NF VALIDATION - Validation of alternative analysis methods
Application to the food industry

Summary report
according to the standard EN ISO 16140-2:2016

Quantitative method

NEOGEN™ Petrifilm™ Rapid Aerobic Count Plate (RAC)
(certificate # 3M 01/17 – 11/16)
for the enumeration of mesophilic aerobic flora in milk powders and dairy products

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Version 0   October 18, 2024

Preamble

- **Protocols of validation:**

- **EN ISO 16140-1 and EN ISO 16140-2 (September 2016): Microbiology of the food chain — Method validation**
  Part 1: Vocabulary.
  Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

- **Requirements regarding comparison and interlaboratory studies for implementation of the standard EN ISO 16140-2 (Version 12).**

- **Reference method:**

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

- **Application scope:**

- **Milk powder and dairy products**.

- **Certification body:**

- **AFNOR Certification** (https://nf-validation.afnor.org/).

Definitions

- ## Method comparison study

The method comparison study is the part of the validation process that is performed in the expert laboratory. It consists of four parts:

- A comparative study of the results of the reference method to the results of the alternative method in a variety of different items (naturally and/or artificially) contaminated samples (so-called relative trueness study).
- A comparative study of the results of the reference method to the results of the alternative method in artificially contaminated samples using replicates of a single item per category. The data are analyzed using the accuracy profile (AP) approach (so-called AP study).
- An inclusivity/exclusivity study of the alternative method.

- ## Relative trueness study

The relative trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method.

The relative trueness is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples.

- ## Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method.

The accuracy profile is the graphical representation of the capacity of measurement of the quantitative method, obtained by combining acceptability intervals and β-expectation tolerance intervals, both reported to different levels of the reference value.

- ## Inclusivity and exclusivity study

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method.

The exclusivity study is a study involving pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.

- ## 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.

The aim of an interlaboratory study is to determine the variability of the results obtained in different laboratories using identical samples.

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1. Introduction

The NEOGEN™ Petrifilm Rapid Aerobic Count Plate was validated on the 25th of November 2016 for milk powders & dairy products according to the EN ISO 16140-2:2016 and the AFNOR Certification technical rules (Certificate number 3M 01/17 - 11/16). The alternative method was renewed in October 2020.

Table 1 summarizes the different steps of the validation that occurred since the initial validation.

Table 1: Steps of the validation AFNOR certification

Date	Study	Expert Laboratory	Standards
November 2016	Initial validation	ADRIA Développement	EN ISO 16140-2:2016
October 2020	First renewal	ADRIA Développement	EN ISO 16140-2:2016
October 2024	Second renewal	Laboratoire Microsept	EN ISO 16140-2:2016

All the results presented in this report were produced during the initial validation tests performed by ADRIA Développement within the framework of the brand NF VALIDATION according to the current requirements.

2. Protocols of the methods

2.1. Alternative method

2.1.1. Principle of the method

The protocols tested for the initial validation study for dairy products are the following:

28 h ± 2 h at 30°C ± 1°C for non-powdered dairy products,
48 h ± 3 h at 30°C ± 1°C for milk powders.

The workflow of the method is set out in Appendix A.

2.1.2. Restrictions

There are no restrictions for use for the Petrifilm™ Rapid Aerobic Count Plate (RAC) system.

2.2. Reference method

The reference method is the 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. PCA + milk (1 g/l) was used for enumeration.

The workflow of the reference method is presented in Appendix B.

3. Method comparison study

3.1. Relative trueness study

3.1.1. Number and nature of the samples

One food category was tested.
A total of 45 samples were thus analyzed and 37 exploited.

The distribution of the samples is presented in table 2.

Table 2 : number and nature of the samples analyzed

Category		Type	Analyzed samples	Exploited results
Milk powders and dairy products	a	Milk	6	6
	b	Dairy desserts	12	10
	c	Milk powders	27	21
		TOTAL	**45**	**37**
		TOTAL	**45**	**37**

3.1.2. Artificial contamination of the samples

Artificial contaminations were realized by inoculation of lyophilized strain for milk powders.
29 samples were naturally contaminated, and 8 samples were artificially contaminated.

The artificial contaminations performed are presented in the appendix C.

3.1.3. Results

The data are classified in three categories (See Table 3):

- Interpretable results with the reference and the alternative methods,
- Results with less than 4 colonies per plate with the reference and/or the alternative method (indicated with "*" in the data) in order to have a more precise result. These results are not included in the calculation.
- Results below or above the quantification limit: according to the ISO 16140- 2:2016, if any result (either reference or alternative method) is below the quantification limit, the data should be plotted using a substituted value of 1 log10 units less than the observed value in case of a lower than value. Similarly, any value greater than the upper limit should be amended by adding 1 log unit. These results are not included in the calculations but also appear on the graphs.

Raw results are shown in appendix D.

The table 3 is a summary of all the results obtained.

Table 3: summary of the results of the relative trueness study

Categories	Types	Samples analyzed	Number of samples <4 CFU/plate	Number of samples below or above the detection limit	Number of samples with no results	Interpretable results
Milk powders and dairy products	a	6	0	0	0	6
	b	12	1	1	0	10
	c	27	3	1	2	21
	TOTAL	45	4	2	2	**37**
TOTAL		**45**	**4**	**2**	**2**	**37**

The samples, which were not used in the calculations, are provided in Table 4.

Table 4: samples which were not used in the calculations

Sample N°	Product	Reference method: ISO 4833-1	Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate
4737	Dairy dessert	>7.48	>7.48
4465	Panna Cotta	1.00	1.60
4461	Infant formula with probiotics	1.30	1.48
4462	Infant formula with probiotics	>2.48	NI
4567	Infant formula with probiotics	<1.00	1.60
5123	Infant formula with probiotics	2.30	1.90
5124	Infant formula with probiotics	2.00	2.11
4566	Infant formula with probiotics	3.15	NI

3.1.4. Statistical interpretation

Raw data are shown in appendix E.
The obtained data were analyzed using the scatter plot.
The graphs are provided with the line of identity "y = x".
The Figure 1 shows the data plotted for the non-powdered dairy products (types a and b).
The Figure 2 shows the data plotted for milk powders (type c). The Figure 3 shows the data plotted for all the products.

Figure 1: scatter plots of reference-method versus alternative-method results for the non-powdered dairy products (types a and b)
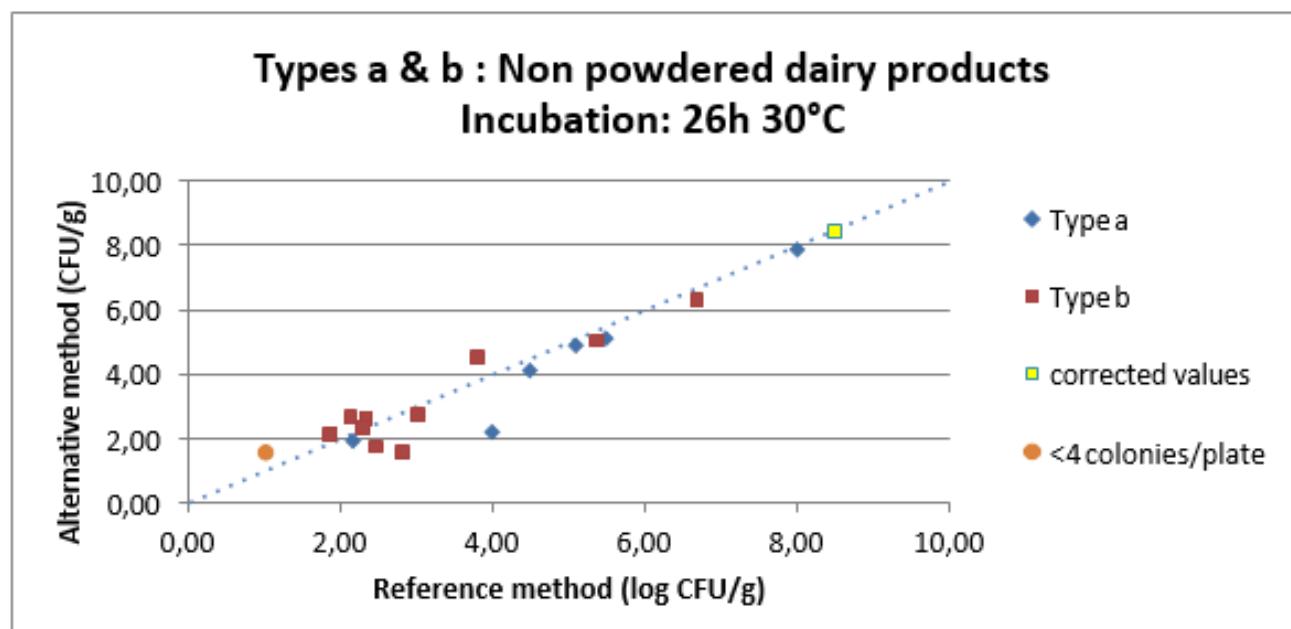
Figure 2: scatter plots of reference-method versus alternative-method results for the milk powders (types c)
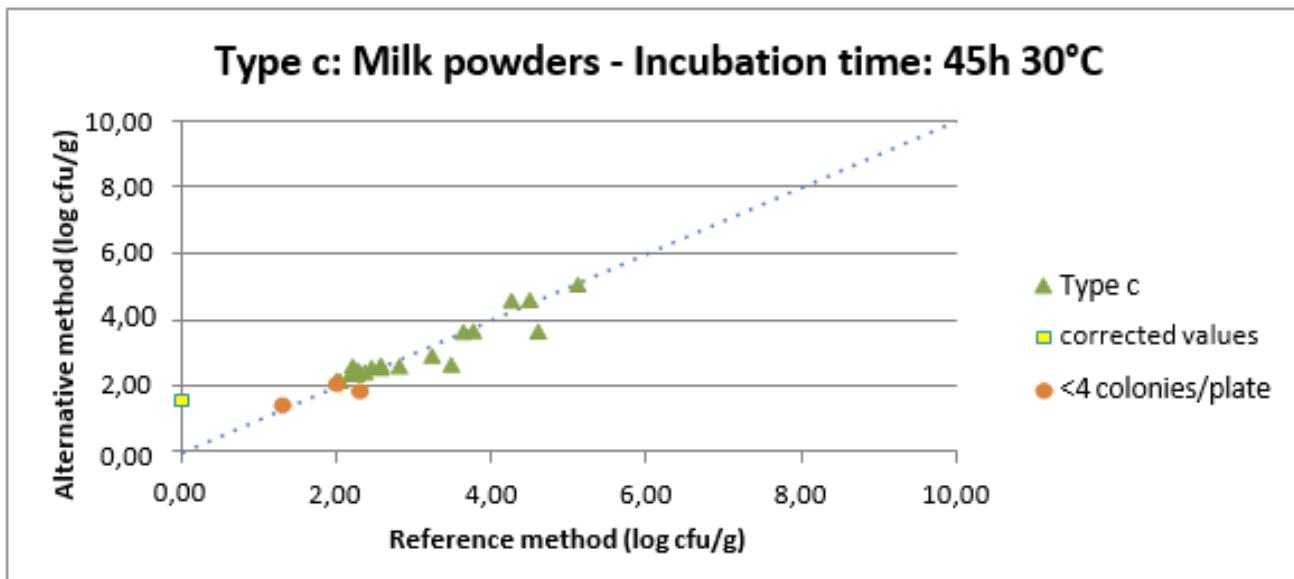
Figure 3: scatter plots of reference-method versus alternative-method results for all products.
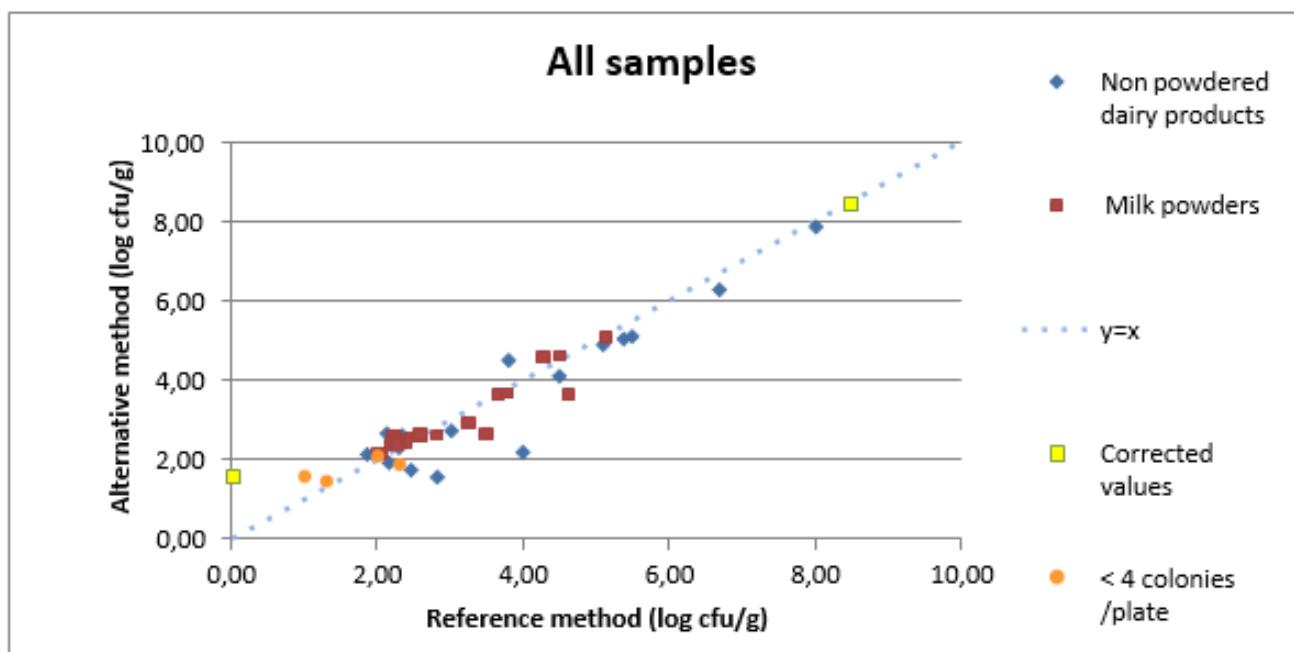
The calculated values for average difference and standard deviation differences per category are provided in Table 5 per incubation protocol.

Table 5: calculated values of the relative trueness study per incubation protocol

Incubation time	Types	n	D	SD	95% lower limit	95% upper limit
26 h	Non-powdered dairy products	16	-0.22	0.63	-1.59	1.16
45 h	Milk powders	21	0.04	0.34	-0.69	0.77
All products		37	-0.08	0.49	-1.08	0.93

The Bland-Altman difference plot for all the samples is given Figure 4.

Figure 4: Bland-Altman difference plot for all the samples.
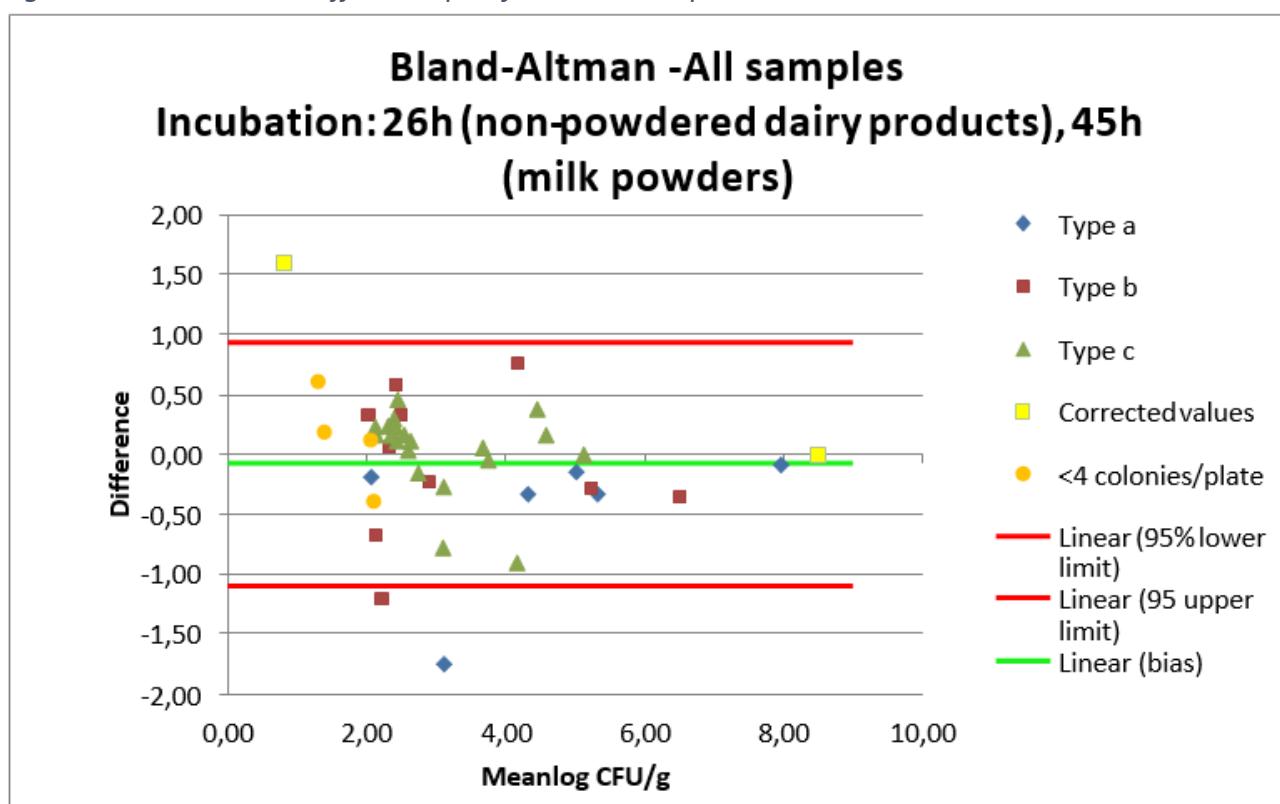
Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in the Table 6.

Table 6: analysis of the data out of the confidence limits

Type	N° Sample	Product	Ref. method	Alt. method	Values before correction (Ref/Alt)	Mean	Diffe-rence	Lower / Upper limits
a	4562	Raw ewe milk	3.98	2.23	/	3.10	-1.75	
b	4738	Pasteurized milk	2.81	1.60	/	2.20	-1.20	-1.08 / 0.93
c	4567	Infant formula with probiotics	0.00	1.60	1.00	0.80	1.60	

Values in green: differences in favor of the alternative method

Values in red: differences in favor of the reference method

Values in black: equivalent enumeration observed with both methods

Values in yellow: corrected value

Three samples showed differences higher than 1 log. One sample had a very low contamination level (4567) with the RAC method and no colony was enumerated on the PCA plates (ISO method). For sample (4562) it was noticed that the colonies on the reference method were micro-colonies.

3.1.5. Storage for one week at -18°C

The raw data are provided in Appendix F.

The following enumerations were done:

-        For non-powdered dairy products, 18 samples were analyzed providing 14 interpretable results by the alternative method.

-        For powdered dairy products, 27 samples were tested for 24 interpretable results.

A first enumeration was done after incubation for 26 h or 45 h at 30°C ± 1°C. A second enumeration was done after storage of the NEOGEN Petrifilm plates for one week at - 18°C (post incubation). For interpretation, the two reading times were compared.

The scatter plots are presented in Figure 5 and the Bland-Altman graph in Figure 6.

Figure 5: storage for one week at -18°C.
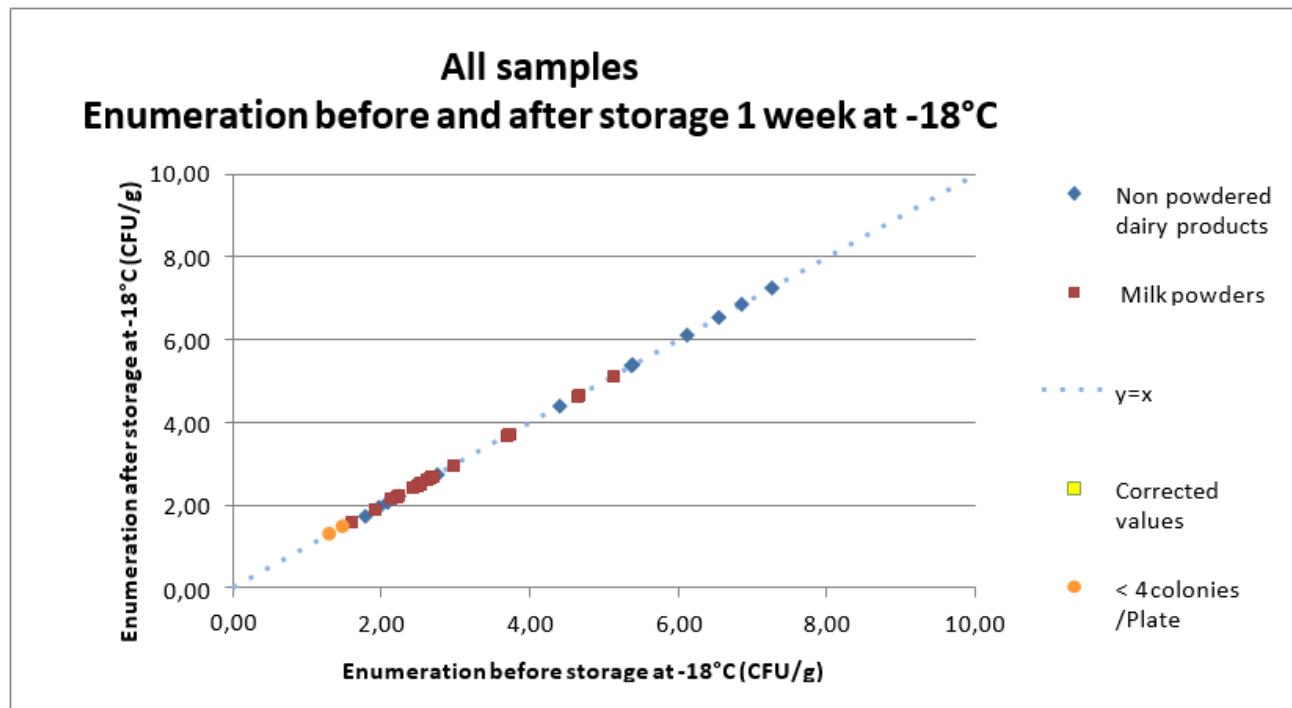
Figure 6: Bland-Altman – all samples- Enumeration before and after storage for one week at -18°C.
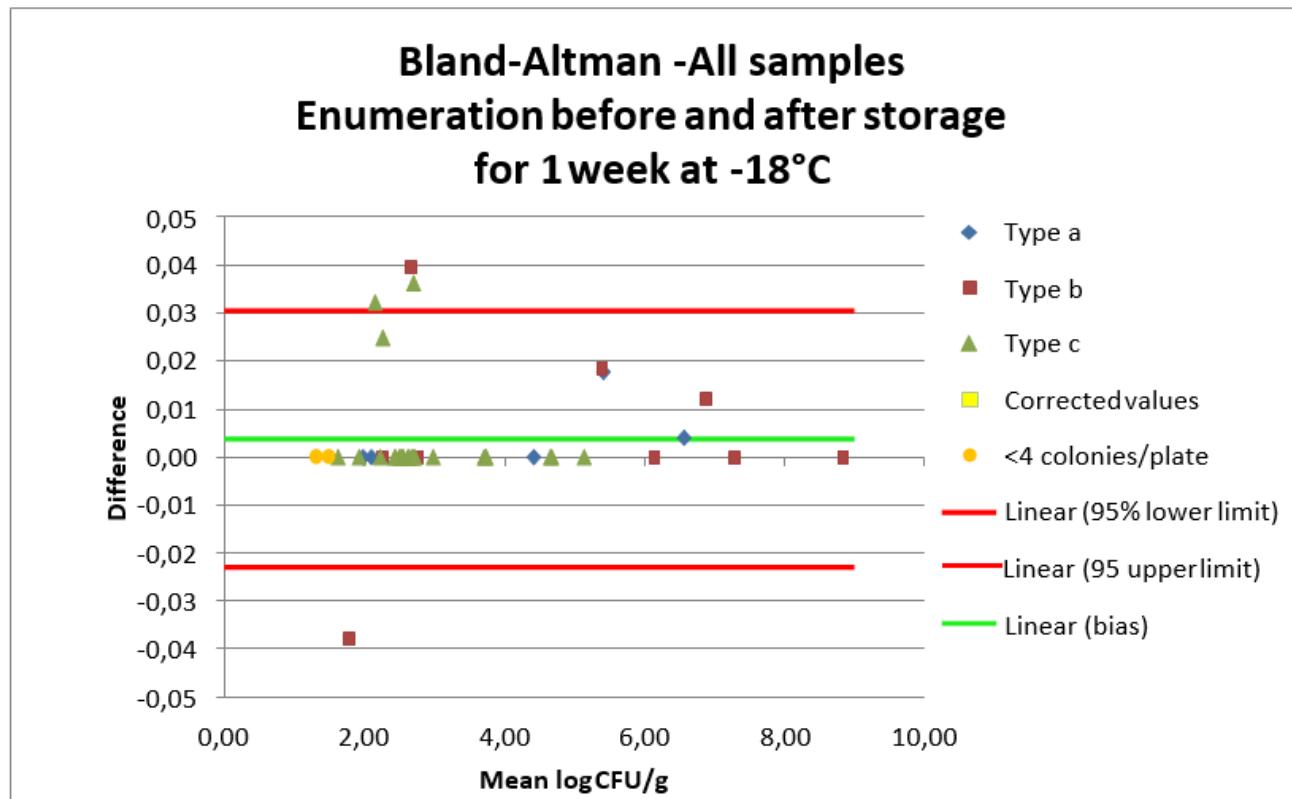
The calculated values for Average difference and Standard deviation differences per category are provided in table 7 per incubation protocol.

Table 7: calculated values per incubation protocol.

Incubation time	Types	n	D	SD	95% lower limit	95% upper limit
26 h	Non-powdered dairy products	14	0.00	0.02	-0.03	0.04
45 h	Milk powders	24	0.00	0.01	-0.02	0.03
All products		38	0.00	0.01	-0.02	0.03

There were no differences observed in results before or after storage of the NEOGEN Petrifilm plates (post incubation). The calculated CL limits are - 0.02 and + 0.03; which are values closed to 0. Four samples are outside the CL limits (See table 6), the difference observed for the samples outside the CL is very low, as seen in the table 8.

Table 8: samples outside the CL

| Type	N° Sample	Product	Reference method	Alternative method	Mean	Difference
b	5573	Ice cream	2.62	2.66	2.64	0.04
b	5575	Rice pudding	1.78	1.74	1.76	-0.04
c	3721	Infant formula	2.66	2.70	2.68	0.04
c	5124	Infant formula with probiotics	2.11	2.15	2.13	0.03

3.1.6. Conclusion

The relative trueness study of the alternative method was satisfied. It is possible to store the NEOGEN Petrifilm plates for one week at - 18°C (post incubation) before proceeding to enumeration.

3.2. Accuracy profiles

3.2.1. Protocols

Two matrices were tested with two different batches per matrix, using 6 samples per type. Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analyzed per matrix. The matrix – strain pairs are presented in table 9.

Table 9: matrix – strain pairs and inoculation protocols for the accuracy profile study

Matrix	Inoculated strain	Protocol tested	Inoculation level		
Infant formula	*Cronobacter sakazakii* Ad1446	**48 h ± 3 h at 30°C ± 1°C**	Lot 1	Level 1 x 5:	300 CFU/g
				Level 2 x 5:	10^4 CFU/g
				Level 3 x 5:	10^6 CFU/g
			Lot 2	Level 1 x 5:	300 CFU/g
				Level 2 x 5:	10^4 CFU/g
				Level 3 x 5:	10^6 CFU/g
Panna cotta	*Bacillus cereus* Ad2117	**28 h ± 2 h at 30°C ± 1°C**	Lot 1	Level 1 x 5:	300 CFU/g
				Level 2 x 5:	10^4 CFU/g
				Level 3 x 5:	10^6 CFU/g
			Lot 2	Level 1 x 5:	300 CFU/g
				Level 2 x 5:	10^4 CFU/g
				Level 3 x 5:	10^6 CFU/g

3.2.2. Results and interpretation

Raw data are provided in appendix G.

The statistical data and the accuracy profiles are shown in figures 7.

Statistical calculations were done according to the Excel spreadsheet named AP calculation tool_ MCS_(clause_6-1-3-3 calculation_and_interpretation_of_accuracy_profile_study) ver. 27-01-2015.xlsx available at http://standards.iso.org/iso/16140.

For the accuracy profiles of all the categories, the probability for the tolerance interval β is set at 80% and the acceptability limits at 0.5.

Figure 7: accuracy profiles

(Food) Category	Category 1
(Food) Type	Non powdered dairy product

Panna cotta

Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
5244-5248	2,65	0,095	-0,247	0,437	YES	YES
5259-5263	2,69	0,129	-0,213	0,471	YES	YES
5249-5253	3,99	0,244	-0,098	0,586	NO	NO
5264-5268	4,00	0,204	-0,138	0,546	NO	NO
5254-5258	5,72	0,147	-0,195	0,489	YES	YES
5269-5273	5,69	0,179	-0,163	0,521	NO	NO

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,072	0,237	YES	+/- 0,500

(Food) Category	Category 1
(Food) Type	Milk powders

Infant formula

Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
6022-6026	2,40	0,064	-0,065	0,194	YES	YES
6037-6041	2,45	0,316	0,186	0,446	YES	YES
6027-6031	4,08	-0,120	-0,250	0,010	YES	YES
6037-6041	4,23	-0,084	-0,214	0,046	YES	YES
6032-6036	6,15	-0,032	-0,162	0,098	YES	YES
6047-6051	6,18	-0,135	-0,265	-0,005	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,061	0,090	YES	+/- 0,500

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For Panna cotta, the upper limit is just above the acceptability limit for two inoculation levels.

If any of the upper or lower limits exceeds the limits and the standard deviation, additional evaluation procedure has to be followed, as described the ISO 16140-2 (2016): new acceptability limits as a function of the standard deviation $Al_s=4$. S_{ref} are calculated.

For the panna cotta, ALs = 4 x 0.072 = 0.288, the new acceptability limit does not change the result.

The alternative method is not equivalent to the reference method for the non- powdered dairy products but note that the enumeration concerns mesophilic aerobic flora, and the enumeration is higher with the alternative method. For a non-selective enumeration, this result shows that in the experiment the NEOGEN Petrifilm Rapid Aerobic Count Plate gives better recovery than the reference method.

For the infant formula, no statistical difference was observed between the two methods.

3.2.3. Conclusion

Even if the observed profiles are not comprised within the acceptability limits for one tested matrix (upper ß-ETI = 0.586, 0.546 and 0.521), it cannot be considered as a concern, as it shows that in those conditions, the NEOGEN Petrifilm plate method tended to recover slightly more microorganisms than the reference method.

The results of the two accuracy profiles are satisfactory.

3.3. Praticability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

Storage conditions, shelf-life and modalities of utilization after first use	Store unopened NEOGEN Petrifilm RAC plates pouches refrigerated or frozen (-20°C to 8°C). After first use, seal the pouch and store in a cool dry place for no longer than one month.			
Time to result	Steps	Reference method	Alternative method	
			Non-powdered dairy products	Powdered dairy products
	Sampling, analysis	Day 0	Day 0	Day 0
	Enumeration	Day 3	Day 1	Day 2
Common step with the reference method	Initial suspension and dilutions			

4. Interlaboratory study

4.1. Organization of the study

Samples were sent to 14 Collaborators.

A dairy based dessert was inoculated *Enterobacter kobei* Ad706, isolated from milk powder.

Samples were prepared and inoculated on Monday 17 October 2016, as described below:

-8 blind coded samples (10 g) for aerobic mesophilic flora enumeration by the NEOGEN Petrifilm Rapid Aerobic Count Plate method and by the reference method: ISO 4833-1,

-1 water flask labelled "Temperature Control" with a temperature probe for temperature measurement at reception and during sample storage.

The targeted inoculation levels were the following:

- Level 0: 0 CFU/g (samples 2 & 5),
- Level 1: 100 – 1 000 CFU/g (samples 3 & 7),
- Level 2: 1 000 – 10 000 CFU/g (samples 1 & 6),
- Level 3: 100 000 – 1 000 000 CFU/g (samples 4 & 8).

Each laboratory received eight samples of 10 g, i.e. two samples per inoculation level.

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

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

Samples were shipped in 24 h to 48 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

The samples were sent on Monday 17 October 2016.

Collaborative study laboratories and the expert laboratory carried out the analyses on Wednesday 19 October 2016 with the alternative and reference methods.

4.2. Control of the experimental parameters

4.2.1. Contamination levels obtained after artificial contamination

In order to evaluate the *Enterobacter kobei* strain viability during transport, bacterial count of samples was checked at different times, i.e. inoculation time and after 24 h and 48 h storage at 2 - 8°C.

Six samples (3 contamination levels x 2 samples) were enumerated at Day 0, Day 1 and Day 2.

Two sets of analyses were run: one before the study (See table 10) and one the same time as the inter-laboratory study (See table 11).

Table 10: strain stability – dataset n°1.

Days of analysis	Enterobacter kobei enumeration (CFU/g)			
	Reference method: ISO 4833		Method alternative: NEOGEN™ Petrifilm™ Rapid Aerobic Count	
	Replicate 1	*Replicate 2*	*Replicate 1*	*Replicate 2*
Day 0	390	550	500	420
	3400	4500	3800	4200
	44000	38000	45000	47000
Day 2	440	400	590	450
	3200	3300	4100	3600
	48000	40000	51000	48000

Table 11: strain stability – dataset °2.

Days of analysis	Enterobacter kobei enumeration (CFU/g)			
	Reference method: ISO 4833		Method alternative: NEOGEN™ Petrifilm™ Rapid Aerobic Count	
	Replicate 1	*Replicate 2*	*Replicate 1*	*Replicate 2*
Day 0	530	530	610	750
	6500	5200	5300	5200
	41000	62000	48000	88000
Day 1	550	490	670	600
	5500	6300	6600	6700
	45000	33000	50000	37000
Day 2	1800	1100	1900	1000
	12000	10000	15000	8300
	53000	76000	66000	81000

For the first dataset, no evolution was observed during storage for 48 h at 2 - 8°C.
For the second dataset, an evolution was observed; the enumerations were increased by a factor 2 between Day 1 and Day 2.

4.2.2. Logistic conditions

The temperatures measured at reception by the Collaborators, the temperatures registered by the thermo-probe, the receipt dates and the analysis date are given in Table 12.

Table 12: sample temperatures at receipt

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time		Analysis date
A	4.0	5.5	19/10/2016	12h00	19/10/2016 (Day 2)
B	3.0	7.6	19/10/2016	11h45	19/10/2016 (Day 2)
C	*Probe not received*	5.8	19/10/2016	10h30	19/10/2016 (Day 2)
D	*Probe not found by the lab.*	**11.7**	19/10/2016	17h30	20/10/2016 (Day 3)
E	/	/	19/10/2016	16h26	*Not run*
F	6.0	**9.9**	18/10/2016	12h00	19/10/2016 (Day 2)
G	1.5	3.4	18/10/2016	15h00	19/10/2016 (Day 2)
H	2.0	3.5	18/10/2016	11h00	19/10/2016 (Day 2)
I	2.5	3.9	19/10/2016	11h30	19/10/2016 (Day 2)
J	3.5	3.0	19/10/2016	10h00	20/10/2016 (Day 3)
K	3.5	8.0	19/10/2016	11h00	19/10/2016 (Day 2)
L	6.0	7.1	19/10/2016	15h30	19/10/2016 (Day 2)
M	*Probe not yet received*	6.8	19/10/2016	11h30	19/10/2016 (Day 2)
N	2.5	4.0	19/10/2016	10h45	19/10/2016 (Day 2)

All the samples were delivered in appropriated conditions except for Lab D and F which received their package above 8.4°C. Temperatures during shipment and at receipt were all correct.

-For Lab D, it was impossible to verify the temperature as they did not find the probe in the package. Note that this Lab run the analysis at Day 3.

-For Lab F, the probe indicated a temperature of 6.0°C at receipt. The results of this lab were kept.

No evolution of the strain has been observed after 48 h of storage at 7°C.

4.2.3. Homogeneity of inoculation

Homogeneity tests were conducted according to the ISO/TS 22117. Ten samples per inoculation level were analyzed in duplicate by the reference method. The test concluded to the homogeneity of the inoculation for the three inoculation levels.

4.3. Calculation, interpretation and summary of data

The raw data are given in Appendix H.

4.3.1. Results obtained by the expert laboratory

The results obtained by the expert Lab. are the following (See Table 13).

Table 13: expert laboratory results.

Level		Reference method		Alternative method	
		CFU/g	log CUF/g	CFU/g	log CUF/g
Level 0	Duplicate 1	< 10	< 1.00	< 10	< 1.00
	Duplicate 2	< 10	< 1.00	< 10	< 1.00
Level 1	Duplicate 1	1800	3.26	1900	3.28
	Duplicate 2	1100	3.04	1000	3.00
Level 2	Duplicate 1	12000	4.08	15000	4.18
	Duplicate 2	10000	4.00	8300	3.92
Level 3	Duplicate 1	53000	4.72	66000	4.82
	Duplicate 2	76000	4.88	81000	4.91

4.3.2. Results obtained by the collaborators

Samples were sent to 14 collaborators

- Lab E did not run the analyses,
- Labs D and J run the analyses at Day 3,
- Lab L analyzed only a part of the samples due to late delivery of the samples.

Finally, results from 10 collaborators were kept for interpretation: A, B, C, F, G, H, I, K, M and N.

A summary of the test results is given in Table 14 (CFU/g) and Table 15 (log CFU/g).

Table 14: summary of data (CFU/g)

Collabo-rators	Blank level				Low level				Medium level				High level			
	Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	<10	<10	<10	<10	730	990	710	970	12000	8600	15000	11000	60000	110000	73000	120000
B	<10	<10	<10	<10	1500	1100	1200	1000	11000	17000	13000	21000	110000	100000	84000	65000
C	70 Ne	<40	50 Ne	<10	1500	1100	1300	1100	9900	20000	11000	21000	81000	85000	120000	400000
F	<10	<10	<10	<10	2300	1700	1700	1500	16000	24000	19000	23000	250000	190000	220000	230000
G	<10	<10	<10	<10	480	1900	660	7800	11000	15000	8200	9200	100000	110000	120000	80000
H	<40	<10	<10	<10	820	1100	1200	1600	9500	6700	11000	12000	66000	6800	160000	76000
I	<10	<10	<10	<10	940	1300	1200	1200	9700	11000	7700	12000	69000	58000	67000	85000
K	<10	<10	<10	<10	690	340	450	300	13000	2700	8100	4000	37000	36000	30000	38000
M	<10	<10	<10	<10	680	660	770	940	9000	8300	8500	8600	77000	61000	96000	88000
N	<10	<10	<10	<10	1000	810	1100	960	10000	15000	16000	15000	91000	89000	120000	100000

Table 15: summary of data (log CFU/g)

Collabo-rators	Blank level				Low level				Medium level				High level			
	Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	<1.00	<1.00	<1.00	<1.00	2.86	3.00	2.85	2.99	4.08	3.93	4.18	4.04	4.78	5.04	4.86	5.08
B	<1.00	<1.00	<1.00	<1.00	3.18	3.04	3.08	3.00	4.04	4.23	4.11	4.32	5.04	5.00	4.92	4.81
C	1.85	<1.60	1.70	<1.00	3.18	3.04	3.11	3.04	4.00	4.30	4.04	4.32	4.91	4.93	5.08	5.60
F	<1.00	<1.00	<1.00	<1.00	3.36	3.23	3.23	3.18	4.20	4.38	4.28	4.36	5.40	5.28	5.34	5.36
G	<1.00	<1.00	<1.00	<1.00	2.68	3.28	2.82	3.89	4.04	4.18	3.91	3.96	5.00	5.04	5.08	4.90
H	<1.60	<1.00	<1.00	<1.00	2.91	3.04	3.08	3.20	3.98	3.83	4.04	4.08	4.82	3.83	5.20	4.88
I	<1.00	<1.00	<1.00	<1.00	2.97	3.11	3.08	3.08	3.99	4.04	3.89	4.08	4.84	4.76	4.83	4.93
K	<1.00	<1.00	<1.00	<1.00	2.84	2.53	2.65	2.48	4.11	3.43	3.91	3.60	4.57	4.56	4.48	4.58
M	<1.00	<1.00	<1.00	<1.00	2.83	2.82	2.89	2.97	3.95	3.92	3.93	3.93	4.89	4.79	4.98	4.94
N	<1.00	<1.00	<1.00	<1.00	3.00	2.91	3.04	2.98	4.00	4.18	4.20	4.18	4.96	4.95	5.08	5.00

Few contaminations were observed on blank samples for two Labs: C and H.

The Figure 8 shows the data points after log10 transformation. The visual inspection shows that the alternative method gives results, which are proportional to those of the reference method, except for 3 samples for which the enumeration results are the following:

-Lab C (sample 8):	ISO 4833: 4.93 log	
	Alternative method: 5.60 log	
- Lab G (sample 7):	ISO 4833: 3.28 log	
	Alternative method: 3.89 log	
- Lab H (sample 8):	ISO 4833: 3.83 log	
	Alternative method: 4.88 log	

Lab H reported some difficulties for enumeration of the PCA plates due to spreading colonies.
For Labs G and H, the result of the alternative method is more closed to the expected enumeration.
The data are distributed closely to the first bisecting line with a slope equal to 1.

Figure 8: visual linearity checking
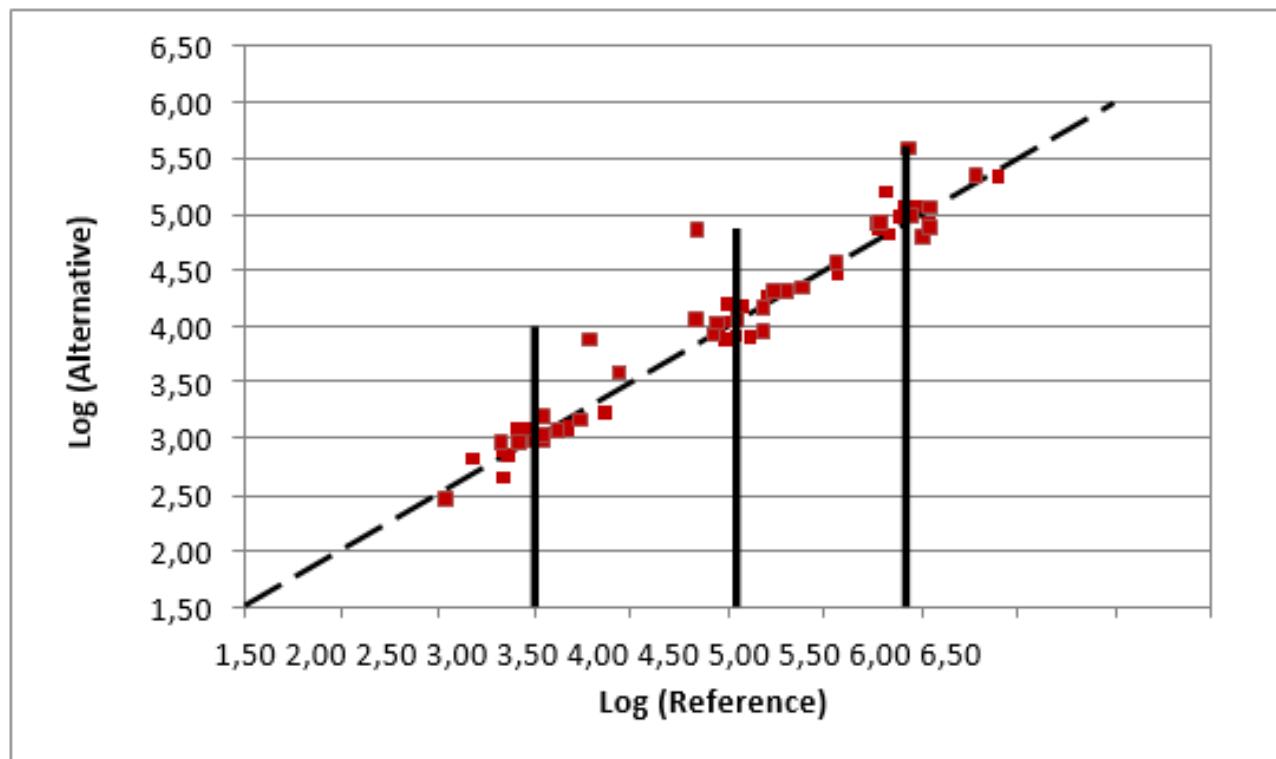
4.3.3. Accuracy profil calculation

Statistical calculations were done according to the Excel spreadsheet named AP calculation tool_ILS_(clause_6-2-3_Calculations_summary_and_interpretation_of_data)_ver14-03-2016.xlsx available at http://standards.iso.org/iso/16140.

A summary of the parameters calculated is presented in the table 16.

Table 16: summary of the statistical test

Accuracy profile

Study Name	NEOGEN Petrifilm Rapid Aerobic Count Plate					
Date	19th October 2016					
Coordinator						
Tolerance probability (beta)	80%	80%	80%			
Acceptability limit in log (lambda)	0,5	0,5	0,5			

	Alternative method			Reference method		
Levels	Low	Medium	High	Low	Medium	High
Target value	2.991	4.041	4.869			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	3.032	4.069	4.998	2.991	4.041	4.869
Repeatability standard deviation (sr)	0.249	0.119	0.158	0.168	0.190	0.232
Between-labs standard deviation (sL)	0.112	0.146	0.208	0.116	0.000	0.219
Reproducibility standard deviation (sR)	0.273	0.188	0.261	0.205	0.199	0.319
Corrected number of dof	18.114	13.295	12.896	16.638	18.625	14.917
Coverage factor	1.368	1.402	1.405			
Interpolated Student t	1.330	1.349	1.351			
Tolerance interval standard deviation	0.2810	0.1959	0.2712	Pooled repro		
Lower TI limit	2.659	3.805	4.631	standard dev		0.247
Upper TI limit	3.406	4.333	5.364			
Bias	0.041	0.028	0.129			
Lower TI limit (beta = 80%)	-0.332	-0.236	-0.238			
Upper TI limit (beta = 80%)	0.415	0.292	0.495			
Lower Acceptability limit	-0.50	-0.50	-0.50			
Upper Acceptability limit	0.50	0.50	0.50			

These values are collected in a graphical representation together with the acceptability limits (AL). This representation is given Figure 9.

Figure 9: accuracy profile
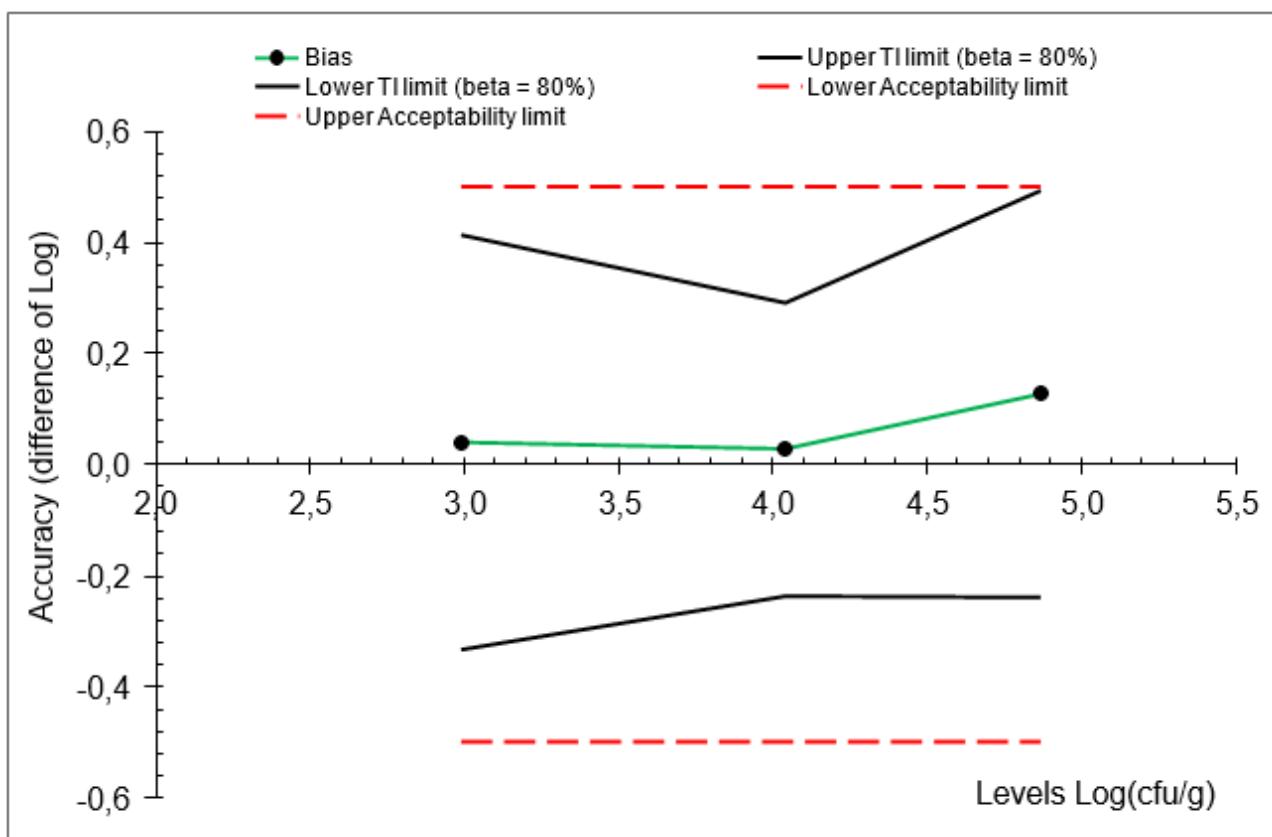
It is observed that for all the levels, the tolerance interval limits of the alternative method are within the acceptable limits of 0.5 log.

In those conditions, the results obtained with the alternative method are not statistically different than those obtained with the reference method.

4.4. Conclusion of the interlaboratory study

As the values for the β-ETI fall within the Acceptability Limits for all levels of contamination, the alternative method is regarded as being equivalent to the reference method.

5. General conclusion of the validation study

5.1. Method comparison study

The observed data and interpretation confirm the performances of the alternative method:

37 samples were tested in the relative trueness study, which clearly satisfied the required criteria for quantitative method comparison according to the EN ISO 16140-2:2016; this study confirms the possibility as well to store NEOGEN Petrifilm plates (post incubation) for one week at - 18°C before reading.

The observed profiles are comprised within the AL actually set at 0.5 Log CFU/g in the EN ISO 16140-2:2016 standard for the powdered dairy products and is just above the AL for the non-powdered dairy products. The method is non-selective, and a slightly better recovery with the NEOGEN Petrifilm Rapid Aerobic Count Plate was demonstrated in those conditions.

5.2. Interlaboratory study

The quality assurance parameters were verified (i.e. inoculation homogeneity, targeted levels, strain stability, logistic conditions, analyses), confirming that the inter-laboratory study was conducted in appropriate conditions.
The data interpretations were done according to the EN ISO 16140-2:2016. For the three contamination levels, the alternative method is accepted as equivalent to the reference method.

The data and their interpretation produced in this renewal report fulfil the requirements of both the EN ISO 16140-2:2016 standard and the version 12 of the Requirements regarding comparison and interlaboratory studies for implementation of the standard EN ISO 16140-2.

Le Lion d'Angers, October 18, 2024

Guillaume MESNARD
Technical deputy manager

François Le Nestour
Head of the Methods Validation studies and R&D

APPENDICES

Appendix A - Flow diagram of the alternative method:

NEOGEN Petrifilm Rapid Aerobic Count Plate

10 g + 90 ml appropriate diluent according to the ISO 6887 parts
Do not use diluents containing citrate, bisulfite or thiosulfate with Neogen Petrifilm RAC Plates; they can inhibit growth.

↓

Stomach

↓

Inoculate 1 mL onto the NEOGEN Petrifilm plate

↓

Incubate

Non-powdered dairy products:

Storage for 1 week at -18°C

28 h ± 2 h at 30°C ± 1°C [2]

Powdered dairy products:

48 h ± 3 h at 30°C ± 1°C [6]

↓
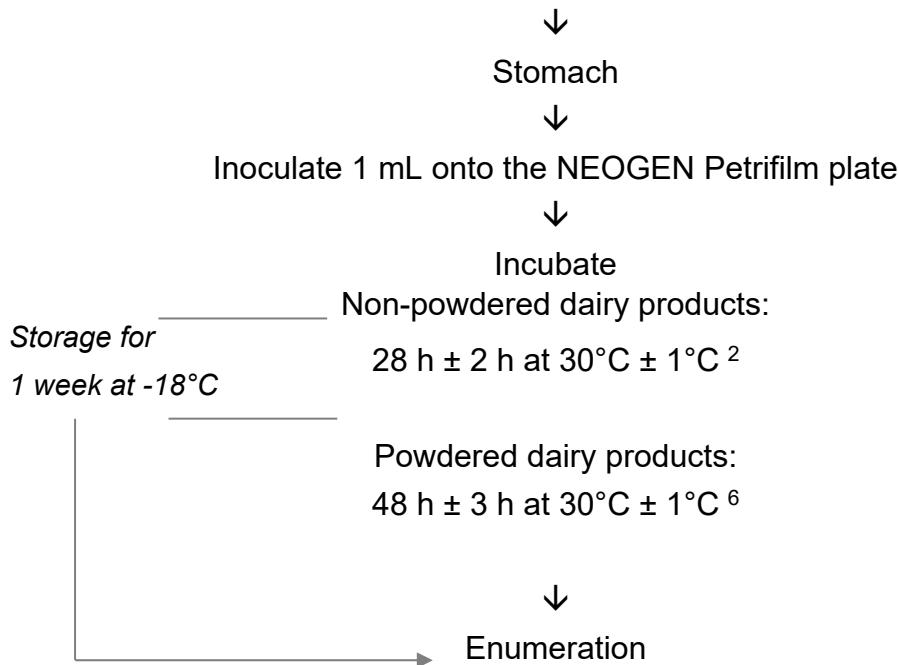
Enumeration

Appendix B – Flow diagram of the reference method test procedure ISO 4833-1:2013 - Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique

10 g + 90 ml appropriate diluent according to the ISO 6887 parts
and ISO 18593 for environmental samples

↓

Stomach

↓

Inoculate 1 plate per dilution

↓

Pour PCA with 1 g/l of skim milk for dairy product
Pour PCA for other products

↓

Incubate at 30°C ± 1°C for 72 h ± 3 h

↓

Reading
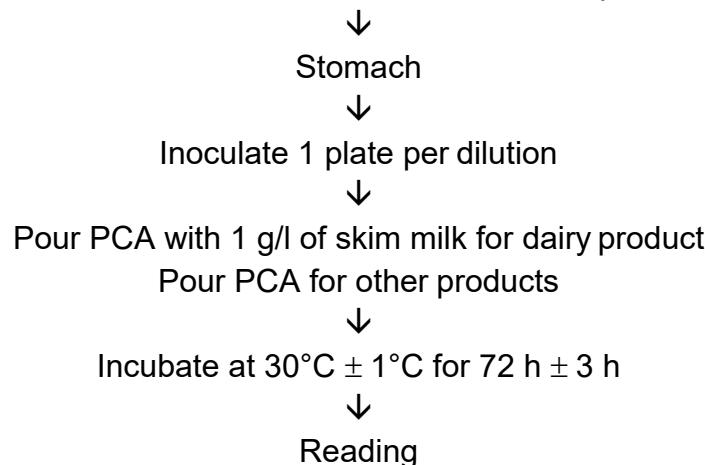
Appendix C – Artificial contaminations of samples

Sample number	Product	Inoculations			Type
		Strain		Injury protocol	
		Reference	Origin		
5125	**Infant formula**	*C. sakazakii* Ad831	Dairy product	Seeding with lyophilized strain-15 days at ambient temperature	c
5126	**Infant formula**	*C. sakazakii* Ad1446	Dairy product	Seeding with lyophilized strain-15 days at ambient temperature	c
5127	**Milk powder**	*B. cereus* Ad1469	Milk powder	Seeding with Lyophilized strain-15 days at ambient temperature	c
5128	**Milk powder**	*B. cereus* Ad1469	Milk powder	Seeding with lyophilized strain-15 days at ambient temperature	c
5129	**Milk powder**	*B. cereus* Ad1848	Milk powder	Seeding with lyophilized strain-15 days at ambient temperature	c
5130	**Milk powder**	*B. cereus* Ad1848	Milk powder	Seeding with lyophilized strain-15 days at ambient temperature	c
5379	**Milk powder**	*C. sakazakii* Ad831	Dairy product	Seeding with lyophilized strain-3 days at ambient temperature	c
5380	**Infant formula**	*B. cereus* Ad1469	Milk powder	Seeding with lyophilized strain-3 days at ambient temperature	c

Appendix D - Relative trueness study: raw data

* : < 4 CFU/plate   NI : non interpretable   Ne: estimated number

Sample number	Product (French name)	Product	pH	Reference method: ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate				Type
								26h at 30°C ± 1°C				
				Dilution	CFU/plate	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	
4463	Lait cru	Raw milk	7.34	1000	278	300000	5.48	1000	142	140000	5.15	a
				10000	48			10000	16			
4464	Lait cru	Raw milk	7.00	100000	>300	99000000	8.00	100000	>300	81000000	7.91	a
				1000000	99		N'	1000000	81		N'	
4561	Lait cru de brebis	Raw ewe milk	7.00	10	11	140	2.15	10	10	91	1.96	a
				100	4			100	0			
4562	Lait cru de brebis	Raw ewe milk	6.99	100	101	9500	3.98	10	13	170	2.23	a
				1000	4			100	6			
4572	Lait cru de vache	Raw milk	7.00	1000	30	30000	4.48	100	140	14000	4.15	a
				10000	3			1000	15			
4573	Lait cru de vache	Raw milk	6.99	1000	120	120000	5.08	1000	86	86000	4.93	a
				10000	15			10000	9			
4465	Panna Cotta fruit exotique	Panna Cotta	7.32	10	1	10*	1.00*	10	4	40	1.60	b
				100	0			100	1		Ne	
4466	Riz au lait	Rice pudding	6.99	100	50	6100	3.79	1000	31	35000	4.54	b
				1000	17			10000	8			
4558	Glace rhum raisin	Ice cream	6.99	10	12	130	2.11	10	46	500	2.70	b
				100	2			100	9			

Table title row: **RAW MILK AND DAIRY DESSERTS**

Sample number	Product (French name)	Product	pH	Reference method: ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate 26h at 30°C ± 1°C				Type
				Dilution	CFU/plate	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	
4559	Glace vanille	Ice cream	6.98	10	4	1000	3.00	10	57	590	2.77	b
				100	10		N'	100	8			b
4560	Glace noix de coco	Ice cream	6.96	10	18	210	2.32	10	41	450	2.65	
				100	5			100	8			b
4563	Riz au lait	Rice pudding	7.01	10	30	280	2.45	10	6	60	1.78	
				100	1			100	0		Ne	b
4564	Crème vanille	Vanilla cream	6.99	10	7	70	1.85	10	13	150	2.18	
				100	1		Ne	100	3			b
4565	Fromage blanc avec coulis	Cheese preparation	6.61	10	18	190	2.28	10	17	220	2.34	
				100	3			100	7			b
4735	Fromage frais	Cheese preparation	6.61	1000	226	230000	5.36	1000	121	120000	5.08	
				10000	31			10000	11			b
4736	Fromage blanc au lait entier pasteurisé	Cheese preparation	6.63	10000	>300	4700000	6.67	10000	210	2100000	6.32	
				100000	47		N'	100000	18			b
4737	Choux à la crème	Dessert with Chantilly	7.00	10000	>300	>30000000	>7.48	10000	>300	>30000000	>7.48	
				100000	>300			100000	>300			b
4738	Lait frais pasteurisé	Pasteurized milk	7.12	10	61	640	2.81	10	4	40	1.60	
				100	9			100	1		Ne	

RAW MILK AND DAIRY DESSERTS

Sample number	Product (French name)	Product	pH	Reference method: ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count				Type
								48h ± 3h at 30°C ± 1°C				
				Dilution	CFU/plate	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	c
3717	Poudre de lait	Milk powder	6.80	10 / 100	29 / 2	280	2.45	10 / 100	33 / 12	410	2.61	c
3718	Poudre de lait	Milk powder	6.74	10 / 100	68 / 2	640	2.81	10 / 100	39 / 10	450	2.65	c
3719	Poudre de lait infantile	Infant formula	6.47	10 / 100	20 / 5	230	2.36	10 / 100	26 / 7	300	2.48	c
3720	Poudre de lait infantile	Infant formula	6.85	10 / 100	38 / 3	370	2.57	10 / 100	35 / 9	400	2.60	c
3721	Poudre de lait infantile	Infant formula	6.76	10 / 100	15 / 3	160	2.20	10 / 100	39 / 12	460	2.66	c
4453	Poudre de lait	Milk powder	6.74	10 / 100	14 / 2	150	2.18	10 / 100	24 / 5	260	2.41	c
4454	Poudre de lait	Milk powder	6.78	10 / 100	18 / 3	190	2.28	10 / 100	29 / 7	330	2.52	c
4455	Poudre de lait	Milk powder	6.74	10 / 100	31 / 10	370	2.57	10 / 100	45 / 8	480	2.68	c
4456	Poudre de lait infantile	Infant formula	6.77	10 / 100	8 / 4	110	2.04	10 / 100	15 / 3	160	2.20	c
4457	Poudre de lait infantile	Infant formula	6.87	10 / 100	17 / 2	170	2.23	10 / 100	32 / 5	340	2.53	c
4458	Poudre de lait infantile	Infant formula	6.83	10 / 100	16 / 4	180	2.26	10 / 100	27 / 7	310	2.49	c
4459	Poudre de lait infantile	Infant formula	6.73	10 / 100	11 / 0	100	2.00	10 / 100	16 / 3	170	2.23	c
4460	Poudre de lait infantile	Infant formula	6.80	10 / 100	16 / 4	180	2.26	10 / 100	23 / 5	260	2.41	

				Reference method: ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count				Type
								48h ± 3h at 30°C ± 1°C				
Sample number	Product (French name)	Product	pH	Dilution	CFU/plate	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	
4461	Poudre de lait infantile avec probiotiques 1er âge	Infant formula with probiotics	6.50	10 / 100	2 / 1	20	1.30* / Ne	10 / 100	3 / 0	30*	1.48* / Ne	c
4462	Poudre de lait infantile avec probiotiques 1er âge	Infant formula with probiotics	7.00	100000 / 1000000	>300 / >300	>300	>8.48	100000 / 1000000	NI / NI	NI	NI	c
4566	Poudre de lait infantile 2e âge avec probiotiques	Infant formula with probiotics	6.96	10 / 100	NI / 14	1400	3.15 / N'	10 / 100	NI / 0	NI	NI	c
4567	Poudre de lait infantile avec probiotiques 1er âge	Infant formula with probiotics	6.54	10 / 100	0 / 0	<10	<1.00	10 / 100	4 / 1	40	1.60 / Ne	c
5123	Poudre de lait infantile 1er âge avec probiotiques	Infant formula with probiotics	6.86	10 / 100	NI / 2	200*	2.30*	10 / 100	8 / 2	80	1.90 / Ne	c
5124	Poudre de lait 2e âge avec probiotiques	Infant formula with probiotics	6.90	10 / 100	NI / 1	100*	2.00*	10 / 100	12 / 2	130	2.11	c
5125	**Poudre de lait infantile 1er âge**	**Infant formula**	6.92	1000 / 10000	39 / 5	40000	4.60	1000 / 10000	5 / 1	5000	3.70 / Ne	c
5126	**Poudre de lait infantile 1er âge**	**Infant formula**	6.90	100 / 1000	23 / 10	3000	3.48	100 / 1000	5 / 1	500	2.70 / Ne	c
5127	**Poudre de lait en poudre écrémé**	**Milk powder**	6.76	100 / 1000	39 / 8	4300	3.63	100 / 1000	48 / 6	4900	3.69	c
5128	**Poudre de lait écrémé**	**Milk powder**	6.73	1000 / 10000	29 / 5	31000	4.49	1000 / 10000	46 / 4	45000	4.65	c
5129	**Poudre de lait écrémé**	**Milk powder**	6.78	1000 / 10000	124 / 14	130000	5.11	1000 / 10000	131 / 14	130000	5.11	c
5130	**Poudre de lait écrémé**	**Milk powder**	6.74	100 / 1000	61 / 3	5800	3.76	100 / 1000	55 / 2	5200	3.72	c
5379	**Poudre de lait écrémé**	**Milk powder**	6.93	100 / 1000	19 / 0	1700	3.23	100 / 1000	10 / 0	910	2.96	c
5380	**Poudre de lait infantile 1er âge**	**Infant formula**	6.84	100 / 1000	170 / 27	18000	4.26	1000 / 10000	45 / 2	43000	4.63	

MILK POWDERS

Appendix E - Relative trueness study: summarized results and calculations

Category	Type	N° sample	Log CFU/g Reference method	Log CFU/g Alternative method	Mean	Diffe-rence	Alternative method <4 CFU/plate	Alternative method <or> threshold corrected values	Mean <4 CFU/plate	Difference <4 CFU/plate	Mean corrected values	Difference Corrected values
1	a	4463	5.48	5.15	5.31	-0.33						
	a	4464	8.00	7.91	7.95	-0.09						
	a	4561	2.15	1.96	2.05	-0.19						
	a	4562	3.98	2.23	3.10	-1.75						
	a	4572	4.48	4.15	4.31	-0.33						
	a	4573	5.08	4.93	5.01	-0.14						
	b	4465	1.00				1.60		1.30	0.60		
	b	4466	3.79	4.54	4.16	0.76						
	b	4558	2.11	2.70	2.41	0.59						
	b	4559	3.00	2.77	2.89	-0.23						
	b	4560	2.32	2.65	2.49	0.33						
	b	4563	2.45	1.78	2.11	-0.67						
	b	4564	1.85	2.18	2.01	0.33						
	b	4565	2.28	2.34	2.31	0.06						
	b	4735	5.36	5.08	5.22	-0.28						
	b	4736	6.67	6.32	6.50	-0.35						
	b	4737	8.48					8.48			8,48	0,00
	b	4738	2.81	1.60	2.20	-1.20						
Average Types a & b						-0.22						
Standard deviation of differences Types a & b						0.63						
1	c	3717	2.45	2.61	2.53	0.17						
	c	3718	2.81	2.65	2.73	-0.15						
	c	3719	2.36	2.48	2.42	0.12						
	c	3720	2.57	2.60	2.59	0.03						
	c	3721	2.20	2.66	2.43	0.46						
	c	4453	2.18	2.41	2.30	0.24						
	c	4454	2.28	2.52	2.40	0.24						
	c	4455	2.57	2.68	2.62	0.11						
	c	4456	2.04	2.20	2.12	0.16						
	c	4457	2.23	2.53	2.38	0.30						
	c	4458	2.26	2.49	2.37	0.24						
	c	4459	2.00	2.23	2.12	0.23						
	c	4460	2.26	2.41	2.34	0.16						
	c	4461	1.30				1.48		1.39	0.18		
	c	4567	0.00					1.60			0,80	1,60
	c	5123	2.30				1.90		2.10	-0.40		
	c	5124	2.00				2.11		2.06	0.11		
	c	5125	4.60	3.70	4.15	-0.90						
	c	5126	3.48	2.70	3.09	-0.78						
	c	5127	3.63	3.69	3.66	0.06						
	c	5128	4.49	4.65	4.57	0.16						
	c	5129	5.11	5.11	5.11	0.00						
	c	5130	3.76	3.72	3.74	-0.05						
	c	5379	3.23	2.96	3.10	-0.27						
	c	5380	4.26	4.63	4.44	0.38						
Average category Type c						0.04						
Standard deviation of differences Type c						0.34						
Average all types				Dall		-0.08						
Standard deviation of differences all types				SDAll		0.49						

	n all	37	
β=95%	T(0.05;70)=	2.028093987	
		1.014471414	Upper limit / Lower limit / Linear
	Average (minimal value)	0.00	0.94 / -1.09 / -0.08
	Average (maximal value)	9.00	0.94 / -1.09 / -0.08

Appendix F - Storage for one week at - 18°C: raw data

				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate								Type
Sample number	Product (French name)	Product	pH	26h or 45h at 30°C ± 1°C				26h or 45h at 30°C ± 1°C and one week at -18°C				
				Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	
5567	Lait cru de brebis	Raw ewe milk	7.03	10 / 100	10 / 3	120	2.08	10 / 100	10 / 3	120	2.08	a
5568	Lait cru de brebis	Raw ewe milk	7.07	10 / 100	10 / 0	91	1.96	10 / 100	10 / 0	91	1.96	a
5569	Lait cru de vache	Raw cow milk	7.12	100000 / 1000000	33 / 5	3500000	6.54	100000 / 1000000	33 / 5	3500000	6.54	a
5570	Lait cru de vache	Raw cow milk	7.10	10000 / 100000	23 / 3	240000	5.38	10000 / 100000	24 / 3	250000	5.40	a
5571	Lait cru de vache	Raw cow milk	7.22	1000 / 10000	23 / 4	25000	4.40	1000 / 10000	23 / 5	25000	4.40	b
5572	Crème glacée menthe chocolat	Ice cream	7.10	10 / 100	46 / 15	560	2.75	10 / 100	46 / 15	560	2.75	b
5573	Crème glacée noix de coco	Ice cream	7.14	10 / 100	43 / 3	420	2.62	10 / 100	48 / 3	460	2.66	b
5574	Crème glacée vanille	Ice cream	7.13	10 / 100	16 / 3	170	2.23	10 / 100	16 / 3	170	2.23	b
5575	Riz au lait	Rice pudding	7.09	10 / 100	6 / 0	60	1.78 Ne	10 / 100	6 / 0	55	1.74 Ne	b
5576	Riz au lait	Rice pudding	7.11	10 / 100	2 / 0	20	1.30*	10 / 100	2 / 0	20	1.30*	b
5577	Riz au lait	Rice pudding	7.07	10 / 100	2 / 0	20	1.30*	10 / 100	2 / 0	20	1.30*	b
5578	Fromage blanc au lait pasteurisé	Fermented milk	6.75	100000 / 1000000	74 / 4	7100000	6.85	100000 / 1000000	75 / 5	7300000	6.86	b
5579	Fromage frais	Fresh cheese	6.72	10000 / 100000	121 / 20	1300000	6.11	10000 / 100000	121 / 20	1300000	6.11	b
5580	Fromage blanc au lait pasteurisé	Fermented milk	6.78	100000 / 1000000	169 / 30	18000000	7.26	100000 / 1000000	169 / 30	18000000	7.26	b

				ALL PRODUCTS								
				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate								Type
Sample number	Product (French name)	Product	pH	26h or 45h at 30°C ± 1°C				26h or 45h at 30°C ± 1°C and one week at -18°C				
				Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	b
5581	Choux à la crème	Pastry	7.14	1000 10000	221 33	230000	5.36	1000 10000	225 35	240000	5.38	b
5582	Choux à la crème	Pastry	7.05	1000000 10000000	>300 65	650000000	8.81 N'	1000000 10000000	>300 65	650000000	8.81 N'	b
5583	Lait 1/2 écrémé pasteurisé	Pasteurized half skimmed milk	7.18	10 100	2 0	20	1.30*	10 100	2 0	20	1.30*	b
5584	Lait 1/2 écrémé pasteurisé	Pasteurized half skimmed milk	7.16	10 100	2 1	20	1.30*	10 100	2 1	20	1.30*	c
3717	Poudre de lait	Milk powder	6.80	10 100	33 12	410	2.61	10 100	33 12	410	2.61	c
3718	Poudre de lait	Milk powder	6.74	10 100	39 10	450	2.65	10 100	39 10	450	2.65	c
3719	Poudre de lait infantile	Infant formula	6.47	10 100	26 7	300	2.48	10 100	26 7	300	2.48	c
3720	Poudre de lait infantile	Infant formula	6.85	10 100	35 9	400	2.60	10 100	35 9	400	2.60	c
3721	Poudre de lait infantile	Infant formula	6.76	10 100	39 12	460	2.66	10 100	41 14	500	2.70	c
4453	Poudre de lait	Milk powder	6.74	10 100	24 5	260	2.41	10 100	24 5	260	2.41	c
4454	Poudre de lait	Milk powder	6.78	10 100	29 7	330	2.52	10 100	29 7	330	2.52	c
4455	Poudre de lait	Milk powder	6.74	10 100	45 8	480	2.68	10 100	45 8	480	2.68	c
4456	Poudre de lait infantile	Infant formula	6.77	10 100	15 3	160	2.20	10 100	15 3	160	2.20	c
4457	Poudre de lait infantile	Infant formula	6.87	10 100	32 5	340	2.53	10 100	32 5	340	2.53	c
4458	Poudre de lait infantile	Infant formula	6.83	10 100	27 7	310	2.49	10 100	27 7	310	2.49	c c
4459	Poudre de lait infantile	Infant formula	6.73	10 100	16 3	170	2.23	10 100	16 4	180	2.26	

				ALL PRODUCTS								Type
				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate								
Sample number	Product (French name)	Product	pH	26h or 45h at 30°C ± 1°C				26h or 45h at 30°C ± 1°C and one week at -18°C				
				Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	Dilution	CFU/Petrifilm	CFU/g	Log (CFU/g)	c
4460	Poudre de lait infantile	Infant formula	6.80	10 / 100	23 / 5	260	2.41	10 / 100	24 / 5	260	2.41	c
4461	Poudre de lait infantile avec probiotiques 1ᵉʳ âge	Infant formula with probiotics	6.50	10 / 100	3 / 0	30*	1.48* / Ne	10 / 100	3 / 0	30*	1.48* / Ne	c
4462	Poudre de lait infantile avec probiotiques 1ᵉʳ âge	Infant formula with probiotics	7.00	100000 / 1000000	NI / NI	NI	NI	100000 / 1000000	NI / ill	NI	NI	c
4566	Poudre de lait infantile 2ᵉ âge avec probiotiques	Infant formula with probiotics	6.96	10 / 100	NI / 0	NI	NI	10 / 100	NI / 0	NI	NI	c
4567	Poudre de lait infantile avec probiotiques 1ᵉʳ âge	Infant formula with probiotics	6.54	10 / 100	4 / 1	40	1.60 / Ne	10 / 100	4 / 1	40	1.60 / Ne	c
5123	Poudre de lait infantile 1ᵉʳ âge avec probiotiques	Infant formula with probiotics	6.86	10 / 100	8 / 2	80	1.90 / Ne	10 / 100	8 / 3	80	1.90 / Ne	c
5124	Poudre de lait 2ᵉ âge avec probiotiques	Infant formula with probiotics	6.90	10 / 100	12 / 2	130	2.11	10 / 100	13 / 2	140	2.15	c
5125	**Poudre de lait infantile 1ᵉʳ âge**	**Infant formula**	6.92	1000 / 10000	5 / 1	5000	3.70 / Ne	1000 / 10000	5 / 1	5000	3.70 / Ne	c
5126	**Poudre de lait infantile 1ᵉʳ âge**	**Infant formula**	6.90	100 / 1000	5 / 1	500	2.70 / Ne	100 / 1000	5 / 1	500	2.70 / Ne	c
5127	**Poudre de lait en poudre écrémé**	**Milk powder**	6.76	100 / 1000	48 / 6	4900	3.69	100 / 1000	48 / 6	4900	3.69	c / c
5128	**Poudre de lait écrémé**	**Milk powder**	6.73	1000 / 10000	46 / 4	45000	4.65	1000 / 10000	46 / 4	45000	4.65	c
5129	**Poudre de lait écrémé**	**Milk powder**	6.78	1000 / 10000	131 / 14	130000	5.11	1000 / 10000	131 / 15	130000	5.11	c
5130	**Poudre de lait écrémé**	**Milk powder**	6.74	100 / 1000	55 / 2	5200	3.72	100 / 1000	55 / 2	5200	3.72	c
5379	**Poudre de lait écrémé**	**Milk powder**	6.93	100 / 1000	10 / 0	910	2.96	100 / 1000	10 / 0	910	2.96	c
5380	**Poudre de lait infantile 1ᵉʳ âge**	**Infant formula**	6.84	1000 / 10000	45 / 2	43000	4.63	1000 / 10000	45 / 2	43000	4.63	

Appendix G - Accuracy profile study: raw data

Panna cotta
Strain: *Bacillus cereus* Ad2117

Matrix	Target inoculation level(CFU/g)	N° sample	Reference method: ISO 4833-1				NEOGEN Petrifilm Rapid Aerobic Count Plate 26h at 30°C ± 1°C			
			Dilution	CFU/plate	CFU/g	log CFU/g	Dilution	CFU/plate	CFU/g	log CFU/g
Panna cotta batch n°1	300	5244	10	36	360	2.56	10	52	540	2.73
			100	4			100	7		
		5245	10	47	450	2.65	10	57	560	2.75
			100	2			100	5		
		5246	10	52	530	2.72	10	79	760	2.88
			100	6			100	4		
		5247	10	43	450	2.65	10	56	560	2.75
			100	6			100	5		
		5248	10	52	490	2.69	10	64	670	2.83
			100	2			100	10		
	10000	5249	100	97	10000	4.00	100	146	14000	4.15
			1000	14			1000	13		
		5250	100	103	10000	4.00	100	182	18000	4.26
			1000	10			1000	18		
		5251	100	93	9700	3.99	100	175	17000	4.23
			1000	14			1000	13		
		5252	100	77	8000	3.90	100	136	14000	4.15
			1000	11			1000	16		
		5253	100	95	9400	3.97	100	173	18000	4.26
			1000	8			1000	23		
	1000000	5254	10000	51	520000	5.72	10000	95	940000	5.97
			100000	6			100000	8		
		5255	10000	38	410000	5.61	10000	73	730000	5.86
			100000	7			100000	7		
		5256	10000	44	470000	5.67	10000	61	590000	5.77
			100000	8			100000	4		
		5257	10000	58	600000	5.78	10000	56	600000	5.78
			100000	8			100000	10		
		5258	10000	52	540000	5.73	10000	74	750000	5.88
			100000	7			100000	8		
Panna cotta batch n°2	300	5259	10	41	450	2.65	10	68	660	2.82
			100	8			100	4		
		5260	10	36	370	2.57	10	69	700	2.85
			100	5			100	8		
		5261	10	48	490	2.69	10	61	650	2.81
			100	6			100	10		
		5262	10	60	580	2.76	10	65	660	2.82
			100	4			100	8		
		5263	10	51	520	2.72	10	55	520	2.72
			100	6			100	2		
	10000	5264	100	87	8800	3.94	100	157	16000	4.20
			1000	10			1000	18		
		5265	100	109	12000	4.08	100	161	16000	4.20
			1000	18			1000	16		
		5266	100	114	11000	4.04	100	178	18000	4.26
			1000	10			1000	16		
		5267	100	100	10000	4.00	100	157	16000	4.20
			1000	11			1000	24		
		5268	100	94	9500	3.98	100	142	14000	4.15
			1000	10			1000	15		
	1000000	5269	10000	44	450000	5.65	10000	65	650000	5.81
			100000	6			100000	7		
		5270	10000	80	770000	5.89	10000	73	760000	5.88
			100000	5			100000	11		
		5271	10000	47	470000	5.67	10000	74	710000	5.85
			100000	5			100000	4		
		5272	10000	48	490000	5.69	10000	80	740000	5.87
			100000	6			100000	1		
		5273	10000	67	750000	5.88	10000	137	1300000	6.11
			100000	16			100000	9		

Infant formula

Strain: *Cronobacter sakazakii* Ad1446

Matrix	Target inoculation level(CFU/g)	N° sample	Reference method: ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate 48h ± 3h at 30°C ± 1°C			
			Dilution	CFU/plate	CFU/g	log CFU/g	Dilution	CFU/Petrifilm test	CFU/g	log CFU/g
Infant formula batch n°1	300	6022	10	23	240	2.38	10	26	290	2.46
			100	3			100	6		
		6023	10	21	240	2.38	10	29	280	2.45
			100	5			100	2		
		6024	10	20	280	2.45	10	13	140	2.15
			100	11			100	2		
		6025	10	22	250	2.40	10	49	500	2.70
			100	5			100	5		
		6026	10	19	260	2.41	10	37	360	2.56
			100	9			100	3		
	10000	6027	1000	11	12000	4.08	100	81	8500	3.93
			10000	2			1000	12		
		6028	1000	11	12000	4.08	100	100	10000	4.00
			10000	2			1000	12		
		6029	1000	12	13000	4.11	100	81	8400	3.92
			10000	2			1000	11		
		6030	1000	15	15000	4.18	100	87	9100	3.96
			10000	2			1000	13		
		6031	1000	10	12000	4.08	100	88	9200	3.96
			10000	3			1000	13		
	1000000	6032	100000	16	1600000	6.20	10000	115	1200000	6.08
			1000000	2			100000	13		
		6033	100000	14	1500000	6.18	10000	126	1300000	6.11
			1000000	2			100000	19		
		6034	100000	11	1100000	6.04	10000	124	1300000	6.11
			1000000	1			100000	15		
		6035	100000	11	1100000	6.04	10000	124	1300000	6.11
			1000000	1			100000	18		
		6036	100000	11	1400000	6.15	10000	112	1100000	6.04
			1000000	4			100000	14		
Infant formula batch n°2	300	6037	10	22	280	2.45	10	53	550	2.74
			100	9			100	7		
		6038	10	22	250	2.40	10	53	560	2.75
			100	5			100	8		
		6039	10	20	240	2.38	10	63	620	2.79
			100	6			100	5		
		6040	10	30	300	2.48	10	66	680	2.83
			100	3			100	9		
		6041	10	31	330	2.52	10	59	580	2.76
			100	5			100	5		
	10000	6042	1000	14	17000	4.23	100	131	12000	4.08
			10000	5			1000	19		
		6043	1000	18	18000	4.26	100	145	15000	4.18
			10000	2			1000	19		
		6044	1000	12	14000	4.15	100	131	13000	4.11
			10000	3			1000	16		
		6045	1000	15	14000	4.15	100	143	15000	4.18
			10000	0			1000	24		
		6046	1000	21	21000	4.32	100	140	14000	4.15
			10000	2			1000	12		
	1000000	6047	100000	15	1700000	6.23	10000	122	1300000	6.11
			1000000	4			100000	20		
		6048	100000	16	1500000	6.18	10000	110	1100000	6.04
			1000000	1			100000	15		
		6049	100000	12	1300000	6.11	10000	119	1200000	6.08
			1000000	2			100000	17		
		6050	100000	21	2000000	6.30	10000	110	1100000	6.04
			1000000	1			100000	9		
		6051	100000	15	1500000	6.18	10000	101	1000000	6.00
			1000000	2			100000	11		

Appendix H - Results obtained by the expert laboratory and the collaborative laboratories

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
ADRIA	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	183	1800	3.26	10	184	1900	3.28
		100	16			100	24		
	7	10	116	1100	3.04	10	99	1000	3.00
		100	9			100	13		
	1	100	117	12000	4.08	100	152	15000	4.18
		1000	10			1000	14		
	6	100	105	10000	4.00	100	81	8300	3.92
		1000	9			1000	10		
	4	1000	51	53000	4.72	1000	67	66000	4.82
		10000	7			10000	6		
	8	1000	74	76000	4.88	1000	82	81000	4.91
		10000	10			10000	7		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
A	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	72	730	2.86	10	72	710	2.85
		100	8			100	6		
	7	10	101	990	3.00	10	98	970	2.99
		100	8			100	9		
	1	100	110	12000	4.08	100	145	15000	4.18
		1000	17			1000	15		
	6	100	82	8600	3.93	100	115	11000	4.04
		1000	13			1000	9		
	4	1000	58	60000	4.78	1000	70	73000	4.86
		10000	8			10000	10		
	8	1000	106	110000	5.04	1000	120	120000	5.08
		10000	14			10000	9		
B	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	100	14	1500	3.18	10	122	1200	3.08
		1000	3			100	15		
	7	100	11	1100	3.04	10	104	1000	3.00
		1000	1			100	10		
	1	100	101	11000	4.04	100	124	13000	4.11
		1000	15			1000	14		
	6	100	173	17000	4.23	1000	20	21000	4.32
		1000	14			10000	3		
	4	1000	111	110000	5.04	1000	85	84000	4.92
		10000	7			10000	7		
	8	1000	98	100000	5.00	1000	64	65000	4.81
		10000	15			10000	7		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
C	2	10	7	70	1.85	10	5	50	1.70
		100	0	Ne	Ne	100	0		
	5	10	1	<40	<1.60	10	0	<10	<1.00
		100	0		detection	100	0		
	3	10	146	1500	3.18	10	122	1300	3.11
		100	17			1000	24		
	7	10	112	1100	3.04	10	108	1100	3.04
		100	9			100	11		
	1	100	105	9900	4.00	100	113	11000	4.04
		1000	3			1000	7		
	6	100	203	20000	4.30	100	211	21000	4.32
		1000	22			1000	19		
	4	1000	82	81000	4.91	1000	122	120000	5.08
		10000	7			10000	12		
	8	1000	83	85000	**4.93**	10000	39	400000	**5.60**
		10000	10			100000	5		
D	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
Analyses at Day 3	3	100	27	2600	3.41	10	248	2500	3.40
		1000	2			100	24		
	7	10	175	1800	3.26	10	192	1900	3.28
		100	20			100	16		
	1	1000	53	51000	4.71	1000	46	47000	4.67
		10000	3			10000	6		
	6	1000	49	51000	4.71	1000	35	36000	4.56
		10000	7			10000	5		
	4	1000	272	270000	5.43	1000	258	260000	5.41
		10000	25			10000	31		
	8	10000	32	310000	5.49	1000	258	260000	5.41
		100000	2			10000	24		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
F	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	240	2300	3.36	10	166	1700	3.23
		100	23			100	21		
	7	10	168	1700	3.23	10	143	1500	3.18
		100	14			100	22		
	1	100	161	16000	4.20	100	183	19000	4.28
		1000	13			1000	26		
	6	1000	26	24000	4.38	100	227	23000	4.36
		10000	0			1000	29		
	4	1000	227	250000	5.40	10000	23	220000	5.34
		10000	45			100000	1		
	8	1000	189	190000	5.28	1000	225	230000	5.36
		10000	17			10000	23		
G	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	48	480	2.68	10	65	660	2.82
		100	5			100	8		
	7	100	19	1900	3.28	100	86	7800	3.89
		1000	2			1000	0		
	1	1000	11	11000	4.04	100	80	8200	3.91
		10000	1			1000	10		
	6	1000	13	15000	4.18	100	92	9200	3.96
		10000	3			1000	9		
	4	1000	102	100000	5.00	1000	120	120000	5.08
		10000	13			10000	14		
	8	1000	118	110000	5.04	1000	78	80000	4.90
		10000	3			10000	10		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
H	2	10	3	<40	<1.60	10	0	<10	<1.00
		100	0		detection	100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
* spreading colonies at (-1)	3	100*	9	820	2.91	10	121	1200	3.08
		1000	0	Ne	Ne	100	16		
	7	100*	12	1100	3.04	10	162	1600	3.20
		1000	0			100	16		
	1	100	90	9500	3.98	100	121	11000	4.04
		1000	14			1000	4		
	6	100	68	6700	3.83	100	121	12000	4.08
		1000	6			1000	13		
	4	1000	62	66000	4.82	1000	155	160000	5.20
		10000	11			10000	23		
	8	100	64	6800	**3.83**	1000	80	76000	**4.88**
		1000	10			10000	4		
I	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	1			100	0		
	3	10	95	940	2.97	10	123	1200	3.08
		100	8			100	14		
	7	10	132	1300	3.11	10	120	1200	3.08
		100	9			100	14		
	1	100	98	9700	3.99	100	71	7700	3.89
		1000	9			1000	14		
	6	100	112	11000	4.04	100	113	12000	4.08
		1000	9			1000	15		
	4	1000	65	69000	4.84	1000	64	67000	4.83
		10000	11			10000	10		
	8	1000	57	58000	4.76	1000	82	85000	4.93
		10000	7			10000	11		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
J	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
Analyses at Day 3	3	100	210	22000	4.34	100	200	22000	4.34
		1000	36			1000	40		
	7	100	173	17000	4.23	100	200	23000	4.36
		1000	18			1000	50		
	1	1000	210	210000	5.32	1000	100	120000	5.08
		10000	20			10000	30		
	6	1000	192	200000	5.30	1000	300	280000	5.45
		10000	28			10000	10		
	4	10000	218	2200000	6.34	10000	100	1100000	6.04
		100000	22			100000	20		
	8	100000	42	4200000	6.62	10000	200	2100000	6.32
		1000000	4			100000	30		
K	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	70	690	2.84	10	43	450	2.65
		100	6			100	6		
	7	10	34	340	2.53	10	30	300	2.48
		100	3			100	3		
	1	100	132	13000	4.11	100	82	8100	3.91
		1000	6			1000	7		
	6	10	264	2700	3.43	100	43	4000	3.60
		100	28			1000	1		
	4	1000	35	37000	4.57	1000	29	30000	4.48
		10000	6			10000	4		
	8	1000	38	36000	4.56	1000	41	38000	4.58
		10000	2			10000	1		

Collaborator	N° sample	Reference method : ISO 4833-1				Alternative method: NEOGEN Petrifilm Rapid Aerobic Count Plate			
		Dilution	CFU/ plate	CFU/g	log (CFU/g)	Dilution	CFU/ Petrifilm	CFU/g	log (CFU/g)
L	2	100	20	2800	<1.00	10	0	<10	<1.00
		1000	10			100	0		
	5								
	3	1000	12	20000	4.30	100	95	9600	3.98
		10000	10			1000	10		
	7								
	1	1000	10	11000	4.04	100	100	10000	4.00
		10000	2			1000	10		
	6								
	4					1000	75	71000	4.85
						10000	3		
	8								
M	2	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	5	10	0	<10	<1.00	10	0	<10	<1.00
		100	0			100	0		
	3	10	62	680	2.83	10	76	770	2.89
		100	13			100	9		
	7	10	64	660	2.82	10	92	940	2.97
		100	8			100	11		
	1	100	87	9000	3.95	100	83	8500	3.93
		1000	12			1000	10		
	6	100	82	8300	3.92	100	87	8600	3.93
		1000	9			1000	8		
	4	1000	77	77000	4.89	1000	96	96000	4.98
		10000	8			10000	10		
	8	1000	56	61000	4.79	1000	85	88000	4.94
		10000	11			10000	12		