MVTC meeting (25/06/21) it was decided that the preportion of isolates to be used should reflect the types of contamination seen in UHT cartons. The type of contamination used in this study was approximately 50% spores and 50% vegetative cells for each item type. During this study, mould and spore forming isolates were inoculated into the cartons as spores.

Artificial contamination of samples was done using a spiking protocol . The samples were inoculated with a selection of organisms capable of growing on a plate count at 30°C. Prior to inoculation, the organisms were treated with an appropriate injury protocol heat treatment e.g. 55°C for 5 min or peroxide stress (100 mM hydrogen peroxide (H_2O_2) for 20 min). The level of injury was determined by enumeration on appropriate selective and non selctive plates with the aim of having more than 0.5log difference for a stress application. For isolates that did not have a specific selective media, a non-selective agar with added salt was used to determine the level of injury of the strain used in the study. Details of the artificial contaminations are presented in Annex D for reference.

All isolates used for artificial inoculations preferably originated from comparable sample types as the ones to be inoculated. Each particular strain was used to contaminate no more than 3 different items in this study. Inoculation of samples was generally at a level of 0.5 -2 cfu/1L sample, with only occasionally at a maximum of 2.8 cfu/1L sample.

3.1.3 Confirmation protocols

A confirmation step is not included in this study due to the wide range of organisms being detected and there is no requirement by the manufactuer for confirmation.

3.1.4 Sensitivity study results

All raw data on the sensitivity study are given in Annex E. Sample numbers in **bold** indicate artificial inoculation of the sample (see Annex D for details on artificial inoculation).

Table 3 shows the summary of results of the reference method and the alternative methods for **the single category at both pre-incubation times 48h and 72h**.

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

Table 3a - Summary of sensitivity study results – with a pre incubation time of 48h

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive Agreement PA (R+/A+) 28	Positive Deviation PD (R-/A+) 2
Alternative method negative (A-)	Negative Deviation (R+/A-) 0	Negative Agreement NA (R-/A-) 30

Table 3b - Summary of sensitivity study results – with a pre incubation time of 72h

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive Agreement PA (R+/A+) 28	Positive Deviation PD (R-/A+) 2
Alternative method negative (A-)	Negative Deviation (R+/A-) 0	Negative Agreement NA (R-/A-) 30

Table 4a – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method) with a pre incubation time of 48h

Category		**Type**		**PA**	**NA[1]**	**PD**	**ND[2]**	**PPNA3**	**PPND3**	**Total**
1	UHT treated milk and dairy alternative drinks	a	UHT Dairy liquid products	10	10	0	0	0	0	20
		b	UHT plant drinks	10	10	0	0	0	0	20
		c	UHT flavoured milks	8	10	2	0	0	0	20
All categories				**28**	**30**	**2**	**0**	**0**	**0**	**60**

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

***Table 4b – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method)* with a pre incubation time of 72h**

Category		Type		PA	NA[1]	PD	ND[2]	PPNA3	PPND3	Total
1	UHT treated milk and dairy alternative drinks	a	UHT Dairy liquid products	10	10	0	0	0	0	20
		b	UHT plant drinks	10	10	0	0	0	0	20
		c	UHT flavoured milks	8	10	2	0	0	0	20
All categories				**28**	**30**	**2**	**0**	**0**	**0**	**60**

[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 6a - Overview calculated sensitivity parameters per Category and Type with a pre incubation time of 48h

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
UHT treated milk and dairy alternative drinks	a	10	10	0	0	0	100.0	100.0	100.0	0.0
	b	10	10	0	0	0	100.0	100.0	100.0	0.0
	c	8	10	2	0	0	100.0	80.0	90.0	0.0
	Total	**28**	**30**	**2**	**0**	**0**	100.0	93.3	96.7	0.0
All categories		**28**	**30**	**2**	**0**	**0**	100.0	93.3	96.7	0.0

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

Table 6b - Overview calculated sensitivity parameters per Category and Type with a pre incubation time of 72h

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
n UHT treated milk and dairy alternative drinks	a	10	10	0	0	0	100.0	100.0	100.0	0.0
	b	10	10	0	0	0	100.0	100.0	100.0	0.0
	c	8	10	2	0	0	100.0	80.0	90.0	0.0
	Total	**28**	**30**	**2**	**0**	**0**	100.0	93.3	96.7	0.0
All categories		**28**	**30**	**2**	**0**	**0**	100.0	93.3	96.7	0.0

3.1.6 Discordant results

Negative deviations are listed in Table 7.

Table 7 - Negative deviations

Category/Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
No negative deviations were observed in this study at either preincubation time				

Positive deviations are listed in Table 8.

Table 8 - Positive deviations for UHT treated milk and dairy alternative drinks cateogry

Category/Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
preincubation time = 48h				
UFIT strawberry protein shake	54	+ve	n/a	*Klebsiella oxytoca*/2.8
Muller Frijj fudge brownie milk	59	+ve	n/a	*Paenibacillus macerans*/ 1.0
preincubation time = 72h				
UFIT strawberry protein shake	54	+ve	n/a	*Klebsiella oxytoca*/2.8
Muller Frijj fudge brownie milk	59	+ve	n/a	*Paenibacillus macerans*/ 1.0

In addition to the analysis with the Soleris commerical sterilty testing vials (NF105), the samples were also plated out on mPCA after the incubation time to determine if viable organisms were present in the sample. Plate counts carried out on the incubated samples revealed that viable colonies grew on the plates at a level of at least 10^1 cfu per ml showing that viable organisms were present in the sample added to the NF105 vials.

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

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

Category	Negative Deviations (ND[1])	Positive Deviations (PD)	ND-PD	Acceptability Limit (AL)	Comment
UHT treated milk and dairy alternative drinks	0	2	-2	3	Pass
Total	0	2	-2	3	Pass

[1] ND: including PPND

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

Category	Negative Deviations (ND[1])	Positive Deviations (PD)	ND-PD	Acceptability Limit (AL)	Comment
UHT treated milk and dairy alternative drinks	0	2	-2	3	Pass
Total	0	2	-2	3	Pass

[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) for both incubation times (48h and 72h pre-incubation).

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 11.

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

Category	Type	Strain	Reference number	Strain origin	Seeding or spiking procedure
UHT treated milk and dairy alternative liquid products	UHT whole milk	*Pantoea agglomerans*	CRA 17030, NCIMB 702072	Pasteurised milk	n/a spiking protocol used – heat stress 55°C for 5min

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 0.5-1cfu per L carton used in order to get 20 test portions providing fractional recovery,
- Higher level: One inoculated between 3-5 cfu per L carton used in order to get 5 test portions contaminated at a higher level.

Artificial contamination of samples was carried out by seeding with heat shocked cells of *Pantoea agglomerans* prior to analysis.

3.2.3 RLOD study results

The tabulated raw data on the RLOD study are given in Annex G.

The RLOD calculations were performed using the Excel spread sheet (version3 15-08-2015) of the international standard as described in ISO 16140-2: 2016.

The RLOD per Category is given in Table 12

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 alternativ method results
UHT treated milk and dairy alternative drinks 48h preincubation	1.315	Confirmation not required with this method
UHT treated milk and dairy alternative drinks 72h preincubation	0.553	Confirmation not required with this method

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.htm

The LOD50 per Category is given in Table 13

Table 12 – Presentation of LOD50 after confirmation of the alternative method results

Type (Category)	**LOD50 cfu per portion**	**Lower confidence limit cfu per portion**	**Upper confidence limit cfu per portion**
UHT treated milk and dairy alternative drinks 48h preincubation	0.821	0.469	1.437
UHT treated milk and dairy alternative drinks 72h preincubation	0.365	0.209	0.637
UHT treated milk and dairy alternative drinks Reference method	0.567	0.328	0.980

3.2.4 Conclusion RLOD study

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for the single category tested at both pre incubation levels (48h and 72h).

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 were freshly cultured in an appropriate media and incubated at an appropriate temperature. Dilutions were made in order to inoculate 1 -5 CFU per 1L carton. Spores were used to inoculate the vials of the isolates known to be spore forming bacteria (isolates 11-25). The study used 1L cartons of whole UHT milk for the pre incubation step.

The alternative method protocol was then performed.

Exclusivity: It was agreed with the MicroVal Techncial Committee meeting 24-25 June 2021 that as the method will detect all organisms capable of growing in a total viable count, that an exclusivity does not need to be carried out for this method.

Results inclusivity study

All raw data on inclusivity and exclusivity are given in Annex F.

A total of 50 strains were tested for **inclusivity**. All 50 of these isolates analysed showed the expected positive result.

3.3.2 Conclusion inclusivity and exclusivity study

The alternative Neogen Soleris commercial sterility testing vials detection method is selective and specific.

4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

In the sensitivity study, the observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for both incubation times (48h and 72h pre-incubation).

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for the single category tested at both pre incubation levels (48h and 72h).

The alternative Neogen Soleris commercial sterility testing vials detection method is selective and specific.

5 Interlaboratory study

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

5.1 Study organisation

Collaborator number
Samples were sent to 16 laboratories in 7 different countries.

Matrix and strain used
In this collaborative study one food type (UHT plant based milk) was evaluated. The milk was obtained from a local retailer and screened for commerical sterility using European Directive 92/46 annex C chapter 2 for ultra-high temperature (UHT) milk, using ISO 4833-1:2013 for the plate count at 30°C reference method. Samples were individually inoculated with *Staphylococcus saprophyticus* CRA 314.

Samples were inoculated on 25/04/2022, as described below:

- 48 blind coded samples per collaborator were prepared for analysis by the Soleris commercial sterility testing vials (NF-105) method and by the reference method European Directive 92/46 annex C chapter 2 for ultra-high temperature (UHT) milk using ISO 4833-1:2013 for the plate count at 30°C.
- 1 water flask labelled “Temperature Control” with the samples to check that the temperature conditions during transit.

All the samples were individually inoculated at the required level in 250ml cartons.

The samples were stored chilled at 4°C and the analyses was started on Monday 02 May 2022. Stability studies had been conducted to show that the required level of target organisms would be present after 7 and 8 days chill storage. The expert lab analysed a set of samples on Monday 02 May 2022.

Sample analysed with the alternative method were pre incubated at 30°C for 48h only prior to addtion into the Soleris vials. The shorter preincubation time was considered to be the most challenging parameters for sample analysis for the ILS.

Inoculation
The target inoculation levels were:

- Level 0: 0 CFU/250ml
- Level 1: 1.1 CFU/250ml
- Level 2: 4.3 CFU/250ml

Each laboratory received 48 samples of 250ml, i.e. 8 samples per inoculation level and method.

Labelling and shipping
Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories. A temperature control flask and a temperature probe were added to the package in order to register the temperature profile during the transport, package delivery. Samples were despatched on Tuesday 25th April and stored in the refrigerator at 2-8°C upon arrival, ready for analysis on Monday 2nd May.

5.2 Experimental parameters controls

5.2.1 Commercial sterility of the matrix before inoculation
In order to detect the presence of organisms in the sample the reference method was performed on six 250ml cartons before the inoculation. All the results were negative.

5.2.2 Strain stability during transport
The stability study was carried out with 18 samples inoculated at L2 and 18 samples inoculated L2 level of contamination (1.0 and 3.3 cfu per 250ml carton). Triplicate samples were removed from storage after 0, 7 days and 8 days storage at 4°C for tesing for commercial sterility with either the reference method or alternative method.

The results from the stability trials are shown in Table 13 below

Table 13 - Commercial sterility in the matrix

L1 samples						
	Reference method (detection) – 1 cfu/sample			Alternative method (detection) – 1 cfu/sample		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Day 0	detected	detected	detected	detected	detected	not detected
Day 7	detected	not detected	detected	detected	not detected	detected
Day 8	not detected	detected	detected	detected	detected	not detected
L2 samples						
	Reference method (detection) – 3.3 cfu/sample			Alternative method (detection) – 3.3 cfu/sample		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Day 0	detected	detected	detected	detected	detected	detected
Day 7	detected	detected	detected	detected	detected	detected
Day 8	detected	detected	detected	detected	detected	detected

5.2.3 Contamination levels

The samples prepared for the ILS were inoculated as follows.

A culture of *Staphylococcus saprophyticus* CRA 314 was grown overnight in Tryptone Soya Broth incubated at 30°C. The levels in the culture were checked by plating out on count agar and the *S. saprophyticus* culture was chilled overnight for use in inoculating samples on 25 April 2022.

The overnight culture was diluted such that L1 samples were inoculated at a level of 1.1 CFU/250ml carton and L2 were inoculated with a level of 4.3 CFU/250ml carton on 25 April 2022.

5.2.4 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 14.

Table 14 - 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	Not received	6	29/04/2022 12:00	Two samples leaking	02/05/2022
2	Not received	8.5	03/05/2022 14:00	Good	03/05/2022
3	Not received	4	28/04/2022 16:00	ok	02/05/2022
4	Samples not received – held at customs				
5	Samples not received – held at customs				
6	Samples not received – held at customs				
7	Data not returned				
8	Not received	6.5	02/05/2022 11:30	ok	02/05/2022
9	5.6	3.5	28/04/2022 11:15	intact	02/05/2022

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
10	Not received	7	28/04/2022 13:00	Samples M32, M39, M44 damaged	02/05/2022
11	Not received	5.6	28/04/2022 14:00	Good	02/05/2022
12	Not received	9.8	28/04/2022 07:00	Fine, no broken samples	02/05/2022
13	Not received	6.8	28/04/2022 12:00	M9, M14, M18 leaking	
14	Samples not received – held at customs				
15	Not received	20.5	02/05/2022 13:00	Sample 14 damaged	02/05/2022
16	5.5	6	29/04/2022 12:00	Two samples leaking	02/05/2022

Four labs did not receive their samples before the experiment start date due to delays at customs. A further three labs also received their samples delayed, but in time for analysis.

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 15.

Table 15– Results obtained by the expert lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	5/8	5/8
L2	8/8	7/8

5.3.2 Results obtained by the collaborative laboratories

- *Commercial sterility determination*

16 collaborators participated to the study. The results obtained by the individual collaborators in the inter-laboratory study are summarised in Table 16 (reference method) and Table 17 (alternative method). Additional analysis carried out to confirm the alternative method was a check of the curve characteristics obtained for each sample. Samples with a detection time of 24h with the shape of curve being typical for *S. saprophyticus* were considered to be a positive result.

Table 16 - Positive results by the reference method (ALL the collaborators)

Collaborator	Contamination level		
	L0	**L1**	**L2**
1	1/8	5/8	8/8
2	0/8	1/8	3/8
3	1/8	7/8	7/8
4	No results received		
5	No results received		
6	No results received		
7	0/8	4/8	5/8
8	0/8	5/8	6/8
9	0/8	5/8	6/8
10	0/8	2/8	2/8
11	1/8	6/8	2/8
12	3/8	3/8	4/8
13	0/8	4/8	7/8
14	No results received		
15	1/8	5/8	8/8
16	0/8	3/8	6/8
TOTAL	**P_0 =** 7/96	**P_1 =** 50/96	**P_2=** 64/96

Table 17 **- Positive results (before and after confirmation) by the alternative method after 48hrs (ALL the collaborators)**

Collaborators	Contamination level					
	L0		**L1**		**L2**	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
1	0/8	0/8	4/8	4/8	8/8	8/8
2	0/8	0/8	5/8	5/8	7/8	7/8
3	0/8	0/8	4/8	4/8	8/8	8/8
4	No results received					
5	No results received					
6	No results received					
7	1/8	1/8	5/8	5/8	8/8	8/8
8	1/8	1/8	2/8	2/8	5/8	5/8
9	0/8	0/8	4/8	4/8	6/8	6/8
10	1/8	1/8	4/8	4/8	7/8	7/8
11	0/8	0/8	2/8	2/8	5/8	5/8

Collaborators	**Contamination level**					
	L0		**L1**		**L2**	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
12	0/8	0/8	5/8	5/8	8/8	8/8
13	0/8	0/8	3/8	3/8	8/8	8/8
14	No results received					
15	4/8	2/8	7/8	7/8	8/8	8/8
16	0/8	0/8	5/8	5/8	8/8	8/8
TOTAL	P_0 = 7/96	CP_0 = 5/96	P_1 = 50/96	CP_1 = 50/96	P_2 = 86/96	CP_2 = 86/96

Labs 10 and 15 were removed from the sample sets retained for interpretation due to the high temperature of samples at receipt for lab 15 and the swapping of sample sets by lab 10.

During the study there were blank samples that gave a positive result for either the reference method or the alternative method. The most probable cause for these results was cross contamination during analysis.

5.3.3 Results of the collaborators retained for interpretation

The results obtained with 10 collaborators kept for interpretation are presented in Table 18 (reference method) and Table 19 (alternative method).

Table 18 - Positive results by the reference method (Without Lab 10 and 15)

Collaborator	Contamination level		
	L0	**L1**	**L2**
1	1/8	5/8	8/8
2	0/8	1/8	3/8
3	1/8	7/8	7/8
7	0/8	4/8	5/8
8	0/8	5/8	6/8
9	0/8	5/8	6/8
11	1/8	6/8	2/8
12	3/8	3/8	4/8
13	0/8	4/8	7/8
14	No results received		
16	0/8	3/8	6/8
TOTAL	**P_0 =** 6/80	**P_1 =** 43/80	**P_2=** 54/80

Table 19 - Positive results (before and after confirmation) by the alternative methods (Without Lab 10 and 15)

Collaborators	Contamination level					
	L0		**L1**		**L2**	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
1	0/8	0/8	4/8	4/8	8/8	8/8
2	0/8	0/8	5/8	5/8	7/8	7/8
3	0/8	0/8	4/8	4/8	8/8	8/8
7	1/8	1/8	5/8	5/8	8/8	8/8
8	1/8	1/8	2/8	2/8	5/8	5/8
9	0/8	0/8	4/8	4/8	6/8	6/8
11	0/8	0/8	2/8	2/8	5/8	5/8
12	0/8	0/8	5/8	5/8	8/8	8/8
13	0/8	0/8	3/8	3/8	8/8	8/8
14	No results received					
16	0/8	0/8	5/8	5/8	8/8	8/8
TOTAL	**P_0** = 2/80	**CP_0** = 2/80	**P_1** = 39/80	**CP_1** = 39/80	**P_2** = 71/80	**CP_2** = 71/80

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 20).

Table 20- Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	92.5%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	97.5%

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) only and this was used for the calculations.

A summary of the results of the collaborators retained for interpretation, and obtained with the reference and the alternative method for Level 1 is provided in **Table 21.**

Table 21- Summary of the obtained results with the reference and the alternative method for L_1

Response	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 21**	Positive deviation (R-/A+) **PD = 19**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 21**	Negative agreement (A-/R-) **NA = 19**

Based on the data summarized in **Table 21**, 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 22**).

Table 22- Sensitivity, relative trueness and false positive ratio percentages

Calculation	Formula	L_1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)}\ x\ 100\% =$	66.7%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)}\ x\ 100\% =$	68.8%
Relative trueness	$RT = \frac{(PA+NA)}{N}\ x\ 100\% =$	50.0%
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 23 for Level 1 and in Table 23 for Level 2.

The positive deviations are listed in Table 25 for Level 1 and in Table 26 for Level 2.

Table 23 **- Negative deviations for Level 1**

Category	**Type**	**Sample nº**	**Alternative method results**	**(additional) Confirmatory test results**	**Inoculation (CFU/Sample)**
Number of negative deviations = 21					
UHT treated dairy and plant drinks	Plant drink	1M35/11	-ve	n/a	1.6
		1M47/23	-ve	n/a	
		3M35/11	-ve	Curve analysis and colony count	
		3M38/14	-ve	n/a	
		3M41/17	-ve	n/a	
		7M47/23	-ve	n/a	
		8M32/8	-ve	n/a	
		8M44/20	-ve	n/a	
		8M47/23	-ve	n/a	
		9M35/11	-ve	n/a	
		9M41/17	-ve	n/a	
		9M44/20	-ve	n/a	
		11M35/11	-ve	n/a	
		11M38/14	-ve	n/a	
		11M20/44	-ve	n/a	
		11M47/23	-ve	n/a	

Category	Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
		12M47/23	-ve	n/a	
		13M35/11	-ve	n/a	
		13M44/20	-ve	n/a	
		13M47/23	-ve	n/a	
		16M32/8	-ve	n/a	

Table 24- Negative deviations for Level 2

Category	Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
Number of negative deviations = 5					
UHT treated dairy and plant drinks	Plant drink	8M42/8	-ve	n/a	4.3
		8M45/21	-ve	n/a	
		9M42/18	-ve	n/a	
		9M48/24	-ve	n/a	
		11M42/18	-ve	n/a	

Table 25 - Positive deviations for Level 1

Category	Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
Number of positive deviations = 18					
UHT treated dairy and plant drinks	Plant drink	1M44/20	+ve	n/a	1.1
		2M26/2	+ve		
		2M35/11	+ve		
		2M44/20	+ve		
		2M47/23	+ve		
		7M41/17	+ve		
		7M44/20	+ve		
		9M38/14	+ve		
		9M47/23	+ve		
		11M32/8	+ve		
		12M26/2	+ve		
		12M29/5	+ve		
		12M32/8	+ve		
		13M26/2	+ve		
		13M32/8	+ve		
		16M35/11	+ve		
		16M41/17	+ve		

Category	Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
		16M44/20	+ve		

Table 26- Positive deviation for Level 2

Category	Type	Sample nº	Alternative method results	(additional) Confirmatory test results	Inoculation (CFU/Sample)
Number of positive deviations = 19					
UHT treated dairy and plant drinks	Plant drink	2M27/3	+ve	n/a	4.3
		2M36/12	+ve		
		2M45/21	+ve		
		2M48/24	+ve		
		3M27/3	+ve		
		7M30/6	+ve		
		7M36/12	+ve		
		7M42/18	+ve		
		8M30/6	+ve		
		9M33/9	+ve		
		9M36/12	+ve		
		11M30/6	+ve		
		11M36/12	+ve		
		11M39/15	+ve		
		11M45/21	+ve		
		12M27/3	+ve		
		12M30/6	+ve		
		12M33/9	+ve		
		12M36/12	+ve		
		13M27/3	+ve		
		16M45/21	+ve		
		16M48/24	+ve		

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 $[(ND - PD)_{max}]$ 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.

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

where

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

Calculations for L_1

Completing the calculation for L_1 where $P_x = 42$, $CP_x = 39$ and $N_x = 80$ gives an $(\text{ND-PD})_{\max}$ of 10.96.

The ND-PD for L_1 of 3 meets the $(\text{ND-PD})_{\max}$ value of 10.96.

Calculations for L_2

Completing the calculation for L_1 where $P_x = 54$, $CP_x = 71$ and $N_x = 80$ gives an $(\text{ND-PD})_{\max}$ of 9.35

The ND-PD for L_2 of -17 meets the $(\text{ND-PD})_{\max}$ value of 9.35.

The ISO 16140-2 (2016) requirements are fulfilled as (ND - PD)max 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 27).

Table 27 **– RLOD**

RLOD	***RLODL***	***RLODU***	***Confidence interval***
0.66	***0.51***	***0.86***	***90%***

6 CONCLUSION

The **method comparison study conclusions** are:

In the sensitivity study, the observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for both incubation times (48h and 72h pre-incubation).

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 2.5 for unpaired studies, for the single category tested at both pre incubation levels (48h and 72h).

The alternative Neogen Soleris commercial sterility testing vials detection method is selective and specific.

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

The data shows no significant differences between the reference method and the alternative method.

The ND-PD value meets the AL criteria of $ND\text{-}PD_{max}$ L_1 level and L_2 level.

The data and interpretations comply with the EN ISO 16140-2:2016 requirements.

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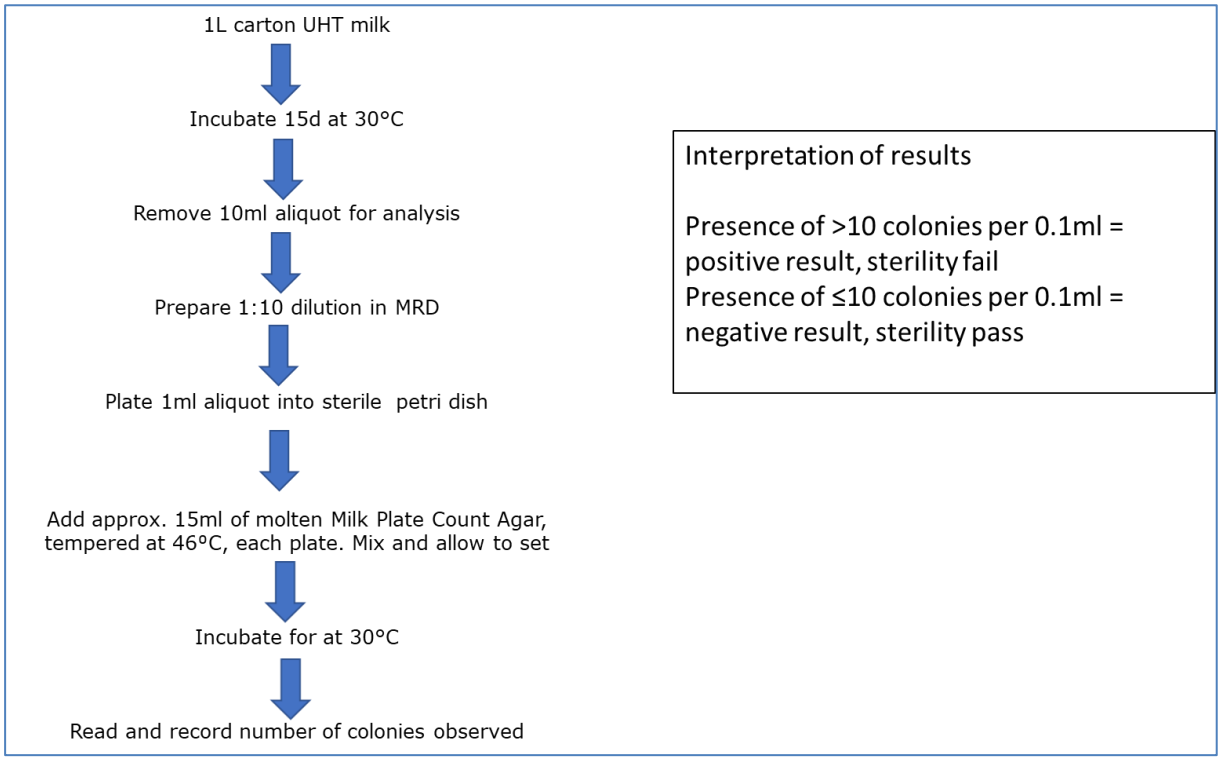
The Neogen Soleris commercial sterility testing vial (NF105) is considered equivalent to the Reference method.

Date, 21 July 2022

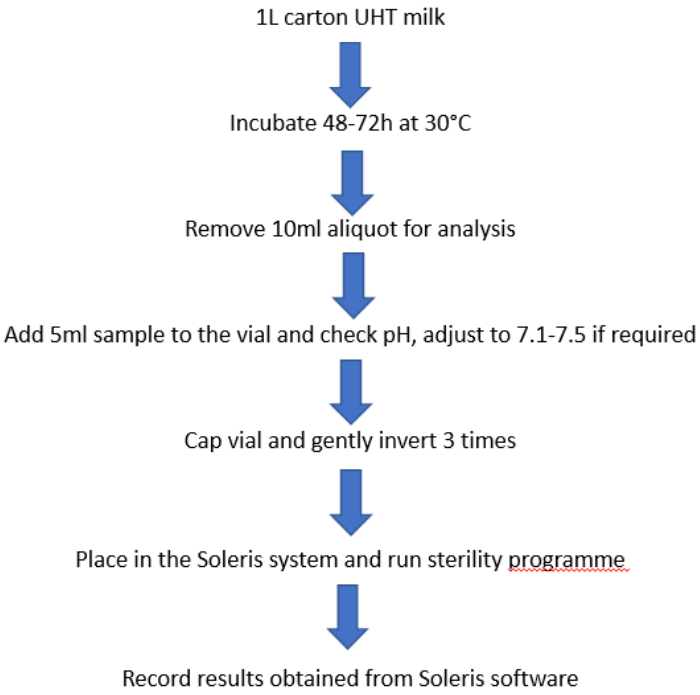
Signature Suzanne Jordan

Dr. Suzanne Jordan, Campden BRI

ANNEX A: Flow diagram of the reference method = European Directive 92/46 annex C chapter 2 for ultra-high temperature (UHT) milk, using ISO 4833-1:2013 for the plate count at 30°C.



ANNEX B: Flow diagram of the alternative method = Soleris commercial sterility vials



ANNEX C: Kit insert(s)

Please refer to separate pdf for details

ANNEX D: Artificial contaminations

item	Sample no	Strain	code	source	Injury protocol	Injury evaluation (log10)	Level inoculated cfu per carton	Ref method final result	Alternative method final result	
									48h	72h
Type = Type = UHT Dairy liquid products										
Delamere dairy sterilised skimmed milk	**11**	*Pantoea agglomerans*	17030, NCIMB 702072	Pasteurised milk	Heat treatment at 55° for 5min	0.5	2.3	+	+	+
Morrisons long life British whole milk	**12**	*Pantoea agglomerans*	17030, NCIMB 702072	Pasteurised milk	Heat treatment at 55° for 5min	0.5	2.3	+	+	+
Cowbelle semi skimmed long life milk	**13**	*Enterococcus malodoratus*	16860	Gouda cheese	Heat treatment at 55° for 5min	1	1.6	+	+	+
Łaciate uht milk	**14**	*Enterococcus malodoratus*	16860	Gouda cheese	Heat treatment at 55° for 5min	1	1.6	+	+	+
Viva whole long life milk	**15**	*Torulaspora delbrukeii*	16154	Spoiled yogurt	Heat treatment at 55° for 5min	1	2.8	+	+	+
Viva skimmed long life milk	**16**	*Bacillus subtilis*	6134	custard	100mM hydrogen peroxide for 10min	0.5	4.0	+	+	+
lactofree whole UHT milk	**17**	*Bacillus subtilis*	6134	custard	100mM hydrogen peroxide for 10min	0.5	4.0	+	+	+
Delamere dairy sterilised whole milk	**18**	*Clostridium perfringens*	15911, NCTC 8239	Salt beef	Heat treatment at 55° for 5min	1	1.8	+	+	+
Dairy Manor skimmed long life milk	**19**	*Bacillus coagulans*	353	beans in tomato sauce	Heat treament 65°C for 10mins	0.3	2.2	+	+	+
Dairy Manor semi skimmed long life milk	**20**	*Bacillus coagulans*	353	beans in tomato sauce	Heat treament 65°C for 10mins	0.3	2.2	+	+	+
Type = UHT plant drinks										
Good hemp creamy seed drink	**28**	*Micrococcus luteus*	3503	Air sample	Heat treament 60°C for 10mins	0.7	1.9	+	+	+

Mighty Pea M.lk unsweetened	**32**	*Micrococcus luteus*	3503	Air sample	Heat treament 60°C for 10mins	0.7	1.7	+	+	+
Mighty Pea M.lk	**33**	*Candida krussei*	CRA629	Yogurt base	Heat treament 65°C for 5mins	1	1.0	+	+	+
Tesco long life soya drink unsweetened	**34**	*Candida krussei*	CRA629	Yogurt base	Heat treament 65°C for 5mins	1	1.0	+	+	+
Morrisons almond UHT	**35**	*Kluyvera ascorbata*	17126	industrial	100mM hydrogen peroxide for 10min	1	1.7	+	+	+
Morrisons savers long life sweetened soya milk	**36**	*Brevibacillus parabrevis*	7750	unknown	Heat treament 65°C for 10mins	1	1.8	+	+	+
Alpro single soya UHT alternative to cream	**37**	*Brevibacillus parabrevis*	7750	unknown	Heat treament 65°C for 10mins	1	1.8	+	+	+
Rude health Organic unsweetened oat drink	**38**	*Byssochlamys fulva*	CRA16668; CBS113245	Pasteurised fruit juice	100mM hydrogen peroxide for 15min	0.5	1.3	+	+	+
Morrisons oat uht	**39**	*Byssochlamys fulva*	CRA16668; CBS113245	Pasteurised fruit juice	100mM hydrogen peroxide for 15min	0.5	1.3	+	+	+
Alpro cashew long life drink	**40**	*Byssochlamys fulva*	CRA16668; CBS113245	Pasteurised fruit juice	100mM hydrogen peroxide for 15min	0.5	1.3	+	+	+
Type = UHT flavoured milks and drinks										
Galaxy chocolate flavoured milk drink	**51**	*Listeria innocua*	3130	Cheese factory	Heat treatment at 55° for 5min	0.7	1.0	+	+	+
For goodness shakes strawberry protein drink	**52**	*Listeria innocua*	3130	Cheese factory	Heat treatment at 55° for 5min	0.7	1.0	+	+	+
For goodness shakes salted caramel protein drink	**53**	*Aeromonas hydrophila*	8388, NCTC 8049	tin of milk with a fishy odour	100mM hydrogen peroxide for 10min	1	2.8	+	+	+
UFIT strawberry protein shake	**54**	*Klebsiella oxytoca*	8387	Water	100mM hydrogen peroxide for 10min	1	2.8	-	+	+

UFIT salted caramel protein shake	**55**	*Klebsiella oxytoca*	8387	Water	100mM hydrogen peroxide for 10min	1	1.8	+	+	+
UFIT chocolate protein shake	**56**	*Bacillus weihenstephanensis*	16578	Pasteurised milk	100mM hydrogen peroxide for 20min	0.3	1.8	+	+	+
UFIT vanilla protein shake	**57**	*Bacillus weihenstephanensis*	16578	Pasteurised milk	100mM hydrogen peroxide for 20min	0.3	2.0	+	+	+
Muller Frijj strawberry milk	**58**	*Bacillus pumilus*	655	chilled chicken in white wine sauce	500mM/10mins	0.5	1.4	+	+	+
Muller Frijj fudge brownie milk	**59**	*Paenibacillus macerans*	16488, DSM 357	Unknown,	Heat treament 65°C for 5mins	1	1.0	-	+	+
Muller Frijj chocolate milk	**60**	*Paenibacillus macerans*	16488, DSM 357	Unknown,	Heat treament 65°C for 5mins	1	1.0	+	+	+

ANNEX E: Raw data sensitivity study

Item	Sample No	Reference method		Alternative method Soleris NF 105 vials					
				48h incubation			72h incubation		
		ISO plate count cfu per *	final result	detection time	Final result	call	detection time	Final result	call
Type = Type = UHT Dairy liquid products									
Tesco skimmed UHT milk	1	<1	-	nd	-	NA	nd	-	NA
Tesco semi skimmed UHT milk	2	2	-	nd	-	NA	nd	-	NA
Waitrose long life skimmed milk	3S	<1	-	nd	-	NA	nd	-	NA
Lactose free semi skimmed UHT milk	4	<1	-	nd	-	NA	nd	-	NA
Creamfields UHT semi skimmed milk	5	<1	-	nd	-	NA	nd	-	NA
Waitrose long life semi skimmed milk	6S	<1	-	nd	-	NA	nd	-	NA
Morrisons long life British semi skimmed milk	7	<1	-	nd	-	NA	nd	-	NA
Delamere dairy sterilised semi skimmed milk	8	<1	-	nd	-	NA	nd	-	NA
M&S long life semi skimmed milk	9S	<1	-	nd	-	NA	nd	-	NA
Morrisons long life British skimmed milk	10	<1	-	nd	-	NA	nd	-	NA
Delamere dairy sterilised skimmed milk	**11**	8.00E+06	+	6.8	+	PA	6.8	+	PA
Morrisons long life British whole milk	**12**	4.40E+08	+	6.8	+	PA	8.8	+	PA
Cowbelle semi skimmed long life milk	**13**	1.70E+06	+	6.8	+	PA	6.8	+	PA
Łaciate uht milk	**14**	2.00E+07	+	22.5	+	PA	6.8	+	PA
Viva whole long life milk	**15**	1.30E+05	+	13.6	+	PA	17.4	+	PA

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Soleris commercial sterility testing vials (NF105) summary report

Item	Sample No	Reference method		Alternative method Soleris NF 105 vials					
				48h incubation			72h incubation		
		ISO plate count cfu per *	final result	detection time	Final result	call	detection time	Final result	call
Viva skimmed long life milk	**16**	1.40E+05	+	6.8	+	PA	6.8	+	PA
lactofree whole UHT milk	**17**	1.50E+07	+	23.3	+	PA	6.8	+	PA
Delamere dairy sterilised whole milk	**18**	2.00E+08	+	6.8	+	PA	6.8	+	PA
Dairy Manor skimmed long life milk	**19**	2.30E+04	+	6.7	+	PA	6.8	+	PA
Dairy Manor semi skimmed long life milk	**20**	1.80E+08	+	6.7	+	PA	6.8	+	PA
Type = UHT plant drinks									
Rice dream original organic drink	21	1	-	nd	-	NA	nd	-	NA
Oatly long life drink	22	<1	-	nd	-	NA	nd	-	NA
Koko dairy free original drink	23	<1	-	nd	-	NA	nd	-	NA
Alpro Junior Growing Up soya drink	24	<1	-	nd	-	NA	nd	-	NA
Alpro almond no sugars long life drink	25	<1	-	nd	-	NA	nd	-	NA
Alpro long life oat drink	26	<1	-	nd	-	NA	nd	-	NA
Alpro coconut long life alt drink	27	<1	-	nd	-	NA	nd	-	NA
Alpro barista almond long life drink	29	<1	-	nd	-	NA	nd	-	NA
Alpro oat no sugars long life drink	30	<1	-	nd	-	NA	nd	-	NA
Alpro plant protein original soya drink	31	<1	-	nd	-	NA	nd	-	NA
Good hemp creamy seed drink	**28**	1.20E+07	+	6.8	+	PA	6.8	+	PA
Mighty Pea M.lk unsweetened	**32**	1.20E+07	+	6.8	+	PA	8.1	+	PA
Mighty Pea M.lk	**33**	2.00E+08	+	6.8	+	PA	6.8	+	PA
Tesco long life soya drink unsweetened	**34**	41	+	10.1	+	PA	6.8	+	PA

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Item	Sample No	Reference method		Alternative method Soleris NF 105 vials					
				48h incubation			72h incubation		
		ISO plate count cfu per *	final result	detection time	Final result	call	detection time	Final result	call
Morrisons almond UHT	**35**	1.10E+03	+	7.5	+	PA	6.8	+	PA
Morrisons savers long life sweetened soya milk	**36**	4.60E+07	+	6.7	+	PA	6.8	+	PA
Alpro single soya UHT alternative to cream	**37**	4.00E+06	+	6.7	+	PA	6.8	+	PA
Rude health Organic unsweetened oat drink	**38**	2.00E+05	+	18.7	+	PA	9.9	+	PA
Morrisons oat uht	**39**	5.00E+04	+	7.5	+	PA	11.6	+	PA
Alpro cashew long life drink	**40**	8.00E+04	+	6.8	+	PA	6.8	+	PA
Type = UHT flavoured milks and drinks									
Oatly chocolate long life drink	41	<1	-	nd	-	NA	nd	-	NA
Alpro plant protein chocolate flavour soya drink	42	<1	-	nd	-	NA	nd	-	NA
Yazoo chocolate milk	43	<1	-	nd	-	NA	nd	-	NA
Yazoo strawberry milk	44	<1	-	nd	-	NA	nd	-	NA
Yazoo banana milk	45	<1	-	nd	-	NA	nd	-	NA
400ml Yazoo chocolate orange	46	<1	-	nd	-	NA	nd	-	NA
400ml Mars milk no added sugar	47	<1	-	nd	-	NA	nd	-	NA
Mighty Shake Chocolate 330M	48	<1	-	nd	-	NA	nd	-	NA
Muller Frijj cookie dough milk	49S	<1	-	nd	-	NA	nd	-	NA
For goodness shakes recovery vanilla flavour	50	<1	-	nd	-	NA	nd	-	NA
Galaxy chocolate flavoured milk drink	**51**	2.20E+08	+	6.8	+	PA	6.8	+	PA
For goodness shakes strawberry protein drink	**52**	6.50E+07	+	6.8	+	PA	6.8	+	PA
For goodness shakes salted caramel protein drink	**53**	3.40E+04	+	6.8	+	PA	6.8	+	PA

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Soleris commercial sterility testing vials (NF105) summary report

Item	Sample No	Reference method		Alternative method Soleris NF 105 vials					
				48h incubation			72h incubation		
		ISO plate count cfu per *	final result	detection time	Final result	call	detection time	Final result	call
UFIT strawberry protein shake	**54**	<1	-	6.8	+	PD	6.8	+	PD
UFIT salted caramel protein shake	**55**	3.90E+07	+	6.8	+	PA	6.8	+	PA
UFIT chocolate protein shake	**56**	6.90E+05	+	6.7	+	PA	6.8	+	PA
UFIT vanilla protein shake	**57**	4.00E+06	+	6.7	+	PA	6.8	+	PA
Muller Frijj strawberry milk	**58**	60	+	24.5	+	PA	6.8	+	PA
Muller Frijj fudge brownie milk	**59**	<1	-	17.3	+	PD	6.8	+	PD
Muller Frijj chocolate milk	**60**	1.30E+04	+	6.7		PA	19.1		PA

ANNEX F Raw data on inclusivity

Sample No	Species	Genus	CRA code	Source	Level inoculated cfu per carton	Alternative method Soleris NF 105 vials detection time/ h	
						48h incubation	72 hour incubation
1	*Raoultella*	*terrigena*	17343	raw milk	3.8	9.7	6.8
2	*Enterobacter*	*cloacae*	1472	dried milk	4.1	6.8	N/A
3	*Klebsiella*	*oxytoca*	8387	Water	4.2	6.8	N/A
4	*Kluyvera*	*ascorbata*	17126	industrial	2.3	9.5	N/A
5	*Escherichia*	*adecarboxylata*	5501	Skim milk powder	2.4	6.8	N/A
6	*Klebsiella*	*trevisanii*	NCIMB 8606	Ropy cream	2.1	6.8	N/A
7	*Pantoea*	*agglomerans*	17030, NCIMB 702072	Pasteurised milk	4.2	6.8	N/A
8	*Aeromonas*	*hydrophila*	8388, NCTC 8049	tin of milk with a fishy odour	4.3	10.9	N/A
9	*Escherichia*	*coli*	1476	Dried milk	3.8	8.1	N/A
10	*Rahnella*	*aqualtilis*	16911	drinking water	4.5	6.8	N/A
11	*Bacillus*	*coagulans*	16586	beans in tomato sauce	4.3	6.7	N/A
12	*Bacillus*	*subtilis*	16597	custard	4.2	6.7	N/A
13	*Bacillus*	*weihenstephanensis*	16578	Pasteurised milk	3.7	6.8	N/A
14	*Bacillus*	*polymyxa*	16652	chilled chicken in white wine sauce	4.6	6.7	N/A
15	*Bacillus*	*cereus*	7746	milk or cream	4.8	6.7	N/A

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Sample No	Species	Genus	CRA code	Source	Level inoculated cfu per carton	Alternative method Soleris NF 105 vials detection time/ h	
						48h incubation	72 hour incubation
16	*Bacillus*	*pseudomycoides*	6317	soil in Sweden	4.1	6.7	N/A
17	*Bacillus*	*pumilus*	16594	chilled chicken in white wine sauce	4.1	6.7	N/A
18	*Lysinibacillus*	*sphaericus*	7757	unknown	3.7	6.7	N/A
19	*Clostridium*	*perfringens*	15911, NCTC 8239	Salt beef	4.7	6.7	N/A
20	*Bacillus*	*licheniformis*	16588	pesto sauce	3.8	31.1	6.8
21	Paenibacillus	macerans	16488	unknown	1	33.8	ND
22	*Brevibacillus*	*brevis*	7748	unknown	2	6.8	6.8
23	*Aneurinibacillus*	*aneurinolyticus*	7751	unknown	2	23.3	N/A
24	*Paenibacillus*	*pabuli*	16605	barley	3	6.8	N/A
25	*Brevibacillus*	*aigri*	16606	unknown	1	6.8	ND
26	*Staphylococcus*	*carnosus*	1123	goat's milk	4.8	6.8	N/A
27	*Listeria*	*ivanovii*	16045, NCIMB 8510	soft cheese	4.3	6.8	N/A
28	*Streptococcus*	*thermophilus*	7675	Pasteurised milk	4.1	6.7	N/A
29	*Lactobacillus*	*acidophilus*	3910	Dairy product	3.2	17.9	N/A
30	*Carnobacterium*	*divergens*	8999	Brie	2.3	6.8	N/A
31	*Staphylococcus*	*saprophyticius*	3503	distilled water environmental	3	6.8	N/A

Sample No	Species	Genus	CRA code	Source	Level inoculated cfu per carton	Alternative method Soleris NF 105 vials detection time/ h	
						48h incubation	72 hour incubation
32	*Micrococcus*	*luteus*	1513	Air sample	2.1	9	N/A
33	*Enterococcus*	*faecalis*	272	Dried milk powder	3.3	6.8	N/A
34	*Staphylococcus*	*cohnii*	409/3026	skin	4	6.8	N/A
35	*Staphylococcus*	*aureus*	314	Slow cheese	2.6	6.8	N/A
36	*Staphylococcus*	*epidermidis*	16030	runway & can seam	4.3	6.8	N/A
37	*Pediococcus*	*pentosaceus*	1100	Brine	4.6	16.2	N/A
38	*Listeria*	*monocytogenes 1/2a*	3130	Stilton	3.6	6.8	N/A
39	*Listeria*	*innocua*	16828	Cheese factory	2.9	6.8	N/A
40	*Staphylococcus*	*hominis*	16029	unknown	3.3	6.8	N/A
41	*Lactococcus*	*lactis*	16659	Green ham	3.8	6.8	N/A
42	*Micrococcus*	*roseus*	7775	water	2.7	26.1	6.7
43	*Streptococcus*	*lactis*	1511	dried milk powder	4	6.8	N/A
44	*Enterococcus*	*malodoratus*	16860	Gouda cheese	2.4	6.7	N/A
45	*Enterococcus*	*pseudoavium*	16852	Cow udder - bovine mastitis	2.6	6.7	N/A
46	*Aureobasidium*	*pullulans*	CRA16148	Soft drinks factory	2.2	6.8	N/A
47	*Byssochlamys*	*fulva*	CRA16668; CBS113245	Pasteurised fruit juice	2.2	23.2	N/A
48	*Candida*	*krussei*	CRA629	Yogurt base	4.7	6.8	N/A

Sample No	Species	Genus	CRA code	Source	Level inoculated cfu per carton	Alternative method Soleris NF 105 vials detection time/ h	
						48h incubation	72 hour incubation
49	*Kluyveromyces*	*marxianus*	CRA 6749	Dairy isolate	3.3	6.8	N/A
50	*Torulaspora*	*delbrukeii*	CRA16154	Spoiled yogurt	3.3	16.4	N/A

ANNEX G Raw data Relative Level of Detection

Sample	Category	Strain	Code	Source	Stress	Inoculum level cfu per carton	Sample no	Alternative method Soleris NF 105 vials				Reference method		
								48 hour incubation		72 hour incubation		Sample no	mPCA	final result
								detection time	final result	detection time	final result			
UHT whole milk	Blank	N/A	N/A	N/A	N/A	N/A	1	ND	-	ND	-	6	<1	-
UHT whole milk	Blank	N/A	N/A	N/A	N/A	N/A	2	ND	-	ND	-	7	<1	-
UHT whole milk	Blank	N/A	N/A	N/A	N/A	N/A	R53	ND	-	ND	-	R55	<1	-
UHT whole milk	Blank	N/A	N/A	N/A	N/A	N/A	4	ND	-	ND	-	8	<1	-
UHT whole milk	Blank	N/A	N/A	N/A	N/A	N/A	5	ND	-	ND	-	10	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	11	ND	-	6.8	+	31	<1	-

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Sample	Category	Strain	Code	Source	Stress	Inoculum level cfu per carton	Sample no	Alternative method Soleris NF 105 vials				Reference method		
								48 hour incubation		72 hour incubation		Sample no	mPCA	final result
								detection time	final result	detection time	final result			
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	12	ND	-	6.8	+	32	400	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	13	6.8	+	N/A	+	33	2	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	14	9.8	+	N/A	+	34	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	15	ND	-	ND	-	35	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	16	44.7	+	7.7	+	36	1.30E+05	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	17	9.9	+	N/A	+	37	8.00E+04	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	18	ND	-	7.4	+	38	2.30E+04	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	19	ND	-	ND	-	39	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	1	20	ND	-	ND	-	40	500	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	21	ND	-	6.8	+	41	1.10E+06	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	22	14.2	+	N/A	+	42	8.00E+06	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	23	ND	-	32.3	+	43	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	24	6.8	+	N/A	+	44	<1	-
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	25	6.8	+	N/A	+	45	1.50E+03	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	26	ND	-	6.8	+	46	8.00E+07	+

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Sample	Category	Strain	Code	Source	Stress	Inoculum level cfu per carton	Sample no	Alternative method Soleris NF 105 vials				Reference method		
								48 hour incubation		72 hour incubation		Sample no	mPCA	final result
								detection time	final result	detection time	final result			
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	27	6.8	+	N/A	+	47	2.40E+08	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	28	6.8	+	N/A	+	48	7.90E+05	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	29	6.8	+	N/A	+	49	7.60E+08	+
UHT whole milk	Low	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	0.65	30	6.8	+	N/A	+	50	1.60E+08	+
UHT whole milk	High	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	3.3	51	6.8	+	N/A	+	56	2.00E+07	+
UHT whole milk	High	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	3.3	52	6.8	+	N/A	+	57	1.90E+03	+
UHT whole milk	High	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	4.3	53	6.8	+	N/A	+	58	1.10E+06	+
UHT whole milk	High	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	4.3	54	6.8	+	N/A	+	59	6.70E+05	+
UHT whole milk	High	*Pantoea agglomerans*	17030	Pasteurised milk	Heat - 55 degrees/5mins	4.3	55	6.8	+	N/A	+	60	1.00E+09	+